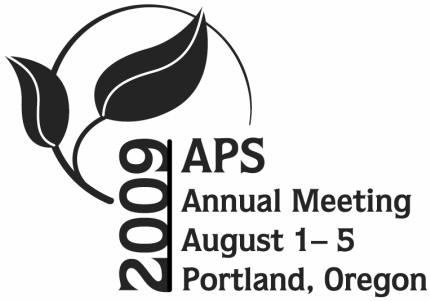

2009 APS Annual Meeting
Abstracts of Presentations

Abstracts submitted for presentation at the APS 2009 Annual Meeting in Portland, Oregon, August 1–5, 2009 (including abstracts submitted for presentation at the 2009 APS Pacific Division Meeting). The abstracts are arranged alphabetically by the first author's name.

Field assessment of non-toxigenic *Aspergillus flavus* strain K49 in competitive displacement of toxigenic isolates
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Phytopathology 99:S1

Non-toxigenic strains of *Aspergillus flavus* offer the potential to control aflatoxin contamination by competitive displacement of indigenous populations of *A. flavus* colonizing corn. Two sets of experiments were conducted to assess the competitiveness of strain K49 when challenged against two toxigenic isolates (F3W4 or K54) using a pin-bar inoculation technique. In 2007, corn ears were inoculated with six ratios of strain K49 and F3W4 in two experimental sites. A second study assessed the ability of equal densities of K49 when challenged with toxigenic strains F3W4 and K54 in 2007 and 2008. In the Stoneville site, when K49 comprised 10% of the inoculum, aflatoxin concentrations were reduced to ~500 ppb compared to 3500 ppb when inoculated with 100% F3W4. At the Elizabeth site, a 30% and 90% reduction in aflatoxin was observed when 10% and 50% of the inoculum was non-toxigenic strain K49, respectively. Strain K49 was capable of reducing aflatoxin contamination by ~90% when challenged with the high producing strain K54 or the moderate producer F3W4. In challenges with either F3W4 or K54, greater than 85% of isolates recovered were non-toxigenic, while 100% of the isolates were toxigenic when inoculated with F3W4 or K54 alone. These studies indicate that competition is affected by location and environmental conditions associated with the study, however, strain K49 had a similar degree of efficacy in competition with two toxigenic strains of varying potential for aflatoxin production.

Suppression of Phytophthora blight of cucumber and bell pepper with AG3 phosphonate
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Phytopathology 99:S1

Phytophthora blight or crown or root rot (*Phytophthora capsici* Leonian) is an important disease of a wide range of plant species including peppers, tomatoes, cucumbers and other cucurbits. AG3 phosphonate (Calirus 150, Bromine Compounds Ltd., Beer Sheva, Israel) is a new liquid formulation effective against Pythium damping-off of cucumber and clubroot of bok choy and cabbage. In this study, the effectiveness of AG3 phosphonate to suppress Phytophthora damping-off and root rot of cucumber or Phytopthora blight of bell pepper was determined in a peat-based mix or sandy-loam soil artificially infested with soil inoculum of *P. capsici* under growth room conditions. In an infested peat-based mix, AG3 phosphonate preplant amendment or postplanting drench treatments (0.05, 0.1, and 0.2% a.i.) and a 10-min seed-soak treatment (10.45% a.i.) significantly increased the percentage of healthy cucumber seedlings and reduced damping-off and root rot severity. The seed-soak and 0.2% treatments were the most effective treatments that consistently suppressed damping-off and root rot of cucumber even in a peat-based mix infested with high inoculum levels of *P. capsici*. The bell pepper transplants grown in an infested peat-based mix or a sandy-loam soil and received the single drench applications of AG3 phosphonate (0.05, 0.1, and 0.2% a.i.) showed significantly less incidence and severity of Phytophthora blight compared to the control plants receiving no treatment.

Quantitative trait loci associated with seedling and adult-plant resistance to oat crown rust caused by *Puccinia coronata*
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Phytopathology 99:S1

Crown rust is an economically important disease of oat worldwide, causing yield loss, reduction of test weight and seed quality, and increased lodging. Genetic resistance is an effective method to control crown rust. The oat line MN841801 has shown disease resistance to diverse populations of *P. coronata* for more than 30 years. The objective of this study was to identify and map the resistance in MN841801 at seedling and adult-plant stages using a population of 150 $F_{6:8}$ recombinant inbred lines of the cross of MN841801-1/'Noble-2'. The population was evaluated for crown rust resistance at the seedling stage using isolates avirulent on MN841801-1 and virulent on 'Noble-2'. Partial adult-plant resistance (APR) was evaluated in field and greenhouse experiments using two isolates virulent on both parents at the seedling stage but giving low infection on adult plants of MN841801-1. The seedling tests identified three loci for resistance on linkage groups MN3, MN6 and MN26. A total of nine quantitative trait loci (QTLs) were associated with APR. The two major APR QTLs overlapped with regions on MN3 and MN26 associated with seedling resistance. Comparison of our results with other crown rust resistance mapping studies indicates that seedling and APR resistance loci may be clustered in specific regions of the oat genome.

Evolutionary epidemiology of *Beet necrotic yellow vein virus* (BNYVV) in North America
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Phytopathology 99:S1

Analysis of the genetic structure of BNYVV populations from North America indicated that, before commercialization of resistant *Rz1*-cultivars, a wild type viral haplotype encoding the ACD p25-motif (RNA3) predominated in susceptible sugarbeets nationwide. Recently, rhizomania has emerged in resistant cultivars. This outbreak was associated with evolution of BNYVV from ACD to ALD (avirulent) to VLE (resistance breaking) p25-motifs in

'Imperial Valley' of California. However, this evolutionary trajectory has not been found in other pathosystems, which suggests an alternative mechanisms of BNYVV to overcome *Rz1*. Field root-sampling indicated that most asymptomatic *Rz1*-plants were subliminally infected by BNYVV. Avirulent isolates were 2–3 times more genetically heterogeneous than wild type isolates infecting susceptible plants, and most of their variability was within isolates. By contrast, resistance breaking isolates were also highly heterogeneous, but mainly among isolates. Serial passages of an ACD-isolate reproduced its genetic stasis in susceptible plants and its high variability in resistant plants. In addition, these assays revealed that virus diversification was greater in plants with stronger restriction to virus accumulation. Moreover, the ratio between intraplant-interplant viral nucleotide diversity was directly correlated with virus fitness to specific host environments.

Assessing resistance in wheat to *Xanthomonas translucens* pv. *undulosa*
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Phytopathology 99:S2

Bacterial leaf streak (BLS) caused by *Xanthomonas translucens* pv. *undulosa* (Xtu), is a re-emerging disease of wheat in the northern Great Plains of the United States. Planting resistant cultivars offers the best approach to control BLS in the absence of effective bactericides. However, currently grown wheat cultivars appear to have inadequate level of resistance to control BLS. This study was conducted to determine genetic relationships among 39 strains of Xtu using repetitive sequence-based PCR (rep-PCR) and insertion sequence-based PCR (IS-PCR) primers and evaluate the reaction of wheat cultivars, land races, and advanced breeding lines to BLS in a greenhouse. The results suggested that the strains were highly diverse and similarity coefficients based on the three primers and clustering by UPGMA revealed four clusters. One cluster consisted of 30 strains from both wheat and barley. The other clusters contained a few strains or a single strain. Wheat accessions showed a wide range of susceptibility to BLS. Four wheat cultivars and breeding lines with the low disease scores were identified and these will be evaluated further for their utility in breeding wheat for resistance to BLS.

***Tsn1*-mediated host response to Ptr ToxA**
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Phytopathology 99:S2

The sensitivity gene, *Tsn1*, interacts with the virulence determinant *ToxA* effector from *Pyrenophora tritici-repentis* in an inverse gene-for-gene interaction and confers cell death in sensitive wheat. We used the Affymetrix GeneChip® wheat genome array to investigate the physiological and molecular responses of sensitive wheat to *ToxA*. Functional clustering revealed regulation of genes involved in defense-related, signal transduction, oxidative stress, cell wall associated, protein turnover, and phenylpropanoid pathways. Nine candidate genes were selected from defense-related and signal transduction classes and gene expression profiles were validated using quantitative real-time PCR. These results suggest that following perception of *ToxA* by *Tsn1* induces transcriptional re-programming events that are of commonly associated with disease resistance reactions following *Avr-R*.

A method for the identification of RNA viruses of miscanthus and switchgrass
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Phytopathology 99:S2

Miscanthus × *giganteus* and *Panicum virgatum* (switchgrass) are two potential biomass crops being evaluated for cellulosic ethanol production. Viral diseases are potentially significant threats to these crops. *M.* × *giganteus* is sterile, and is vegetatively propagated through the use of rhizomes, thus biomass production could be reduced by spread of viruses by planting infected rhizomes. Therefore, identification of viruses infecting these crops is important for quarantine purposes, virus resistance breeding, and production of virus-free planting materials. Here, we report a new generic method that was used to detect known and unknown RNA viruses. The method involves partial virus purification of viruses from infected leaf tissue, nuclease digestion of unencapsidated nucleic acids, extraction of encapsidated viral RNA, random amplification, cloning, sequencing of amplicons, and BLAST searches for similar sequences in GenBank. Using this method, we identified a possibly new *Marafivirus* in *P. virgatum* that is 76% identical to the nucleotide sequence of *Maize rayado fino virus* (MRFV), its closest relative in GenBank. *Sugarcane mosaic virus* (SCMV) from *M.* × *giganteus* and *Maize dwarf mosaic virus* (MDMV) from Johnsongrass also were identified. The method is being optimized for the detection of additional RNA and DNA viruses from different genera and families.

Nutritional requirements of *Xylella fastidiosa* that causes bacterial leaf scorch of blueberry
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Phytopathology 99:S2

Two undefined media (PW and CS20) and one defined medium (XF-26) were compared for their abilities in supporting the primary isolations of *Xylella fastidiosa* (Xf) from tissues of four diseased blueberry plants. One gram of stem tissues from each plant was sterilized with 15% Clorox for 3 min before being rinsed 3 times in sterile water. Each tissue was minced in 3 mL of PW broth; 0.1 mL of the minced sap was used for a 10-fold serial dilution in PW broth to 10^{-8}. One tenth of one mL of each cell suspension from the following dilutions was placed onto the three agar media and spread with an L-shaped glass rod: 10^{-3}, 10^{-4}, and 10^{-5}. CFUs per plate per dilution were counted and were then converted to CFUs per gram of tissues. The average CFUs/g tissues were 8.6×10^6, 8.5×10^6, and 9.0×10^5 for PW, CS20, and XF-26 respectively. Of the 17 amino acids in XF-26 medium, single amino acid elimination from the medium was conducted to elucidate the essentiality of each amino acid for the *in vitro* growth of blueberry strains of Xf. Seven serial sub culturing weekly in each medium were performed and the results indicated that gly, met, ser, asn, and his were essential while ala, arg, cys, gln, ile, leu, lys, phe, pro, thr, try, and val were nonessential for the growth of blueberry Xf in define medium XF-26. Other ingredients included in XF-26 were K_2HPO_4, $(NH_4)_2HPO_4$, $MgSO_47H_2O$, trisodium citrate, disodium succinate and potato starch.

Incidence of Tomato double streak virus disease in Varamin region
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Phytopathology 99:S2

During the years 2006 to 2008, a survey was conducted through different tomato fields in Varamin city. A suspicious disease with stem necrosis streaks and necrotic spots symptoms on fruits recorded on tomato plants in some tomato plants. Using electron microscopic observation filamentous virus-like particles were observed within diseased tomato crude extracts. In order to evaluating the biological properties of suspected viral agents, indicator herbaceous plants were also mechanically inoculated. Symptomatic tomato leaf, stem and fruit samples were collected and serologically tested by DAS-ELISA method for the presence of ToMV, TMV and PVX viruses by means of Agdia commercial polyclonal antibodies (Agdia, Inc, U.S.A.). According to the results obtained by DAS-ELISA method, ToMV TMV and PVX viruses were detected alone or in mixed infection among the tomato plants showing stem necrotic streak symptoms. Symptoms appeared on inoculated indicator plants were in concordance with what had been reported before for the assayed viruses two weeks after virus inoculation. Using RT-PCR molecular method and specific primers designed for the coat protein sequence, presence of the TMV and ToMV confirmed for the ELISA positive tested tomato plants. All tested samples were amplified a 700 bp, 695 and 340 bp fragments for TMV, ToMV and PVX tested samples. This is the first report of tomato infecting double streak virus disease in Varamin tomato plantation.

Molecular diversity and recombination in a foveavirus infecting grapevine
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Phytopathology 99:S2

Rupestris stem-pitting associated virus (RSPaV; genus *Foveavirus*; family *Flexiviridae*) is widely distributed in grape-growing countries worldwide. We studied the genetic diversity of RSPaV in Pacific Northwest (PNW) vineyards. The coat protein (CP) and a portion of the replicase (Rep) from seventy three isolates were amplified, cloned, sequenced and phylogenetically compared with corresponding sequences of RSPaV isolates deposited in GenBank from other countries. In pair wise comparisons, the CP sequences from 54 isolates showed nucleotide sequence identities ranging from 79 to 100% and amino acid sequence identities ranging from 86–100%. The Rep

sequences from 53 isolates also showed similar range of identity values. Seven of the 53 isolates showed intra-isolate genetic diversity in the CP, indicating mixed infection of diverging viral variants within a single grapevine. This was observed most frequently in grafted vines. Phylogenetic incongruence (an indicator of recombination) was observed based on the position of some RSPaV isolates in the CP- and Rep-based phylogenetic groupings. Further analysis of these isolates by Recombination Detection Program version 3 (RDP3) Beta 27 indicated more recombination events in the CP sequences than in the Rep sequences. These results indicate the quasispecies nature of RSPaV and provide evidence for the first time of the occurrence of recombination in the genus *Foveavirus*.

Molecular characterization of two novel soybean-infecting begomoviruses from Nigeria

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Phytopathology 99:S3

Two begomoviruses infecting soybean (*Glycine max* L. Merr.) from Nigeria were molecularly characterized. They are provisionally named as Soybean mottle mosaic virus (SbMMV) and Soybean yellow mosaic virus (SbYMV). SbMMV produced mottled mosaic symptom and SbYMV produced yellow mosaic symptom in soybeans. SbMMV is a monopartite with a DNA-A component of 2768 nucleotides (nt), whereas SbYMV is bipartite with a DNA-A component of 2708 nt and a DNA-B component of 2647 nt. DNA-A of SbMMV has an AV2 open reading frame (ORF), a signature ORF present in 'Old World' begomoviruses, and the DNA-A of SbYMV contains no AV2 ORF. A comparison of DNA-A components showed 62% identity among the two viruses. SbYMV showed a maximum of 74% nt sequence identity with *Cowpea golden mosaic virus* and SbMMV showed a maximum of 65% nt identity with *Mungbean yellow mosaic India virus*. Phylogenetic analysis revealed that both viruses clustered within the unique clade formed by legume-infecting *Begomovirus* species. Preliminary survey using PCR-based diagnosis of the two viruses showed widespread occurrence of SbYMV, compared to SbMMV across soybean-growing regions of Nigeria.

Genetic diversity of endogenous plant pararetorival sequences associated with dahlia mosaic from geographically diverse sources of dahlia

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Phytopathology 99:S3

Three distinct caulimoviruses have been reported to infect dahlia (*Dahlia variabilis*): *Dahlia mosaic virus* (DMV), Dahlia common mosaic virus (DCMV) and an endogenous plant pararetrovirus (DMV-D10). To better understand the genetic diversity of DMV-D10, the dsDNA genome of a DMV-D10 isolate from Lithuania was cloned, sequenced and compared to those previously reported from the US (DMV-D10), Mexico (WDMV-D10) and New Zealand (DMV-NZ). DMV-Lithuania had 90% nucleotide (nt) sequence identity with DMV-D10 and WDMV-D10 and shared 88% identity with DMV-NZ when the complete genomes were compared. When the nt sequences of ORF I, ORF III and ORF VI were individually compared, the sequence identity between DMV-D10 from Lithuania and DMV-D10 from Mexico and the US ranged from 88% to 96%. Information on the genetic diversity of endogenous plant pararetroviral sequences in cultivated and wild dahlia species would provide increased understanding of the evolutionary pathways of these reverse transcribing viral elements.

Monitoring host responses to zebra complex disease on potatoes

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Phytopathology 99:S3

Zebra Complex (ZC) is a newly emerged disease whereby affected plants display a variety of foliar symptoms that resemble those caused by other known pathogens. The only reliable way to determine presence of ZC is a chip-frying test at the end of the growing season. Therefore, there is a need to develop reliable methods to correctly identify ZC affected plants at early disease stages. For this purpose we initiated DNA and protein based tests towards characterization of ZC and its recently found associated *Ca.* liberibacter. Multiplex PCR was developed to assess both the presence of *Ca.* liberibacter and the DNA quality of either ZC affected plants or the suspected potato psyllid vector. In addition, protein profile comparisons were performed on several ZC and healthy (H) plants. Results showed that ZC plants contained more protein than H plants and the ZC stem protein profile resembled a tuber protein profile. Using mass spectrometry we identified two proteins BT3 and cyclophilin, which accumulated in a ZC-dependent manner. Moreover, ZC appeared to modify the identity of the stem since it was showing physiological tuber properties, as was verified by monitoring levels of starch upon lugol staining of different tissues showing accumulation of starch in stems only of ZC plants. The developed tests and the ZC characteristic host responses will be implemented in studies of ZC and related syndromes, i.e. psyllid yellows, to help understanding these potato diseases.

Analysis of ribosomal DNA-ITS region for grouping of *Rhizoctonia* species isolated from turfgrass in Maryland and Virginia

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Phytopathology 99:S3

More than 400 *Rhizoctonia* isolates were collected from diseased turfgrass leaves from five geographic areas in Virginia and Maryland. A random sample of 54 isolates was selected and their anastomosis groups (AGs) were determined by hyphal fusion reactions with *Rhizoctonia* tester strains. The internal transcriber spacer (ITS) region of the ribosomal DNA of the isolates was sequenced and compared to known sequences of *R. solani*, *Waitea circinata var. zeae* (Wcz), and *Waitea circinata var. circinata* (Wcc). *R. solani* isolates anastomosed with AG1-1A, AG2-2IIIB and AG5. Seven isolates which did not anastomose with *R. solani* tester strains were identified as bi-nucleate *Rhizoctonia* species (*Ceratobasidium sp*.) by comparing with GenBank deposited sequences. Most isolates of Wcz and Wcc anastomosed with tester strains of each other. The cladistic analysis of ITS sequences supported five monophyletic groupings for the isolates tested. There was strong evidence for separation of *Waitea circinata*, *Ceratobasidium sp*. and *Rhizoctonia solani* into different clades. The phylogenetic analysis of ITS corresponded well with previously known AG sequences by grouping similar AG sequences together. Overall, anastomosis by hyphal fusion supported genetic relatedness of the isolates as determined by sequence analysis.

Resistance to respiration inhibitor fungicides in *Monilinia fructicola* field isolates from South Carolina and Georgia

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Phytopathology 99:S3

Respiration inhibitor (RI) fungicides, i.e. quinone outside inhibitors and carboxamides, are major components of current spray programs for peach brown rot management. From 2006 to 2008, a total of 157 *M. fructicola* isolates were collected from an orchard that had never been sprayed with RI fungicides and from orchards with multiple years of RI exposure in South Carolina and Georgia. The sensitivity of isolates to azoxystrobin, pyraclostrobin, boscalid, and pyraclostrobin+boscalid was determined in conidial germination and mycelial growth tests. Mean EC_{50} values from ten South Carolina and five Georgia populations to azoxystrobin, pyraclostrobin, and boscalid were significantly higher ($P = 0.01$) compared to the mean EC_{50} value of the baseline population. The mean EC_{50} values of isolates collected from 2006 to 2008 increased 4- and 3-fold for boscalid and azoxystrobin, respectively, indicating a shift in isolate sensitivity. Ten isolates with different EC_{50} values were selected for fungicide efficacy tests on detached fruits. There was a strong correlation between disease incidence and in vitro sensitivity (EC_{50} value) to azoxystrobin ($r = 0.81$; $P = 0.005$), pyraclostrobin + boscalid ($r = 0.79$; $P = 0.01$), and boscalid ($r = 0.74$; $P = 0.01$). Isolates with high EC_{50} values were not fully controlled with the field rates of common RI fungicides. Our results indicate that RI-resistant *M. fructicola* populations are building up in commercial peach orchards of the Southeast.

Ecophysiological determinants of three important peach pathogens: *Monilinia fructicola*, *Rhizopus stolonifer*, and *Gilbertella persicaria*

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Phytopathology 99:S3

Environmental factors favouring natural niches of plant pathogens are key components for designing management strategies. In this study, *Monilinia fructicola*, *Rhizopus stolonifer*, and *Gilbertella persicaria,* three important pathogens of peach fruit were investigated for their ability to assimilate different carbon (C) and nitrogen (N) sources and for the effect of water activity (a_w), pH and temperature on their germination and growth. The fungi were grown on a liquid medium containing lactose, glucose, sucrose, fructose, dextrose, galactose, maltose, or raffinose as C sources and urea, NH_4NO_3, $NaNO_2$, glycine, bovine serum albumin, peptone, beef extract or glutamine as N sources. Biomass yield of *M. fructicola, R. stolonifer*, and *G. persicaria* was the highest on raffinose, sucrose, and galactose, respectively. All species yielded the highest biomass when beef extract was used as a N source. *M. fructicola* germinated and grew between pH 2 and 7. *R. stolonifer* and *G. persicaria* were inhibited at pH < 3 but grew up to pH 10. Germination and mycelial growth of *M. fructicola, G. persicaria*, and *R. stolonifer* were

completely inhibited at a_w levels of 0.96, 0.92, and 0.89, respectively at 20 or 30°C, and completely inhibited at 5°C regardless of the a_w. The differential ability of these peach pathogens to develop under different conditions will help in understanding their epidemiology and in developing best control strategies for each species.

Transposable elements in *Verticillium dahliae* and *V. albo-atrum*
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Phytopathology 99:S4

The genomes of the filamentous fungi *Verticillium dahliae* and *V. albo-atrum* have recently become available. Transposable elements have been implicated in fungal genome evolution previously and we therefore set out to characterize elements found in *Verticillium* species. In *V. dahliae* we identified long terminal repeat (LTR) retroelements, including two of the copia class and a group of three highly related gypsy class elements, as well at least one DNA transposon. Partial sequences corresponding to these retroelements have been identified in *V. albo-atrum.* The identification and characterization of other *V. albo-atrum* elements is in progress. Southern hybridization analysis is being done to assess the distribution of the *V. dahliae* retroelements within various North American strains of *Verticillium.* A large variation was found in copy number between strains, suggesting that some or all of these elements are active. ESTs corresponding to each element have been identified, and further analysis of gene expression is being conducted. Variation of retroelement distribution between strains will be presented with respect to genome evolution and the potential use of these elements as diagnostic markers.

Transformation of *Phomopsis viticola* with the green fluorescent protein
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Phytopathology 99:S4

Phomopsis viticola is the causal agent of Phomopsis cane and leaf spot on *Vitis* spp., which is a serious and economically important disease in temperate regions. Here we describe the transformation of this fungus with two different constructs (pBHt2_sGFP and pIGPAPA) containing the green fluorescent protein (GFP) and the hygromycin B resistance gene (*hph*). Protoplast mediated transformation yielded three fluorescent transformants (DA-2, DA-4, and DA-5) and multiple non-fluorescent transformants. All transformants obtained were mitotically stable. The growth rates of DA-4 and DA-5 were slightly, but significantly ($P < 0.05$), less than DA-2 and OH-22 (wild type); however, transformation resulted in no change in sporulation rate, log spore production, and virulence on grape internodes and leaves compared to the wild type. These transformants will be useful tools for elucidating fungal penetration of host plants, invasive growth, and the nature of its host association.

Regulation of c-di-GMP intracellular levels in *X. fastidiosa*
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Phytopathology 99:S4

Xylella fastidiosa is a bacterium that lives in the xylem of hundreds of plant species and causes a number of economically important plant diseases, including Pierce's disease (PD) of grapevine. PD symptoms are related to water stress due to the occlusion of the xylem by extensive bacterial colonization, extracellular polysaccharide production and biofilm formation. Our project focuses on characterizing of the role that cyclicdiguanylate (c-di-GMP) signaling system plays in mediating biofilm formation and virulence of *X. fastidiosa.*c-di-GMP is a second messenger that regulates bacterial biological processes including biofilm formation, motility and virulence in several bacterial pathogens. This molecule is synthesized by diguanylate cyclase enzymes (DGCs) and is degraded by phosphodiesterases (PDEs). DGC activity resides in the GGDEF domains of these proteins and PDE reside in proteins containing EAL or HD-GYP domains. In the *X. fastidiosa* genome there are 6 genes that are predicted to encode proteins that contain the conserved GGDEF, EAL and/or HD-GYP domains. We predict that these domains control the intracellular c-diGMP concentration and hence c-di-GMP signaling. We will present results describing the effect of overexpressing *X. fastidiosa* genes encoding GGDEF, EAL or HD-GYP on c-di-GMP intracellular levels. In addition, we will present results describing the effect that altered levels of intracellular c-di-GMP exerts on biofilm formation, cell aggregation and movement of *X. fastidiosa* in vitro.

A hypersensitive response in *Nicotiana* species within the Alatae section is elicited by the *Tomato bushy stunt virus* coat protein p41 gene
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The *Tomato bushy stunt virus* (TBSV) P22 protein elicits a hypersensitive response (HR) in *Nicotiana glutinosa* and *N. edwardsonii*, whereas the P19 protein elicits an HR in *N. tabacum*. However, little is known about the resistance to TBSV in many other *Nicotiana* species. To explore this topic, we inoculated TBSV virions to 18 *Nicotiana* species representative of 10 of the 14 taxonomic sections in the genus. We found that in addition to the three mentioned species, eight other species reacted with HR: *N. arentsii*, and *N. undulata* (Undulatae section); *N. langsdorfii*, *N. longiflora*, and *N. bonariensis* (Alatae section); *N. rustica*, (Rusticae section); *N. repanda*, (Repandae section); and *N. sylvestris*, (Sylvestres section). Four species were resistant without involving HR, and three were susceptible. We subsequently agroinfiltrated constructs expressing TBSV P22 and P19 proteins into leaves of the 11 species that responded with HR. *N. glutinosa* and *N. edwardsonii* reacted with HR to P22, whereas *N. tabacum*, *N. sylvestris*, and *N. bonariensis* reacted with HR to P19. To elucidate if other TBSV proteins could elicit HR in the other six HR-dependent *Nicotiana* species, we created new constructs for agroinfiltration of the TBSV p41 coat protein, and the p33 and p92 replicase genes. We found that *N. langsdorfii*, *N. longiflora*, and *N. bonariensis* (Alatae section) developed HR to the TBSV P41 protein. This study shows that agroinfiltration can be used to identify new avirulence targets and resistance genes across a plant genus.

The effects of chemical control, cultivar resistance and root system on black shank incidence, caused by *Phytophthora parasitica* var. *nicotianae*
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Black shank, caused by *Phytophthora parasitica* var. *nicotianae* (*Ppn*), is a major disease of tobacco. The effects of chemical control, cultivar resistance and root system on black shank incidence were investigated. The fungicide mefenoxam, alone or in combination with dichloropropene+chloropicrin, was applied at certain rates and tobacco growth stage in naturally infected fields in North Carolina. The effectiveness of these fungicides depends on cultivar, predominant black shank race and time of application. When race 1 was predominant, the best control was offered to the planted cv. K346 by applying 0.117 l m^{-2} of mefenoxam at the first cultivation and layby. Regarding the resistance of flue-cured cultivars against race 0 and 1, the highest control to both races was provided by the line RJR75. In addition, the root system structure of cultivars with or without the *ph* gene that provides immunity to race 0 of *Ppn* was studied. NC196 and SP227, cultivars carrying the *ph* gene, produced fewer side roots (roots emerged at the stalk base) than cultivars with partial resistance (K326, K346). This result explains the mechanism of resistance to Ppn that is avoidance of infection by *Ppn* zoospores due to smaller root system. Based on our results, application of mefenoxam before the appearance of black shank symptoms and the use of cultivars with high partial resistance or possessing the *ph* gene when race 1 or race 0 is predominant, respectively, appear to be the most critical factors in controlling *Ppn*.

Investigating the mechanism of pathogenesis of *Phytophthora parasitica* var. *nicotianae* transformed with GFP on various tobacco cultivars
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Three races of *Phytophthora parasitica* var *nicotianae* (*Ppn*) have been reported in North Carolina: race 0, 1 and 3. The predominant race in the flue-cured tobacco-growing areas is race 0. Race 1 occurs under selection pressure resulting from continuous cultivation of completely resistant cultivars (*ph* gene) against race 0. Due to the rapid increase of race 1 in tobacco fields, the mechanisms of pathogenicity of the *Ppn* races against tobacco cultivars are under investigation. The cytological biomarker green fluorescent protein (GFP) was used to transform isolates of *Ppn* races to study its pathogenesis, penetration and ramification into plant tissues, and to quantify the grown fungal-like biomass into tobacco cultivars. The pABH6 and pTHnptII linearized plasmid constructs carrying the GFP cassette and G418 resistance, respectively, were used to transform *Ppn* protoplasts with the *gfp* gene. Transformed fungal-like strains homogeneously expressing GFP were visualized using an epi-fluorescent microscope with UV light at 490 nm. A

pathogenesis test for the *gfp* mutants was carried out to verify whether transformation affects their infection ability in comparison to the corresponded parental isolate. Differences revealed according to the completed time-series test for the pathogenesis-ramification process in tobacco tissues of all *Ppn*-GFP races and by the biomass of a pathogen expressing GFP in infected tobacco cultivars that was quantified via fluorometer device counts at 490 nm, are discussed.

The alternate major effector AvrXa7 in bacterial blight of rice evades host resistance by targeting an alternate major host susceptibility gene
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Xanthomonas oryzae pv. oryzae (*Xoo*), the causal agent of bacterial blight of rice depends on type III transcription activator like (TAL) effectors for effective pathogenicity and host colonization. Previous studies in our lab have demonstrated that one of the TAL effectors PthXo1 targets host susceptibility gene *Os8N3*. Here we present that another TAL effector AvrXa7 targets another member of the same MtN3 family, *Os11N3*. The expression of *Os11N3* is induced several fold during infection with strains carrying *avrXa7* in susceptible IR24 plants but not induced in plants containing broad recessive resistance gene *xa5*. *xa5* is an allele of *TFIIAg5*, which encodes the g subunit of eukaryotic transcription complex TFIIA. The results indicate that the induction of *Os11N3* by Avrxa7 requires the presence of wild type *TFIIAg5* allele. Knocking down of *Os11N3* through RNA interference made the plants resistant to strains with *avrXa7* and not *pthXo1*. Rice plants were inoculated with *Xoo* strains with FLAG-tagged AvrXa7 and chromatin immuno-precipitation assay done using monoclonal FLAG antibody resulted in an enrichment of *Os11N3* promoter and not *Os8N3* promoter.

The RdRp gene of *Velvet tobacco mottle virus*
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Velvet tobacco mottle virus (VTMoV) occurs as a natural infection in *Nicotiana velutina* (common name: Velvet tobacco), a native of the arid region of central Australia. VTMoV is distinguished by its narrow host range and transmissibility by the mirid *Cyrtopeltis nicotianae* [Hemiptera; Miridae]. It is classified as a *Sobemovirus* from its morphology, cellular localisation of virions, and general nucleic acid features, including encapsidation of a type of virusoid RNA specific to sobemoviruses. We aim to; 1) sequence the positive sense ssRNA genome of VTMoV; 2) compare multiple isolates for sequence variation and 3); determine whether mirid transmission is associated with any specific sequence motifs. Over half the genome of one VTMoV isolate has been assembled into a single continuous fragment using an RT-PCR based primer walking strategy, combining both degenerate sobemovirus primers and specific VTMoV primers. This sequence includes part of the coat protein and VpG gene sequences, as well as the full RNA dependent RNA polymerase (RdRp) gene. Analysis of the RdRp sequence supports classification of VTMoV as a *Sobemovirus*. Currently a range of VTMoV isolates are being evaluated for variation in the RdRp gene. The RdRp gene will be used as a descriptor of the molecular ecology of VTMoV, a wild plant virus occupying an ecological niche unconstrained by agricultural practices.

Global gene flow of *Verticillium dahliae* affecting lettuce in California
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Verticillium dahliae causes severe losses in lettuce and other vegetables in California's Salinas (SV) and Pajaro (PV) Valleys. Since 1995, in parallel with the expansion in spinach production, Verticillium wilt emerged on lettuce in this region. The disease has since caused significant lettuce crop failures. Although Verticillium wilt occurred in SV and PV prior to 1995, lettuce was immune. All vegetable seed is imported from US and international sources for vegetable production in SV and PV. To investigate potential sources of *V. dahliae* in lettuce, we characterized 22 polymorphic simple sequence repeats (SSR) by analyzing the genome of *V. dahliae* isolate Ls17 from lettuce. From the SSRs, negligible differentiation was measured between *V. dahliae* populations from lettuce plants (n = 60) and US and European spinach seed (n = 43). Conversely, significant differentiation was measured with a tomato population (n = 65) from the San Joaquin Valley, where lettuce seed is produced. Significant admixture was observed between lettuce and spinach populations, whereas the tomato population formed a separate cluster with little evidence of admixture. Elevated symmetrical migration of isolates was measured between lettuce and spinach, but significantly smaller migrations were identified between these two crops and the tomato. These findings suggest a potentially significant impact on lettuce production from imported spinach seed infected with *V. dahliae* into the SV and PV.

Mutation of *avrXg1* and *flgC* genes affect motility and virulence of *Xanthomonas axonopodis* pv. *glycines*
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Mutations in two predicted functional domains, the 4th central repeat and an acidic activation domain, of *avrXg1* in *Xanthomonas axonopodis* pv. *glycines* (*Xag*) resulted in enhanced virulence and bacterial population on resistant (Williams82) and susceptible (Spencer) cultivars. Virulence factors, cellulase and pectate lyase were increased in the *avrXg1* mutant resulting in 20 and 11% increase in severity of bacterial pustule on susceptible and resistant cultivars, respectively. Furthermore, the *avrXg1* mutant exhibited enhanced swimming motility suggesting a possible role for plant genes in bacterial motility. The effect of swimming motility on virulence and fitness was studied further by constructing a *flgC* deletion mutant. This resulted in a dominant-negative effect on *Xag* virulence on soybean. Reduced biofilm production and complete loss of disease induction were observed suggesting that functional flagella are essential for motility and virulence. This is the first study to demonstrate a role for motility in virulence of *Xag* on soybean.

Potential alternative hosts for a powdery mildew on pea
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Powdery mildew of pea (*Pisum sativum*) is an important disease in the field and in the greenhouse. The most widely documented powdery mildew on pea is *Erysiphe pisi,* but *E. trifolii* and *E. baeumleri* have also been reported. From greenhouse-grown peas, we obtained powdery mildew samples with rDNA ITS sequences nearly identical to previously deposited sequences of *E. trifolii*. Because detailed studies on host range of this pea powdery mildew in the US Pacific Northwest were lacking, we tested common legume plants from the region as potential alternative hosts. Eleven species were used in greenhouse cross inoculation studies: *Lens culinaris*, *Glycine max, Melilotus albus, M. officinalis, Medicago polymorpha, M. lupulina, M. scutellata, Lathyrus latifolius*, *Trifolium pratense, Vicia cracca,* and *V. faba*. Except for *Glycine max,* all the plant species tested developed powdery mildew lesions in 10–14 days after inoculation. Susceptibilities of two of these species (*L. culinaris* and *M. albus*) were also confirmed with detached leaf assays. Results showed that all the above legumes (except soybean) are potential alternative hosts for the *E. trifolii* found on pea. Powdery mildews found on wild legumes (*Meliotus albus* and *Medicago lupulina*) were also confirmed to be *E. trifolii*, suggesting that the wild legumes could be inoculum sources of powdery mildew on greenhouse pea plants during winter months. These findings have implications in managing powdery mildew of pea.

Systemic movement of fungicides in peanut plants in the field
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Systemic movement of fungicides within plants can affect disease control and needs to be understood. Three terminal fully expanded leaves of a primary lateral branch of 90-day old Tifrunner peanut were treated with prothioconazole + tebuconazole (Provost, 0.58 kg/ha a.i.), azoxystrobin (Abound, 0.88 kg/ha a.i.), or flutolanil (Moncut, 0.79 kg/ha a.i) to determine fungicide movement. Leaves and pods on the same branch below the treated leaves were sequentially numbered from 1 to 3, with 1 being the closest to the treated foliage. Nontreated numbered leaves, stems, new leaves, and pods were sampled 4, 8, and 12 days after treatment for bioassay with *Sclerotium rolfsii*. Provost was acropetally translocated to the new leaves 12 days after treatment, and leaves 1 to 3 also had reduced colonization of *S. rolfsii* 8 days after treatment. The proximal pods had 57% less colonization 12 days following treatment, but distal pods were similar to the nontreated. Moncut was highly translocated to new leaves 12 days after treatment, and showed moderate reductions of colonization on lower leaves. Abound was readily translocated to the new leaves but showed little activity on stems and proximal leaves, and no activity on pods and distal leaves. Movement of fungicide to the lower foliage clearly is occurring. Whether this downward movement is systemic or is simply wash off and physical relocation of fungicide residues is unclear, but it may improve control of soilborne diseases.

Inhibition of grapevine powdery mildew by improved vineyard sunlight exposure

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The commonly observed phenomenon that severity of grapevine powdery mildew, caused by *Erysiphe (Uncinula) necator*, is lower on tissues exposed to full sunlight than those in the shade has received little formal study. We measured vine canopy density using an expanded Point Quadrat Analysis technique and showed strong correlations between increased canopy density/shading and disease severity in both sprayed and upsprayed vineyards. Through manipulations of the vineyard canopy to regulate sunlight exposure, we decreased disease severity on fruit and leaves by up to 50% and 87%, respectively, by increasing illumination. Mesoclimate environmental monitoring showed no differences in ambient air temperature or relative humidity in the exposed versus shaded portions of the canopy. Relative to shaded leaves, sunlight exposure raised mid-day leaf surface temperatures 5–15°C, frequently into a range detrimental to fungal development or survival. Furthermore, ultraviolet radiation (UV-B) levels in the shaded areas averaged approximately 90% less than in the direct sun. UV-filtering material placed over sun-exposed vines led disease severity on leaves and clusters to be intermediate between shaded (most severe) and sun-exposed (least severe) vines. Implementing our findings via leaf removal around clusters at fruit set within a vertical-shoot-positioned training system reduced fruit disease severity by 35% relative to Umbrella-Kniffen trained vines without leaf removal.

Monitoring the sensitivity to boscalid of *Alternaria alternata* populations from California pistachio orchards

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Phytopathology 99:S6

The sensitivity to boscalid of approximately 700 single-spore isolates of *Alternaria alternata* was assessed using a rapid screening based on the mycelial growth test in agar media amended with the discriminative dose of 10 ppm boscalid. The isolates were isolated from infected pistachio tissues collected during the 2005, 2006, 2007, and 2008 growing seasons from 47 commercials and untreated pistachios orchards located in six California Counties. The radial growth of the tested isolates was recorded directly after 7 days incubation. In each County, the frequencies of boscalid resistant isolates in the sampled populations were established. The *A. alternata* isolates were grouped into two populations of sensitive and resistant isolates (EC_{50} > 10 ppm) with high resistance levels observed in fields with a history of boscalid exposure. Preliminary cross resistance studies, carried out with 70 boscalid-resistant showed these resistant isolates were also resistant to penthiopyrad, but remained sensitive to fluopyram.

Late-season chasmothecium production by *Uncinula necator* on grape leaves in Michigan

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Many epidemiological aspects of powdery mildew of grape, caused by *Uncinula necator*, have been studied to date. However, questions regarding timing of production of overwintering inoculum remain to be explored. Funnels were placed below the canopy of 'Chardonel' and 'Pinot noir' grapevines in Clarksville and Traverse City, MI, respectively. Rainwater samples were collected weekly, filtered and microscopically examined for chasmothecia (also known as cleistothecia) from 19 August until 9 October, 2008. A peak in the number of chasmothecia trapped in mid-September at both sites was correlated with the highest precipitation level observed during the sampling period. Average peak chasmothecium counts were 489 in Clarksville and 4907 in Traverse City, this increase may be due to higher disease pressure. Additionally, leaves of 'Chardonel' vines were sampled weekly from 4 September until 2 October to study chasmothecium development. A production peak was evident by 25 September (33 ascocarps/cm^2) on the abaxial surface of the leaves. Over the entire period, more chasmothecia were found on the abaxial surface (27 ascocarps/cm^2 on average) than on the adaxial surface (3 ascocarps/cm^2), presumably because upper leaf surfaces are more exposed to rain events. The epidemiological contribution of chasmothecia on lower leaf surfaces is not clear and needs further investigation.

Chemical control of Phytophthora blight (*Phytophthora capsici*) of pumpkin in Illinois

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Phytophthora blight, caused by *Phytophthora capsici*, has become one of the most important threats to production of cucurbit crops in Illinois. Management of this disease in cucurbit fields in Illinois requires intensive use of effective fungicides. In the past eight years, more than 50 fungicides with potential effects on Phytophthora diseases were tested for their efficacy for control of *P. capsici* in cucurbit fields. The most effective fungicides for control of Phytophthora vine infection and fruit rot in processing pumpkin fields were Forum 4.16SC (dimethomorph), Gavel 75DF (zaxomide + mancozeb), Maestro 80DF (captan), Presidio 4SC (fluopicolide), Ranman 400SC (cyazofamid), Revus 2.09SC (mandipropamid), and Tanos 50DWG (famoxadone + cymoxanil). Spray applications of the fungicides began at the first sign of the disease and continued on a weekly schedule until two weeks to harvest. Tank-mix of the fungicides with a copper compound increased their efficacy in protecting vine and fruit against the pathogen. Spray applications at 10-day intervals did not provide adequate protection of vines and fruit against *P. capsici*. Percent fruits infected with *P. capsici* were significantly lower in plots that received season-long fungicide applications than those of control plots and the plots that did not receive season-long fungicide sprays. A spray-volume of 469 liter per hectare was needed to provide thorough coverage of canopy with fungicides.

Influence of weed species and time of glyphosate application on Rhizoctonia root rot of barley

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Rhizoctonia solani AG-8 causes root disease in wheat, barley, canola and other small grains in the dryland inland Pacific Northwest. The pathogen survives between crops on roots of volunteers and grassy weeds. Destroying this green bridge with herbicides such as glyphosate is a common tactic to control this disease. But little is known about the optimal time between glyphosate application and planting, to allow enough time for *Rhizoctonia* inoculum to be degraded by other soil microorganisms to reduce disease severity in the following crop. The impact of pre-plant glyphosate application times to control weeds on incidence and development of Rhizoctonia root rot disease of barley (*Hordeum vulgare* L.) was investigated under a controlled environment. Wild oat (*Avena fatua* L.), Italian rye grass (*Lolium multiflorum*), and downy brome *(Bromus tectorum L.)* were planted in soil infested with *R. solani* AG-8. Pots were sprayed with glyphosate at 6 wks, 4 wks, 2 wks, 1 wk, and 2 days before planting with barley. Plant measurements were taken three weeks after planting. Weed species, time of application and treatment interactions had significant ($P < 0.05$) effects on shoot length, root length, disease rating fresh weight and dry weight. The highest disease ratings were seen at 2 wks after spaying, but declined at 4 and 6 wks. The timing of glyphosate application and weed species are crucial factors in managing the greenbridge.

Spatial colonization of *Xylella fastidiosa* in the foregut of glassy-winged sharpshooter supports two types of egestion in the inoculation mechanism

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There has been an increase in incidence of Pierce's Disease in California following introduction of the glassy-winged sharpshooter (GWSS), a vector of *Xylella fastidiosa* (*Xf*). Research into host plant resistance is hampered by lack of understanding of vector acquisition and inoculation. During acquisition, *Xf* colonizes the precibarium and cibarium. Subsequent inoculation of *Xf* occurs by an unidentified feeding process. Previous research supports that inoculation occurs through salivation combined with egestion, i.e. outward flow of fluid from the precibarium. To test this hypothesis, we studied colonization of the precibarium and cibarium by GFP-expressing *Xf* over 1 - 6 day acquisition access periods (AAP's) of GWSS on infected grape. Field-collected GWSS (heavily colonized by non-GFP-*Xf* prior to the AAP) were compared with clean, lab-reared GWSS (not colonized prior to the AAP). After the AAP, confocal laser scanning microscopy was used to visualize GFP-*Xf in situ* within the undissected foregut. *Xf* colonies were examined via scanning electron microscopy. Lab-reared GWSS acquired large amounts of GFP-*Xf*, first into the cibarium, then gradually into the precibarium. In contrast, field-collected GWSS acquired fewer GFP-*Xf* into areas of the precibarium critical for inoculation. Both groups of insects also showed de-colonization of the

precibarium over time, with spatial patterns of colony retention suggesting physical flushing of the bacteria from walls of the precibarium via two types of egestion.

Surfactin production by strains of *Bacillus mojavensis*
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Bacillus mojavensis RRC 101 is an endophytic bacterium patented for control of fungal diseases in maize and other plants. Culture extracts and filtrates from this bacterium were antagonistic to the pathogenic and mycotoxic fungus *Fusarium verticillioides*. The identity of the inhibitory substance was recently determined to be the biosurfactant surfactin using HPLC-MS analysis of the culture filtrate. The absolute structure of this cyclic lipopeptide was determined from collisional ion dissociation (CID) analysis to be a cyclic heptapeptide linked to a β-hydroxy fatty acid. Further CID analysis of the peptide moiety was established by deduction, which indicated that the peptide sequence consisted of two acidic amino acids and five hydrophobic amino acids with a sequence of Leu-Leu-Asp-Val-Leu-Leu-Glu. These spectra indicated that B. *mojavensis* RRC 101 produced Leu^7-surfactin. This isomer of Surfactin was determined to be toxic to *F. verticillioides*. We examined 24 additional strains of this species for production of this cyclic lipopeptide, which was quantitated by HPLC-MS analysis. This is the first report for the production of this very powerful biosurfactant but environmental friendly biopesticides from this endophytic species. Thus, this study supports the concept that surfactin is characteristic of the genus *Bacillus*.

Managing peanut stem necrosis disease in Groundnut: A transgenic approach
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Phytopathology 99:S7

Groundnut or Peanut (*Arachis hypogaea* L.) is an important oilseed crop of India. Its cultivation has recently been decimated in the country by an unusual necrosis disease known as peanut stem necrosis disease (PSND). It is characterized by necrosis of terminal leaflets, petioles, stem and buds. Thrips transmitted Ilarvirus-*Tobacco streak virus* (TSV) has been established as the causal agent of the disease. Various cultural and chemical management strategies evaluated in recent years as control measures was not found satisfactory. Hence a transgenic approach was attempted in groundnut by incorporating viral gene to contain the damage caused by TSV. Transgenic lines with sense and antisense coat protein (CP) gene of TSV were generated using *Agrobacterium*- mediated transformation of de-embryonated cotyledons of groundnut cultivar JL-24. Approximately 200 days were required for hardening of transformants after co-cultivation of explants. The putative transformants were analyzed for the presence of viral gene by PCR and genomic Southern hybridization. Transgenic lines each containing one and two copies of the CP gene were identified. Northern analysis indicated the presence of CP transcript in transgenic lines carrying CP gene in sense as well as antisense orientation. CP gene expression was observed in transgenic lines with sense CP gene (S3, S15) by ELISA. Evaluation of the transgenic lines for resistance to TSV in further generations is in progress.

Biological and molecular characterization of Iris yellow spot virus from diverse hosts and geographic regions
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Phytopathology 99:S7

Thrips-transmtted Iris yellow spot virus (IYSV) (genus *Tospovirus,* family *Bunyaviridae*) is a major constraint to the production of onion bulb and seed crops in the US. The genome of IYSV comprises of Large (L), Medium (M) and Small (S) RNAs. The virus was reported from several states in the US and most recently from Nevada and northern California. The response of several commonly used indicator plants to mechanical inoculation by IYSV was evaluated. *Datura stramonium, Nicotiana benthamiana, and Vigna unguiculata* produced symptoms following mechanical inoculation with IYSV. Availability of indicator plants that produce local and/or systemic infection would aid in biological characterization of IYSV. The L and M RNAs of an IYSV isolate from Washington State were cloned and sequenced. The nucleocapsid (N) gene sequence was used to assess the genetic diversity of IYSV isolates reported from different hosts and regions of the world. Phylogenies showed clustering of IYSV isolates into three major clades with lineages based on the source of the isolate.

Monitoring onion thrips (*Thrips tabaci*) and Iris yellow spot virus in bulb and seed onion crops: A potential IPM component for mitigating IYSV epidemics
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Iris yellow spot virus (IYSV), a member of the genus *Tospovirus* and family *Bunyaviridae*, causes an economically important disease in bulb and seed onion crops in several parts of the US. The Pacific Northwest of the US has been particularly affected by serious outbreaks of this virus. Onion thrips are the only known insect vector for IYSV. Developing tools to monitor and manage viruliferous onion thrips could provide an effective management tool to mange this disease. An ELISA-based assay was developed to identify onion thrips that could be potential transmitters of IYSV. As part of a multi-year study, beginning in 2008, onion thrips were monitored in two field plots at the Oregon State University's Hermiston Agricultural Research and Extension Center, on a weekly basis using the full-plant count technique. Each week, at least 20 onion thrips were collected from each field and from each sampling site. Preliminary data showed that onion fields planted next to overwintering onions, a potential source of onion thrips for the following season, did not increase the mean number of onion thrips per plant per week in the field planted adjacent to it. Using antiserum specific to the NSs protein of IYSV, thrips were tested using ELISA to identify potential virus transmitters (viruliferous thrips). The seasonal dynamics of viruliferous thrips could be useful in refining thrips management practices for reducing the impact of IYSV in onion.

Cyberinfrastructure challenges to multi-regional, multi-scale weather forecasting for crop disease early warning systems
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Phytopathology 99:S7

Rapid synoptic and mesoscale weather-based crop disease forecasting requires that the model workflow rely on integration of real-time data services from multiple sources, executed over a pool of high performance computing resources. We present our team's initial steps to create, implement, and validate a multi-scale, multi-crop, multi-regional crop disease forecasting system funded by the USDA. Spatially explicit weather forecast model runs are initiated at the Linked Environments for Atmospheric Discovery (LEAD) portal and sent to run on TeraGrid using cyberinfrastructure funded by NSF. Model results include hourly values for microclimate variables critical for crop disease management and can be directly input into decision support systems. The primary cyberinfrastructure design challenges encountered in the initial development will be discussed and results from our first growing season model runs will be presented. Potato late blight in the Great Lakes, Fusarium head blight of barley in the northern Great Plains, and leaf spot of peanut in the Southeast are used as case studies.

Plant signaling compounds alter secondary metabolite production among antagonistic *Streptomyces*
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Phytopathology 99:S7

Streptomycetes have been implicated in the control of soil-borne plant pathogens, and are known to produce an extensive array of antimicrobial secondary metabolites. We investigated the hypothesis that plants manipulate the production of secondary metabolites by streptomycetes. We tested a collection of diverse *Streptomyces* isolates for responses to potential signaling molecules produced by plants, including plant hormones, flavonoids, sesquiterpene lactones, and crude root exudates. Secondary metabolite production was investigated with the use of high performance liquid chromatography (HPLC) and assays for inhibitory activity. We found evidence that streptomycetes respond to plant-produced compounds with altered patterns of secondary metabolite production. *Streptomyces* isolates in our study had the ability to chemically modify and produce close analogs of plant-derived compounds. The production of similar chemical compounds may facilitate cross-kingdom communication. Our work suggests the potential for plants to manipulate the activities of soil microbial communities, which may confer a selective advantage in suppression of plant pathogens. These

results concur with studies from many different systems showing that microbial activity is tightly linked with the health and functioning of higher organisms.

Loblolly pine decline on Ft. Benning: An analysis of potential underlying causes

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Phytopathology 99:S8

The putative loblolly pine decline syndrome occurring at the Ft. Benning, Georgia military base is being studied in the context of similar declines reported in some conifer forests in the southeastern United States. Loblolly pine is a prolific colonizer of abandoned agricultural sites and has been extensively planted and managed on many soil types from Texas through Virginia. Ft. Benning undergoes unique environmental impacts and constraints which are compounded by limitations imposed by federal requirements for restoring and maintaining habitat for the red cockaded woodpecker. The current decline and mortality in existing mature loblolly pine stands threatens habitat restoration and endangered species recovery goals on this military base. Several interacting factors involving soil conditions, age class of existing loblolly pine stands, root disease causing fungi (e.g., *Heterobasidion*, *Leptographium*), insects, and silvicultural treatments (e.g., prescribed burning) are proposed as potential contributing agents. Given the involvement of these factors in loblolly pine mortality, researchable questions that should be addressed involve both short term and long term studies to determine if the decline is statistically confirmable, and not just a perception of persons focused on the endangered species issues. The highly degraded soils resulting from previous agricultural use may represent an edaphic environment so altered that historic growth patterns are no longer achievable.

Effects of the *Fusarium verticillioides* mycotoxin, fumonisin B1, on maize stomatal behavior

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Phytopathology 99:S8

Fusarium verticillioides is a non-obligate plant pathogen causing a number of maize diseases and is responsible for the production of fumonisin B1 (FB1), a potential human carcinogen and agent of fatal farm animal diseases. The effect of FB1 on the health and development of maize seedlings was recently evaluated, and FB1 was shown to disrupt biosynthesis of complex sphingolipids by inhibiting ceramide synthase. Such inhibition caused the accumulation of sphingoid bases (phytosphingosine and sphinganine) and their 1-phosphates in the roots of maize seedlings. Others have demonstrated that in *Arabidopsis thaliana* sphingosine-1-phosphate (S1P) and phytosphingosine-1-phosphate (Phyto1P) regulate stomatal behavior. Our objectiveis were to evaluate physiological effects of *F. verticillioides* on maize seedlings, including whether FB1 alters maize transpiration and stomatal aperture behavior. Multiple load cells within a growth chamber were used to determine differential transpiration rates between inoculated and control treatments over a two week period. Stomatal conductance was also measured using a porometer. The data sets will serve as a guide for subsequent evaluation of FB1 alone without fungal inoculation. More detailed observations of stomatal behavior will be evaluated *in vitro* using an epidermal peel assay. These data will provide insight into possible downstream effects of FB1 disruption of ceramide biosynthesis and resulting elevated sphingoid base concentrations in maize tissues.

Prevalence of esca and Petri diseases of grape in Iran

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Phytopathology 99:S8

During summer and spring various irrigated vineyards were visited in Iran and samples from declining vines with Petri and esca symptoms were collected. Isolations were made from affected wood tissues from branches, trunks and crowns. Based on anamorph morphology and molecular characteristics (PCR-RFLP and partial beta-tubulin genes) the following species were identified: *Phaeoacremonium aleophilum* (Pm), *Phaeomoniella chlamydospora* (P) (with esca and Petri symptoms) and less frequently *Pm. parasiticum* and *Pm. inflatipes* (with Petri symptoms). The latter species is a new record for Iran. Among grape cultivars, Askari an early cultivar is the most susceptible to Petri disease which is a prevalent disease in Iran. Pathogenecity tests carried out under field and greenhouse conditions using *Pm. aleophilum* and *P. chlamydospora*, resulted in wood discoloration and yellowing of foliage respectively under field and greenhouse conditions ten months after inoculation. The former species caused larger lesions. Within 2 months after inoculating grapevine seedlings under greenhouse conditions by *Pm. aleophilum*, *Pm. parasiticum*, *Pm. inflatipes* and *P. chlamydospora*, symptoms developed as reduced growth, chlorotic leaves, severe defoliation, and wilting. The pathogens were re-isolated from all inoculated plants after symptoms observed.

Common and dwarf bunt of wheat: One or three species?

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Phytopathology 99:S8

Tilletia contraversa, the wheat dwarf bunt pathogen, is differentiated from *T. caries* and *T. laevis*, the common bunt pathogens, by host symptomology, teliospores enveloped in a thick gelatinous sheath, and a requirement for low temperature (optimum 3–8°C) and light for germination of teliospores. Germination typically occurs in 3–6 weeks. Identification of the wheat bunt species has historically been based on teliospore morphology and host stunting but recent studies indicate that these morphological species may not be evolutionarily informative. For example, a combined phylogenetic analysis of elongation factor 1 alpha and RNA polymerase II among 31 wheat bunt isolates revealed two clades, neither of which contained exclusively common or dwarf bunt species. The objective of this study is to develop more informative loci to test the hypothesis of three phylogenetic species of wheat bunt pathogens. Specific primers for Sequence Characterized Anonymous Regions (SCAR markers) were developed from sequenced polymorphic loci amplified using random 10-mers. SCARs were hybridized to chromosomes to select a set of unlinked markers for analysis of 60 wheat bunt isolates from North America, Eurasia and Australia. Results of a multilocus phylogenetic analysis utilizing SCAR loci and mapping of morphological characters including teliospore morphology and germination parameters will be presented for this broad geographic sampling of isolates.

Environmental impact of two potato late blight management strategies in Ecuador

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Phytopathology 99:S8

Potato late blight (PLB) caused by the oomycete pathogen, *Phytophthora infestans,* causes global damages estimated at about five billion USD, with much of this due to frequent fungicide applications. However, excessive fungicide application may not only result in economic loss to the farmer but also creates health and environmental risks in the local agro-ecology. We used the environmental impact quotient (EIQ) to compare conventional and ecological potato production systems in Ecuador. Each conventional production treatment consisted of one of three commonly-grown cultivars with its respective local disease management practices, which had been determined by surveys. Ecological production was based on the use of early-maturing, PLB-resistant cultivars and integrated pest management (IPM) tactics. Production systems were compared in two locations in Ecuador. PLB was controlled in both systems, but the cumulative EIQ values of conventionally produced potatoes were between 400 and 1000, depending on the trial location and cultivar, while those of ecologically produced potatoes were always below 150. Fungicide application for PLB was the primary factor that determined the cumulative EIQ. The use of the EIQ and other sustainability indicators is discussed in the context of potato production in developing countries.

Green tomato fruits are predisposed to sour rot when congested with water

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Geotrichum candidum (sour rot) failed to initiate progressive lesions in wounds on green tomato fruit in storage (25°C), whereas slowly enlarging lesions consistently formed on red fruit. Wounds on green fruit usually contained signs of the pathogen and the pathogen could be readily isolated but individual wounds were frequently surrounded by a thin layer of firm darkened tissues in a condition termed arrested necrosis. Even as the green fruit ripened, lesions failed to develop. By contrast, when wounded green fruit were submerged in tap water for 18 to 24 h prior to or after inoculation, progressive lesions developed within 48 h of storage. The weight increase associated with the 24-h submergence ranged from 4 to 10% of the initial fruit weight. Radial cracks were frequently observed at the edges of the wounds, evidence for tissue expansion. The water congestion treatment also enhanced

sour rot development on red fruit. Water congestion associated with water pressure (submerge in water and expose to air pressure in a chamber) similarly predisposed fruit to infection.

Simultaneous occurence of bacterial pathogens, *Agrobacterium vitis*, *A. tumefaciens* and *Xylophilus ampelinus*, on the same grapevine and various cultivars

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Phytopathology 99:S9

Crown gall agents, *Agrobacterium vitis* and *A. tumefaciens*, and bacterial blight and canker, *Xylophilus ampelinus* are the most important bacterial diseases of Vitis vinifera cultivars. Crown gall and bacterial blight agents were obtained from 14 various grapevine cultivars and 25 different locations, and 280 bacterial strains from totally 309 plant samples in Central Anatolia in 2007 and 2008. The pathogens isolated from the lesions and galls on leaves, branches and trunks on Roy and Sasser, PDA, YPGA and King's B mediums, and identified on the basis of biochemical, morphological, physiological and molecular tests. The PCR primers chosen from vir genes have also been used in identification of *A. tumefaciens* (virD2) and *A. vitis* (virA) and primer set S3/S4 from sequences in the ITS sequence of ribosomal operon for *X. ampelinus*. Three bacterial pathogens, *A. tumefaciens, A. vitis* and *X. ampelinus*, were investigated on the same plant and 103 grapevine samples (mostly on cv. Cardinal and cv. Sultani). *A. tumefaciens* and *A. vitis* were identified on 115 samples and *A. tumefaciens* and *X. ampelinus* on only 7 plants. There was no *A. tumefaciens* alone and *A. vitis* and *X. ampelinus* together on any cultivar. While *A. vitis* was determined alone on 55 plants from various cultivars, *X. ampelinus* was obtained from cv. Source Black on 57 plant samples.

Investigation of thrips population and Tomato spotted wilt virus incidence in processing tomatoes in the Central Valley of California

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Phytopathology 99:S9

Tomato spotted wilt disease caused by Tomato spotted wilt virus (TSWV; genus Tospovirus; family *Bunyaviridae*) has recently emerged as an economically important disease of processing tomatoes in California. TSWV is effectively transmitted by western flower thrips (WFT; *Frankliniella occidentalis*), and replicates in both the thrips vectors and the plant hosts. To develop an improved understanding of this disease in California, population densities of WFT and TSWV incidence were monitored in processing tomato transplant-producing greenhouses and associated fields in the Central Valley of California in 2007 and 2008. Thrips were monitored in tomato flowers and with yellow sticky cards, whereas as TSWV incidence was assessed with sensitive indicator plants and field surveys. Transplants had low populations of thrips and no evidence of TSWV infection. In the field, thrips populations were extremely low during the winter and gradually increased in the spring (March/April) then peaked in May-July. Thrips populations in 2008 were four-fold greater than in 2007. TSWV did not appear until late April to mid-May, but appeared in all monitored fields, though at relatively low incidences (3–19%). Surveys for reservoir hosts revealed low rates of TSWV infection in weeds (<0.1%), with higher rates observed in potential bridge crops, such as radicchio. Based upon these findings, an integrated pest management strategy for TSWV in the Central Valley of California is proposed.

Limited population structure of *Armillaria mellea* throughout coastal California suggests gene flow through basidiospore dispersal

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Armillaria mellea is a fungal pathogen in the orchards, forests, and urban areas of California (CA). Diploid mycelia spread vegetatively belowground among host roots. Basidiospores are not thought to infect planted hosts and haploid mycelia are not collected in nature. We tested the hypothesis that A. mellea populations are spatially structured, based on an assumed limited capacity for spore dispersal. Collections were made from forests and urban areas at five locations (from North to South: St. Helena-N, St. Helena-S, Berkeley, San Jose, Los Angeles), separated by linear distances of 0.3, 80, 78, and 500 km (658 km total). A total of 59 isolates, representing 59 somatic incompatibility groups, were genotyped with nine microsatellite loci. Global differentiation across locations was insignificant (F_{ST} = 0.011, P > 0.05). Pairwise comparisons of locations revealed significant genetic differentiation (F_{ST} = 0.008, P < 0.05) between only the most distant locations (St. Helena-N v. Los Angeles). Assignment tests in STRUCTURE grouped isolates from all locations into one cluster. Our findings of limited population subdivision and no geographic clustering of isolates suggest that spore dispersal prevents geographic differentiation, with the exception of somewhat limited gene flow between opposite ends of CA. The existence of one contiguous, interbreeding population is consistent with the presence of a relatively continuous range of hosts between northern and southern CA.

Sensitivity of *Clavibacter michiganensis* subsp. *michiganensis* to different disinfectants

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Phytopathology 99:S9

Bacterial canker, caused by *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), is a damaging disease of greenhouse-grown tomatoes. The pathogen enters the host plant through wounds and natural openings such as stomata, lenticels, and hydathodes. Cmm can spread mechanically via pruning knives and shears during de-leafing, and by tools, machinery and workers' hands and clothing during crop handling. Commercial disinfectants were examined for efficacy in eliminating Cmm from cutting tools that dispensed the disinfectants onto the cutting surface during pruning operations. Both in vitro experiments using pure cultures of Cmm, and experiments with tomato plants showed that Greenhouse Guardian, KleenGrow and Virkon S killed Cmm in vitro and on cutting tools at the recommended concentrations. These disinfectants can be used to reduce or eliminate the spread of Cmm when used in tools that deliver the disinfectant directly to the cutting surface.

Evaluation of fungicides and biorational products for management of *Pythium* damping-off of vegetable seedlings

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Damping-off, caused by *Pythium aphanidermatum*, can result in significant losses in transplant production in protected environments. Experiments were performed in a greenhouse to determine the efficacy of fungicides and biorational products against *Pythium* damping-off in cabbage and pepper. Treatments applied were the fungicides Ranman, Ridomil Gold, Previcur Flex and Thiram, the botanical products Thymol and Neem oil, the biocontrol agents SoilGard and Actinovate, and the biorational fungicides ProPhyt and OxiDate. Emergence was significantly reduced compared to the untreated control by Thymol (0.3%), SoilGard (0.5, 1, 2 lb/100 gal), Actinovate (4, 6 oz/100 gal), Ridomil Gold (1 pt/20 gal) and OxiDate (1.25 fl oz/gal) indicating phytotoxicity to seed. In peppers, all of the treatments except the 2 pt/100 gal rate of ProPhyt significantly reduced post-emergence damping-off compared to the inoculated control, although there were no significant differences between the effective treatments. In cabbage, all of the treatments were effective. The Ranman treatments were among the most effective. In peppers, the 1.5 oz/100 gal rate of Ranman, the 1 pt/100 gal rate of ProPhyt, and all three rates of Previcur Flex significantly increased the percentage of emerged and healthy seedlings compared to the untreated, inoculated control. Similar results were observed in cabbage, except that the 32 fl oz/100 gal rate of Previcur Flex did not significantly increase the number of healthy seedlings compared to the control.

Exogenous choline contributes to *Pseudomonas syringae* fitness on leaves of field-grown host and nonhost plants

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Phytopathology 99:S9

The foliar bacterial pathogen *Pseudomonas syringae* appears to be adapted to choline-rich environments based on its production of a suite of transporters that maximally transport choline at relatively high concentrations. After uptake, choline and the related compounds carnitine and glycine betaine, which are also transported, may serve as nutrients, protectants from environmental stresses, or precursors for cellular components. We evaluated the contribution of these compounds to the establishment and maintenance of phyllosphere populations of *P. syringae* pv. syringae B728a and *P. syringae* pv. tomato DC3000 by examining the fitness of various mutants on leaves under field conditions. In competition fitness assays involving co-inoculation of kanamycin-marked mutants and parental strains, B728a and DC3000

mutants that lacked all of the transporters for these compounds were significantly reduced in fitness on nonhost plants, suggesting a role for these compounds in epiphytic fitness. This role was supported by the reduced fitness of a triple transporter mutant (*opuC betT bccXWV*) of the more epiphytically fit strain, B728a, but not by DC3000 *opuC betT bccXWV*, on host plants. The double transporter mutant DC3000 *opuC bccXWV,* which could transport choline but not carnitine or glycine betaine, was similar to the parental strain in fitness. This finding demonstrates that choline uptake, in particular, is important to epiphytic fitness.

Biological control of forest weeds using selected *Phoma* spp.: An evaluation of efficacy and risk

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Phytopathology 99:S10

The most common forest weeds in Canada are deciduous perennials that colonize rapidly after disturbances such as harvesting and fire. These shrubs, including *Rubus spectabilis* and *Gaultheria shallon,* are often managed in conifer reforestation sites using chemical herbicides. Because these plants are also valuable native plants, an inundative biocontrol strategy that uses indigenous pathogens of these weeds would be a prudent alternative. Laboratory and greenhouse screening of these pathogens has indicated the potential of selected isolates of *Phoma argillacea* and *Phoma exigua* for use as biocontrol agents for their respective host plants. PCR-DNA based genetic markers have been developed for detection and identification of these isolates of *Phoma* on naturally infected host plants, as well as for monitoring of infection in biocontrol applications. These molecular genetic markers also provide information about genetic variability within populations of *Phoma*, and permit the lead biocontrol isolates to be detected and differentiated. Ongoing plant trials are focused on the optimization of conditions for infection by biocontrol isolates, and the verification of infection by the selected isolates *in planta* using genetic markers. Efficacy of treatments will be compared to chemical and untreated controls. Plants in untreated controls will also be tested by PCR-DNA for the presence of the biocontrol genotypes in order to assess the risk of non-target application.

Monitoring resistant populations of *Xanthomonas citri* subsp. *citri* and epiphytic bacteria on young citrus trees treated with copper or streptomycin

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Since Florida's citrus canker (*Xanthomonas citri* subsp. *citri*, Xcc) eradication program was halted in 2005 attention has focused on management strategies that include the use of bactericides such as copper and streptomycin for disease control. Widespread use of these chemicals in citrus industries elsewhere in the world has led to development of resistant strains of Xcc. Cu and Sm resistance were monitored in Xcc and epiphytic bacterial populations on citrus trees repeatedly sprayed with these chemicals for control of citrus canker. Copper hydroxide (Cu, Kocide 3000) or streptomycin sulphate (Sm, Firewall) were sprayed on foliage of young 'Ray Ruby' grapefruit every 21 days from March to October 2008. Mature canker-symptomatic and non-symptomatic leaves were sampled monthly to assay for resistant Xcc and epiphytic bacteria, respectively. Leaves were washed with MGY broth + 1 mg/L of $CuSO_4$ for 2 hrs using 10 mL of liquid medium/g of leaf and plated on semi-selective medium MGY-KCC + Cu or Sm for isolation of resistant Xcc or on MGY + Cu or Sm for resistant epiphytic bacteria isolation. No Cu or Sm resistant strains of Xcc were isolated. No major differences in total epiphytic bacterial population were observed among treatments over time in comparison to the check. However, Cu and Sm sprays increased the ratio of epiphytic bacterial population with resistance to these chemicals. Overall, the Sm resistant bacterial populations were proportionally lower than Cu resistant bacterial population.

Pathogenic and genotypic variation of Iranian isolates of *Fusarium oxysporum* f. sp. *betae*

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The *Fusarium* wilt and yellows caused by *Fusarium oxysporum* f. sp. *betae* is an economically important fungal disease of sugar beet that characterized by wilting of the foliage, vascular discoloration and interveinal chlorosis of the leaves. In order to study pathogenic variability and genetic diversity of the pathogen 25 isolates of *Fusarium oxysporum* from diseased sugar beet were collected from different regions of Iran. Pathogenicity of all *Fusarium* isolates was determined using a root-dip assay. The results of the pathogenicity tests classified the isolates in four highly, moderate, weakly and non virulent (control) groups. A total of fifteen RAPD selected primers were used for analysis. The cluster analysis was performed by using UPGMA. The results showed a great degree of genetic diversity among the isolates and clustered them in six groups. There was a relationship between RAPD groups and geographical origins of the isolates.

Characterizing fungal disease resistance QTL in near-isogenic maize lines by differences in histology, host gene expression, and disease specificity

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Quantitative disease resistance, despite widespread use, remains poorly understood. We previously generated 252 near-isogenic lines (NILs) in the background of the commonly used maize inbred line B73. Each NIL has 1-5 of 12 resistance quantitative trait loci (QTL) for the fungal maize pathogen *Cochliobolus heterostrophus*, causal agent of southern corn leaf blight. Presented here is a 2-phase study with the ultimate aim of characterizing the physiological basis of our QTL. The first phase investigates QTL disease specificity by field testing 236 NILs for adult plant resistance to 5 fungal maize pathogens. The second phase uses growth chamber juvenile plant trials to compare the interactions between *C. heterostrophus* and 6 select lines - B73, the 2 resistant parent NILs, a major-gene resistant line (B73rhm), and 2 derived NILs - by quantifying spore germination and penetration efficiency, hyphal growth, and host expression of the pathogenesis related genes *PR1* and *PR5*. Germination and penetration efficiency did not differ significantly between lines ($P \leq 0.058$ for all significant effects). *PR1* and *PR5* were significantly upregulated at 15 and 24 hours post-inoculation in all lines, although relative *PR* gene expression between lines did not uniformly correspond with relative resistance. The field data identified QTL with significant multiple disease resistance and susceptibility effects. Experiments are underway to further clarify how these loci affect disease. Results will be presented.

Culture-independent association of fungal and oomycete populations with damping-off disease incidence in soils

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Fungal and oomycete populations contributing to damping-off (DO) of tomato and soybean were identified by terminal restriction fragment length polymorphism (TRFLP) of the internal transcribed spacer region. Soils from three organic transition strategies differed in DO incidence of subsequent tomato and soybean crops. Principal component analysis (PCA) of the TRFLP data revealed distinct communities in response to management and DO incidence. Six terminal restriction fragments (TRF) consistently contributed to the variation in the first three PC across experiments. Non-parametric analysis of variance showed a subset of TRF significantly more abundant in transition strategies with high DO incidence. TRF H116 and H312, were significant in more than one experiment. Pair-wise correlations between the abundance of individual TRF and DO incidence revealed three (H99, H118 and H128) and five (H116, H128, H316, H323 and H329) TRF positively correlated with DO incidence of tomato and soybean, respectively. TRFPL profiles of fungal and oomycete isolates obtained from soils of the same field site were determined. From these, genera that matched the size of TRF associated with DO incidence include *Fusarium, Chaetomium*, *Alternaria*, *Pythium* and *Phytophthora,* genera with well known species of tomato and soybean soilborne pathogens. Hence, members from these genera likely contributed to DO in the studied system. This study provides an alternative method to study diseases caused by multiple pathogen populations in soil.

Soil treatments against *Fusarium oxysporum* f. sp. *vasinfectum* race 4

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Few economically feasible disease management options are available for California cotton producers with fields infested with race 4 of *Fusarium oxysporum* f. sp. *vasinfectum*. For treating soil to reduce inoculum levels, past studies indicate that solarization and fumigation with metam-sodium may be affordable, yet effective solutions. To test their applicability to race 4 in cotton, we compared four soil treatments: a six-week-long summertime solarization, metam-sodium (75 gal/acre), methyl bromide-chloropicrin

(50:50, 350 lbs/acre, tarped) and 1,3-dichloropropene-chloropicrin (40:60, 31.5 gal/acre, tarped). The treatments were applied in plots in a field naturally infested with race 4, using a split-plot design, with soil treatment as the whole plot factor and cotton cultivar as the subplot. Four cultivars representing a range of susceptibilities to race 4 were used to evaluate the treatments. We will present observations from the first season indicating that soil solarization worked as well as more costly, tarped fumigants (methyl bromide-chloropicrin and 1,3-dichloropropene-chloropicrin) as evidenced by plant survival.

Seed transmission of '*Candidatus* Liberibacter asiaticus' in citrus without typical huanglongbing

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Citrus huanglongbing (HLB) is one of the most devastating diseases of citrus worldwide. In the USA, HLB is associated with '*Candidatus* Liberibacter asiaticus' (Las) which is transmitted by the insect vector, Asian citrus psyllid (*Diaphorina citri*), by grafting and by dodder (*Cuscuta campestris*). However, the seed transmissibility of Las in citrus remains undetermined. We have evaluated seedlings grown from the seeds of typical HLB-affected and atypical HLB-affected citrus trees of sweet orange (*Citrus sinensis*), Pummelo (*C. maxima*) and trifoliate (*Poncirus trifoliate*) during 2007–2009. HLB-like symptoms, such as yellow shoot, blotch mottle and vein corky on the leaves were observed in a low percentage of the seedlings, primarily on trifoliate plants. Using various primer sets that target different genetic loci of the bacterial genome, Las was detected from all three species of citrus, ranging from 2.0% to 41.7% using PCR, nested PCR and quantitative PCR. It is important to note that the Las bacterium detected from the seedlings remains at a very low titer, unlike that in graft- or psyllid-transmitted HLB-affected citrus plants, and most, if not all, of the Las-positive seedlings have not developed typical HLB disease over a three year period. The molecular mechanism of the low-titer, non-lethal but seed-transmissible Las is under investigation.

Effect of soil fumigation and compost application on strawberry verticillium wilt

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Phytopathology 99:S11

Verticillium wilt, caused by *Verticillium dahliae*, is an important disease affecting strawberry (*Fragaria* × *ananassa*). Currently, pre-plant soil fumigation with metham sodium is commonly used to control the disease but implies serious risks for health and the environment. Moreover, soil fumigation often leads to the eradication of beneficial organisms and to a negative shift in the biological equilibrium. Application of certain composts is known to provide natural biological control against several diseases and appears as an interesting alternative to soil fumigation for the control of strawberry verticillium wilt. The objective of the study was to evaluate the effect of compost application and fumigation on fruit yield and verticillium wilt severity of field-grown strawberry plants. Two commercial composts (produced from bovine manure and marine residues) were applied at different rates to *V. dahliae* naturally infected field plots fumigated or not with metham sodium and planted with strawberries (cv. Seascape). The results indicate that fumigation significantly decreased fruit yield and did not significantly reduce wilting severity while application of marine compost significantly decreased wilting severity.

Early warning system against forest invasive alien fungal species on live plant material

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An early warning system based on a random sampling of asymptomatic live plant material arriving in Canada is used to detect alien fungal pests. Forty-six sample lots collected by Canadian Food Inspection Agency (CFIA) inspectors from the province of Quebec were analyzed by cloning the fungal ribosomal ITS present in the plant tissues. We obtained 101 fungal species associated with 36 different host plants from the USA, France, the Netherlands and Thailand. Six fungal species found in this study could have a low to moderate potential impact and 11 could have a low potential impact for Canadian forests. Another 14 species could not be assessed given the limited scientific information available. In all cases, the potential impact evaluations of these 31 species originate from the fact that these species are new to science and/or belong to genera and families where pathogenic species are common. The alien fungal introductions with a potential to affect Canadian forests were found at a significant frequency (12.4%) and were present in every sample lot sent by CFIA. The 70 other species found in this study were non-pathogenic fungi; weak to moderately virulent, common and cosmopolitan species; or virulent species found on tropical hosts only.

Effect of foliar fungicide application timing on foliar diseases and yield of soybean in Iowa

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Phytopathology 99:S11

Iowa soybean growers may decide to apply foliar fungicides for a couple of different reasons. Growers either follow current integrated pest management recommendations and apply fungicides when disease pressure or risk of disease warrants them. Or they apply fungicides as recommended by some agribusinesses that include in a tank mix with the last application of glyphosate (typically near growth stage R1), as a "plant health" application (around R3), or tank mixed with insecticide when pest thresholds are reached. The effect of pyraclostrobin, trifloxystrobin + prothioconazole, and flusilazole applied at growth stages R1 or R3 on foliar disease severity and yield were compared at six locations across Iowa. Percent foliar disease severity of brown spot, Cercospora leaf blight, and frogeye leaf spot was assessed at growth stage R6. Disease pressure was less than 15% at all six locations for all foliar diseases in the non-sprayed control. Fungicides reduced brown spot severity at both R1 ($p = 0.081$) and R3 ($p = .069$). Fungicides did not reduce the severity of Cercospora leaf blight and frogeye leaf spot had less than 1% severity in all treatments. While not significantly higher at all locations, mean yields were greatest for R3 applications (54.3 bu/ac) compared to R1 applications (52.3 bu/ac) and non-sprayed control (50.2 bu/ac). Differences between fungicides for disease management and yield response were significant at some locations.

***Dactylaria pseudomanifesta* a new species of a Dematiaceous fungus from the Atlantic Rain Forest of Bahia, Brazil**

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Phytopathology 99:S11

Mitosporic fungi from the leaf litter of Southern Bahia Atlantic Rain Forest were studied in three conservation units. Collects were made from December 2007 to July 2008. Among 52 taxa from 39 genera a new species of the genus *Dactylaria* was found and named *D. pseudomanifesta* J.L. Bezerra & D.M.A. Magalhães, spec. nov., having the following characteristics: effuse colonies and immersed mycelium; conidiophores simple, brown, septate, smooth; cylindrical, 55 – 188 × 5 – 6 µm; conidiogenous cells terminal, integrate, denticulate, 35 – 40 × 5 - 6 µm; denticles apical, in number of three to five, 1 – 2 µm long; conidia obclavate to turbinate, light brown, three-septate, 17 – 25 × 3.5 – 4.0 µm, having a hyaline distal cell. On fallen leaves of *Manilkara maxima* and *Harleyodendrum unifoliolatum*, endemic trees of the Southern Bahia Atlantic Rain Forest, collected in the municipalities of Una (Herbarium Cepec 1459) and Uruçuca (Herbarium Cepec 1417). The genus *Dactylaria* comprises 169 species of which the *D. manifesta* Castañeda & Kendrick is the most similar one but having hyaline conidia of different size.

Testing a whole-genome macroarray for detection of soilborne pathogens of almond and strawberry

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Phytopathology 99:S11

Of the many methods available for detection of pathogens, only array-based methods can afford specific detection and quantification of many microbes simultaneously. It was demonstrated previously that a nylon-bound array of whole genomic DNA from bacterial species hybridized to DNA from environmental samples with species-level specificity and good sensitivity. Our goal is to develop such a system for routine detection of soilborne prokaryotic and eukaryotic pathogens of fruit and nut crops. Genomic DNA from four Oomycetes, five fungi, and two *Prunus* rootstocks was extracted using a modified CTAB method. Four subsamples of the DNA (200 ng per spot) were spotted on nylon membranes and bound covalently. To test the specificity and sensitivity of the array, whole genomic DNA samples of target organisms were labeled with digoxigenin using nick translation, and 50 ng of labeled DNA was hybridized with the arrayed DNA in all pair-wise combinations at 65°C. After stringent washing and blocking, Fab fragments from anti-digoxigenin antibodies conjugated with alkaline phosphatase were attached to the labeled, hybridized DNA. The labeled DNA was quantified by measuring

chemiluminescence of CDP-*Star* on X-ray films. Under the tested conditions, genus-specific detection of the target pathogens was observed. Experiments are in progress to optimize hybridization conditions for attaining species-level specificity as well as high sensitivity and for validation of the macroarray-system using field samples.

Identification and evaluation of *Fusarium* species associated with root disease of soybean and corn in Minnesota

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Fusarium root rot is one of the most common diseases of soybean and corn in the U.S. and leads to substantial yield reduction. Corn-soybean rotations are common and reports indicate that root rot can be associated with the same *Fusarium* species on both hosts; however, information regarding the pathogenicity and distribution of these *Fusarium* species is limited. In 2007 and 2008, soybean and corn samples were collected from 15 fields in 10 counties representing major corn-soybean production areas in Minnesota. Root tissue was plated and *Fusarium* was the most frequently isolated genus in all locations. Three hundred isolates representing nine different *Fusarium* species were identified using morphological characteristics and sequences of the EF-1 gene. Among these, *F. oxysporum*, *F. solani*, *F. acuminatum*, and *F. proliferatum* were most prevalent. *Fusarium* isolates (n = 600) from soybean and corn have been tested for pathogenicity on soybean seedlings in a greenhouse. Isolates of each *Fusarium* species incited root rot symptoms on seedlings, including isolates of *F. oxysporum* and *F. proliferatum* from corn. *Fusarium* isolates that caused severe root rot were also tested in field plots where damping-off and root rot were observed. Additional pathogenicity tests to evaluate the potential of these *Fusarium* species to incite root rot are in progress. These findings suggest the involvement of multiple *Fusarium* species in the development of root rot on both soybean and corn.

The infection and diversity of *Diplodia pinea* in asymptomatic *Pinus patula* trees

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Phytopathology 99:S12

Diplodia pinea (= *Sphaeropsis sapinea*) is a common latent pathogen in Pine trees in many parts of the world, including South Africa. The aim of this study was to determine the distribution and frequency of endophytic *D. pinea* infections in *Pinus patula* trees, seedlings and seeds. The diversity of the endophytic isolates from trees was then determined using 13 microsatellite markers. Isolations of the fungus were successfully made from the main stem, branches and cones of asymptomatic *P. patula* trees. The genotypic diversity of these isolates was high, ranging between 43% to 68%. The haplotypes in each tree were not spatially grouped, but randomly distributed throughout the tree, indicating numerous individual infections over time. Seedlings grown in areas where mature pines were absent (either in nurseries or in the open field) contained low levels or no *D. pinea* infection, while those grown in close proximity to mature trees had a 40% incidence of infection. Furthermore, the fungus could only be isolated from 2–3% of surface disinfected seeds. These data indicate that seeds play a limited role in dissemination of *D. pinea*, although the large amount of seed moved internationally increases the changes of dispersal. The fungus infects plants repeatedly from a young age from inoculum produced on surrounding trees. Latent infections can then persist in the trees for long periods as local infections throughout the tree, even deep in heart of the main stem.

Rapid detection and quantification of *Verticillium dahliae* in soil

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Phytopathology 99:S12

Verticillium dahliae has a wide host range and can cause significant losses in highly susceptible crops such as strawberry. With modification of soil preplant fumigation practices due to the phase out of methyl bromide alternative fumigants may not be as effective in controlling the pathogen. Having an accurate real-time PCR procedure for soil quantification will reduce the time needed for soil assays from 6–8 weeks to days, thereby providing growers more lead time to make planting decisions. The pathogen survives in the soil as microsclerotia, which can present challenges for disruption and extraction of intact DNA. Three different DNA extraction kits were tested and compared using high pressure cycling technology (Barocycler) and the FastPREP tissue homogenizer to disrupt microsclerotia. DNA was further purified using paramagnetic particles and inhibition of PCR amplification was quantified using an internal control template. Species specific primers and a TaqMan probe for *V. dahliae* were designed from the multicopy rDNA. Infested soil (75 microslerotia previously determined by culture) was diluted in non infested soil to create a dilution series of microsclerotia to assess the detection limit, consistency of the assay and potential utilisation of this tool with field soil. Using this approach low inoculum levels were consistently detected; regression analysis describing the relationship between inoculum density and real-time PCR results will be discussed.

Involvement of type IV secretion in *Lysobacter enzymogenes* pathogenesis of fungal and algal hosts

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Phytopathology 99:S12

Type IV secretion systems (T4SS) enable gram negative bacterial pathogenesis of eukaryotic hosts by suppressing defense mechanisms and by facilitating intracellular colonization of host cells. The bacterial biocontrol agent *Lysobacter enzymogenes* is known as an antagonist of other microbes and an intracellular pathogen of a broad range of lower eukaryotes such as nematodes, fungi and algae. Recent analysis of the near-complete genome sequence revealed that *L. enzymogenes* possesses an *Agrobacterium*-like T4SS, composed of *virD4* and the *virB* operon. In this study, we asked whether the T4SS plays a role in *L. enzymogenes* pathogenesis of two lower eukaryotes: the filamentous fungus, *Cryphonectria parasitica,* and the chlorophyte alga, *Chlamydomonas reinhardtii.* A deletion of genes encoding core T4SS machinery resulted in an *L. enzymogenes* mutant strain that was dramatically delayed in the onset of intracellular proliferation within both the fungal and algal hosts. In addition, *C. reinhardtii* cells treated with the *L. enzymogenes* T4SS^{-} mutant strains produced elevated levels of reactive oxygen species compared with those treated with the wildtype strain. These results imply type IV secretion is important for *Lysobacter* pathogenesis of fungal and algal host cells, and may function by suppressing the oxidative burst response produced by host cells during initial stages of infection.

Available nitrogen levels influence *Colletotrichum coccodes* infection severity of Russet Burbank potato roots

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Phytopathology 99:S12

The fungus *Colletotrichum coccodes* is a soil-borne agent that enters potato plants through their roots. *C. coccodes* is the causal agent of black dot in *Solanum tuberosum*, which results in financial losses to potato growers. Edaphic factors are a critical aspect to infection efficacy of *C. coccodes*. Evaluating specific nitrogen (N) levels in the plant rhizosphere and within the plant, disease severity can be correlated to N nutrition. Russet Burbank potatoes were inoculated with *C. coccodes* and grown hydroponically at varying levels of N (5, 40, 160, 640 ppm in hydroponic solution). Infection severity was assessed by plating roots on potato dextrose agar (PDA) and through quantitative RT-PCR. Root infections on PDA were analyzed by area under the disease progress curve (AUDPC), while qRT-PCR was used to generate infection coefficients (IC) from respective pathogen-host nucleic acid abundance. The IC values from differing treatments were compared to assess disease severity. The reduction of *C. coccodes* pathogenicity by increased available N was statistically significant among treatments 5, 40, and 160 ppm of N. A surplus of N at 640 ppm resulted in a slight increase in *C. coccodes* but it was not significantly different than 160 ppm N. These data provide strong evidence that N nutrition is important in suppression of *C. coccodes* and that the amount of available soil N can affect plant health through decreasing disease susceptibility.

Field plot trials in North Dakota and South Dakota using *Bacillus* strain 1BA for biological control of Fusarium Head Blight on wheat and barley

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Phytopathology 99:S12

Management of Fusarium Head Blight (FHB) can include use of fungicides alone or in combination with biological control agents that antagonize the

fungus. *Bacillus* strain 1BA has the ability to reduce different measures of FHB. Field plot trials were conducted in 2008 in both Langdon, North Dakota and Brookings, South Dakota to further assay the efficacy of *Bacillus* strain 1BA. The Langdon trial involved spray application of 1BA at Feekes 10.51 to Howard hard red spring wheat; and the Brookings trial had spray application at Feekes 10.51 for Briggs spring wheat, and at full head emergence for Robust spring barley. All trials included application of Prosaro plus Induce NIS, alone and in combination with 1BA. In the Langdon hard red spring wheat trial, the co-application of 1BA with Prosaro and Induce NIS resulted in a deoxynivalenol (DON) level of 2.5 ppm, compared to 3.2 ppm for Prosaro and Induce alone; and 4.0 ppm for the untreated control. In the Brookings spring wheat trial, results from application of 1BA alone or with Prosaro plus Induce were not significantly different from untreated controls. However, in the barley trial, co-application of 1BA with Prosaro and Induce resulted in significantly lower DON levels and lower disease incidence in barley than for the untreated control. The trials demonstrated that 1BA when co-applied with Prosaro and Induce can sometimes reduce DON levels in grain more than can application of Prosaro and Induce alone.

Evaluation of wild sunflower species for resistance to *Sclerotinia* stalk rot

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Phytopathology 99:S13

One of the most important diseases affecting sunflower production in the United States is stalk rot caused by *Sclerotinia sclerotiorum*. Genetic resistance to *Sclerotinia* has gradually improved in commercial sunflower cultivars, but better levels of resistance are still needed. A greenhouse screening method was developed, using soil-applied *Sclerotinia*-infested millet as inoculum. Greenhouse screening helps to facilitate the rapid screening of much larger plant populations than could be managed in field trials. With this approach, susceptible germplasm can be filtered out, making better use of the follow-up field trials. In 2008, 255 accessions were evaluated in the greenhouse, including all available accessions from the diploid annual species of *H. argophyllus*, *H. debilis*, *H. exilis*, *H. neglectus*, and *H. praecox*, 45 accessions of wild *H. annuus* and five accessions from the perennial species *H. resinosus*. Accessions with superior wilt resistance were identified in all species except for *H. exilis*. Field trials showed that the three most resistant accessions were *H. resinosus* (PI 650079 and PI 650082) and *H. argophyllus*, (PI 649863) with 100% and 94% survival respectively. These findings suggest that resistant germplasm can be readily identified that could contribute toward improved *Sclerotinia* stalk rot resistance in cultivated sunflower.

Evaluation of fungicide sensitivity of *Typhula ishikariensis* and *Typhula incarnata* to fludioxonil, propiconazole and chlorothalonil

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Phytopathology 99:S13

Gray snow mold caused by *Typhula* spp. is a major problem on golf courses where snow cover persists for periods exceeding 60 days. Even though the disease is primarily managed by preventive fungicide applications in the fall prior to permanent snow cover, the pathogen still produces varying degrees of damage to the golf course. Gas chromatography/mass spectrometry analysis indicated that residues of chlorothalonil (10–100 ug/g) and fludioxonil (1–5 ug/g) did not diminish in the verdure through the winter, yet many plots treated with these fungicides, either alone or in combination, often had more than 50% turf damage. In-vitro studies were undertaken to determine sensitivity of *T. incarnata* and *T. ishikariensis* to fludioxonil, propiconazole and chlorothalonil. *Typhula* isolates collected from fungicide treated plots were grown on half-strength potato dextrose agar amended with varying fungicide concentrations. Growth of most *T. ishikariensis* isolates were suppressed by more than 50% on agar amended with 10 µg/ml chlorothalonil, propiconazole and fludioxonil whereas growth of some *T. incarnata* isolates were not suppressed by more than 50% on agar amended with 10 ug/ml chlorothalonil (compared to a non-amended control). This suggests that variability in fungal sensitivity to these fungicides may be as important as fungicide persistence in influencing snow mold development.

Field susceptibility of quince hybrids to fire blight in Bulgaria

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Phytopathology 99:S13

Spread of fire blight in Bulgaria during the last 20 years has nearly eliminated commercial production of pear and quince. Damage has increased in both nurseries and orchards, yet susceptible cultivars continue to be planted. Quince is the host most frequently attacked by *Erwinia amylovora* in Bulgaria, where it is widely cultivated in private yards, and infected trees provide a permanent source of inoculum. The importance of quince as a fire blight host encouraged a 10-year breeding program which subjected 274 hybrid progenies (3 replicates per selection on BA29) to natural fire blight infection. During the epidemic years 2003 and 2005, we identified 18 progenies that exhibited significant resistance to fire blight, with less than 15 blossom or shoot infection points per tree, and less than 5% blighted canopy. New quince genotypes have been selected that combine resistance to fire blight and high fruit quality. These selections will enable continued quince production while reducing disease incidence. Production of other pome fruit crops will also benefit from reduced inoculum levels.

Does the Horsfall-Barratt scale for disease severity estimation affect our ability to test for treatment differences?

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Phytopathology 99:S13

A simulation model designed to sample two diseased populations was used to investigate hypothesis testing with disease severity data from visual estimation to the nearest percent, and the Horsfall-Barratt scale. The relative distance separating mean severities in the two populations, the population standard deviation and rater ability all influenced the probability of a Type II error (failure to reject H_0 when H_0 is false). If populations had the same mean, both methods had equal probability to accept H_0. Gross difference between means or imprecise raters resulted in similar probability for both methods to reject H_0 when it was false. However, if these criteria were not met, the H-B scale had higher probability of causing a Type II error, particularly at severities 20–50%. Larger sample size reduced the probability of Type II error, but invariably the sample size required to reject H_0 at $P = 1.0$ was smaller for direct estimation. Approx. 50% more samples were required for the H-B scaled data to reject H_0 with the same probability as data estimated to the nearest percent. The structure of the H-B scale predisposes hypothesis testing to greater risk of Type II error compared to estimation to the nearest percent. Direct estimation using the 1–100% ratio scale is preferable when assessing plant disease, but the H-B scale, and other scales, can save time, and can be easy to learn and apply, but if used, sufficient sample size is needed to minimise risk of Type II errors.

Efficacy of Cankerguard® sprays for effective decontamination of Citrus canker

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Phytopathology 99:S13

Citrus canker (*Xanthomonas citri* subsp. *citri, Xcc*) is endemic in Florida. We used grapefruit leaf surfaces to explore the efficacy of the personnel decontaminant Cankerguard® to kill inoculum. In three experiments plants in flush (leaves 3/4 expanded) were sprayed with inoculum (2×10^4-9×10^5 CFU/ml). Immediately after inoculation, plants were passed through standard personnel decontamination spray hoops (0, 1, 2, 3, and 6 times) with spray nozzles, ensuring good droplet coverage with each pass. Leaves were sampled at 0, 10 and 20 min after decontamination and tested for viable bacteria of *Xcc* by dilution plating. There was a large and rapid decline in the quantity of live bacteria with one pass through the spray hoop (5 to10 fold decrease in CFU), and multiple sprays (up to six) resulted in a 100 fold reduction in the population to complete decontamination. Presumably better coverage with multiple sprays killed remnant bacteria, although the first spray invariably killed the most bacteria. Based on these results, decontamination with Cankerguard® is efficacious at reducing the quantity of surface inoculum, and even a single spray hoop can cause high mortality, but multiple sprays improve kill of *Xcc*. Inoculated plants were incubated and development of symptoms on plants from all treatments suggested that infection took place at, or very soon after, inoculation as decontamination did not reduce disease incidence or severity.

Analysis of apple (*Malus*) responses to bacterial pathogens using an oligo microarray

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Phytopathology 99:S14

Fire blight is a devastating disease of apple (*Malus* × *domestica*) caused by the bacterial pathogen *Erwinia amylovora* (Ea). When infiltrated into host leaves Ea induces reactions similar to a hypersensitive response (HR). Type III (T3SS) associated effectors, including DspA/E, are suspected to have a major role in eliciting the HR-type response in apple. To understand the mechanism of disease establishment we first compared apple responses to challenges by Ea and the incompatible pathogen *Pseudomonas syringae* pv. *syringae* strain B86-6 (Pss). In a second experiment we compared the responses to Ea, a T3SS and a DspA/E mutant. Gene expression profiles were accessed using a 39,412 long-oligo (70-mer) *Malus* microarray. Leaf tissue was harvested at 6 h.p.i. from apple shoots of susceptible 'Malling 26' rootstock inoculated with the different treatments. Several protocols were tested to optimize transcript labeling and microarray hybridization. A total of 430 apple genes responded similarly to both compatible and incompatible interactions. Approximately 24% of those genes coded for defense or stress related proteins, including Mal d 1, LRR-rich proteins, and dehydrins. In the second experiment we identified a core of 80 genes associated with general responses (MAMP) to Ea, 320 genes associated with responses to T3 effectors of Ea, and 24 genes associated with DspA/E-specific responses. A detailed annotation of the genes identified in our study will provide a comprehensive view of *Malus* defense responses.

Occurrence of coagulase positive staphylococci in horses with reference to organized and non-organized Equi farm

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Phytopathology 99:S14

181 *Staphylococci* were isolated from the upper respiratory tract and skin of 168 healthy horses and donkey reared at organized and non-organized farms in the Bikaner. In order 98 isolates were obtained from horses and 83 were collected from donkeys. Identification of the 181 isolates resulted in the following species distribution: *Staphylococcus aureus, 89* isolates (49.17%); *S. epidermidis* (subspecies not identified), 39 (21.54%); *S. intermedius,* 31(17.12)*; S. lugdunensis* (subspecies not identified), (5.52%) 10. No difference was found between the upper respiratory tract and the skin regarding *staphylococcal* species distribution. This suggests that for the most part *S. aureus and S. epidermidis* comprise the staphylococcal flora in horses.

Determination of the small RNA biogenesis components DCL and RDR in *Phytophthora* oomycetes

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The oomycetes are a distinct lineage of eukaryotes that contain important pathogens of plants. Recently the genomes of four *Phytophthora* species and *H. arabidopsidis* were sequenced. Most eukaryotes have RNA silencing systems that use small RNAs to suppress a wide range of genes, genetic elements, and viruses. We identified the core small RNA biogenesis components and effectors in three *Phytophthora* species, *P. sojae*, *P. ramorum*, and *P. infestans*. Each species has two DCL, one RDR, and five to ten AGO homologs. Previous analysis of high-throughput sequencing data revealed two major size distributions of sRNA sequences, which is consistent with the presence of two DCL homologs, each one specific for a different size class. Protein domain prediction programs indicated the presence of different domains between the two DCL homologs. Both DCLs have two RNase 3 domains at the 3' end of the gene, but in the 5' end DCL1 has DEAD/DEAH box helicase and dsRNA binding domains, whereas DCL2 has a PAZ domain. DCL2 additionally has a dsRNA-binding motif at the extreme 3' end. cDNA sequence analysis has confirmed the coding sequence for DCL2, which has two short introns at the 5' end of the gene. Preliminary analysis of DCL1 suggests there are no introns. The RDR homolog contains the conserved RDRP domain, and also appears to have a Helicase C domain and either a DEAD/DEAH box helicase or ResIII domain.

Management of Rhizoctonia root rot of sugarbeet – fungicide efficacy and identification of environmental parameters for disease development

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Rhizoctonia solani AG 2-2 is the causal agent of Rhizoctonia root and crown rot in sugarbeet. This disease has recently been increasing in occurrence and severity in sugarbeet production areas in the Red River Valley of Minnesota and North Dakota. Since the intraspecific groups AG 2-2 IIIB and AG 2-2 IV both cause Rhizoctonia root and crown rot and are both prevalent in the Red River Valley, our objectives were to compare disease development of these intraspecific groups at four different soil temperatures under controlled climate conditions. A second objective was to determine the minimum amount of soil moisture required for disease to occur. Finally, a third objective was to determine the efficacy of several fungicides at the temperature and moisture levels determined to be optimal for disease development. Trials were conducted using growth chambers set at four temperature regimes (day time high temperatures of 10, 15.6, 21.1 and 26.7°C). No disease development occurred at 10°C. No above-ground symptoms were seen in plants grown at 15.6°C. Both AG 2-2 IIIB and AG 2-2 IV showed significant disease development at 21.1 and 26.7°C during the two-week post inoculation evaluation period. Efficacy of several classes of fungicides was tested at 26.7°C since this was the temperature most conducive to disease development. Application of azoxystrobin and prothioconazole controlled the disease but difenoconazole was not effective at controlling Rhizoctonia root rot.

Effects of post-dew period temperature on *Phakopsora pachyrhizi* urediniospore production on soybean

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Soybean plants inoculated with *Phakopsora pachyrhizi* were incubated in temperature-controlled growth chambers which mimicked day/night temperature profiles of the U.S. soybean production areas during spring, summer, and fall. At 3-day intervals, urediniospores were collected from each plant and counted. At the end of each experiment, numbers of lesions and leaf areas were determined for each leaf. From the data, numbers of lesions per cm^2, and urediniospores produced both per plant and per lesion for each temperature profile were calculated. Leaf samples from each plant were fixed and stained, and average numbers of uredinia per lesion and uredinium diameters determined. Maximum number of urediniospores produced per lesion and plant was with a 25/12°C day/night temperature regime. When day temperatures peaked at 29, 33, or 37°C, urediniospore production per plant was reduced to 41, 7, and 0.1%, respectively, of that at 25°C. Maximum numbers of lesions were produced when the temperature peaked at 21°C and maximum uredinia per lesion at 25 and 29°C. Daily temperature data was obtained from NOAA for a 4-year period for selected soybean areas of the U.S. While temperatures of mid-western states, e.g. Illinois and Iowa, were usually conducive for urediniospore production during May through September, temperatures for southern states commonly peaked above 33°C during July and August, suggesting that high day temperatures limit urediniospore production.

Screening wheat landraces for resistance to new races of *Puccinia graminis* f. sp. *tritici*

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New races of the stem rust pathogen, *Puccinia graminis* f. sp. *tritici*, have arisen in Eastern Africa and threaten wheat production worldwide. As part of a coordinated effort to discover and deploy resistance to the new races, wheat landraces accessions from the USDA-ARS National Small Grains Collection (NSGC) are being screened against the new races in the field at Njoro, Kenya. The NSGC has about 25,000 wheat landraces and field testing capacity is limited. Thus, for testing in Kenya, priority is being given to 1) landraces of common spring wheat, 2) accessions that have shown adult plant stem rust resistance based on NSGC data from St. Paul MN, and 3) accessions that show seedling resistance against race QFCS in greenhouse tests at Aberdeen ID. Of 1768 landraces screened in Kenya, only 3.3% were resistant. Of the accessions tested, 237 had previously shown resistance in the St. Paul adult tests and among these accessions 6.7% were resistant in Kenya. Similarly, of 741 accessions resistant as seedlings to race QFCS, 5.0% were resistant in Kenya. Among the remaining accessions tested only 0.6% were resistant in

Kenya. Thus, selecting accessions for testing based on US stem rust screening data increases the probability of identifying accessions resistant to the new races. Work is underway to characterize the resistant accessions to determine if their resistance is due to new genes that have not yet been deployed in modern wheat cultivars.

Interactions between tanoak and *Phytophthora ramorum* studied on a microscopic and molecular scale

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Phytopathology 99:S15

The goal of this research is to determine which tissues/cell types of tanoak bark *P. ramorum* colonizes, identify where host defense responses occur in relation to the pathogen, and to determine where elicitins are localized in bark tissue. Studying interactions between *Phytophthora ramorum* and *Lithocarpus densiflorus*, tanoak, is important because tanoak plays a large role in facilitating the continued spread of the pathogen, and the survival of tanoak as a species is threatened by *P. ramorum*. These studies should also offer insight as to how this pathogen so successfully antagonizes this host. Observation of hyphae, defense responses, and elicitins is achieved through the use of fluorescence, confocal, and scanning electron microscopy. Stains are used to enhance observation of hyphae and defense responses. Elicitins are small, unique proteins produced by *Phytophthora* and *Pythium* species that have recently been implicated in pathogenicity on this host. They can be observed within tissues after attachment of a fluorescently labeled antibody for the primary elicitin of *P. ramorum* is achieved through a multistep process. This labeling serves as a specific indicator for the pathogen, and will allow us to better understand the role of the protein.

Do dry conditions at-plant increase yellow dwarf of winter wheat in Alabama?

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One of the most important diseases of winter wheat in Alabama is yellow dwarf disease (YD) caused by *Barley yellow dwarf virus* and *Cereal yellow dwarf virus*. Early infection of winter wheat with either or both of these viruses can substantially reduce yields. Insecticide applications can reduce aphid vectors of these viruses and subsequent disease levels; however, the return on these treatments has been inconsistent. Consistency of control might be improved if conditions most favorable for aphid vectors and YD development were known. Historical analyses of Alabama data on winter wheat varietal trials had indicated that warm dry weather prior to planting led to greater YD intensity. To more precisely evaluate the effects of rainfall near the time of planting, we restricted rain amounts to winter wheat seed beds by covering plots for 2 or 4 weeks before and after planting in Nov. 2007. Work was done at two locations. Levels of YD were assessed the following spring and were generally greater in those plots which were covered rain for longer intervals.

Resources for fast-forward R gene mapping and isolation in the genus *Solanum*

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Phytopathology 99:S15

Solanum encompasses ~1,500 species including potato (*S. tuberosum*), tomato (*S. lycopersicum*), and eggplant (*S. melongena*). Macrosynteny is well documented between these species and it is hypothesized genome regions of common origin across *Solanum* species harbor homologous R genes. More than a dozen *Solanum* R genes have been cloned. In this study, we developed a resistance gene analog (RGA) library of more than 100 candidate R genes for the disease resistant wild potato *S. bulbocastanum*. This resource complements smaller RGA libraries from potato, tomato, and the wild *S. caripense*. We examined species distribution of R gene families via phylogenetic analysis of *Solanum* RGAs and cloned R genes. Further, we explored the distribution of specific R gene families in a broad collection of *Solanum* species using Southern hybridization. Our results confirm RGAs isolated from one *Solanum* species represent R genes found in multiple species. Thus, cross-species, comparative genomics approaches to R gene mapping and cloning are feasible. To capitalize upon this observation, we have collaborated to construct a 3.5X BAC-based physical map of *S. bulbocastanum*. This physical map will be anchored to a genetic map. Finally, we isolated BAC clones harboring candidate R genes and placed them on the *S. bulbocastanum* physical map. Our system of integrated genetic, physical, and R gene maps for *S. bulbocastanum* comprises a valuable resource for mapping and cloning in the genus *Solanum*.

Effect of foliar fungicides on corn with simulated hail damage

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Phytopathology 99:S15

A supplemental label for pyraclostrobin fungicide approved recently by the U.S. EPA states that corn (*Zea mays*) plants applied with pyraclostrobin may have better tolerance to damage caused by hail. To determine the effects of foliar fungicides on hail damaged corn, field research trials were conducted near Champaign, IL in 2007 and 2008. Hail damage was simulated with a gasoline-powered string-mower at the V12 growth stage, which caused injury to leaves and defoliation. At VT, the foliar fungicides azoxystrobin and pyraclostrobin were applied to corn. Control treatments included a non-treated control and a non-damaged control. The simulated hail damage significantly ($P \leq 0.05$) increased gray leaf spot (caused by *Cercospora zeae-maydis*) severity in 2007, but not in 2008. Simulated hail damage also significantly reduced yield compared to the non-damaged control in both 2007 and 2008. Foliar fungicides significantly reduced disease severity compared to the non-treated control in 2007, but not in 2008; however, foliar fungicides did not significantly improve yield in either the damaged or non-damaged plots compared to the non-treated controls. Results from our research trials indicated that foliar fungicides provided very little benefit to corn injured by simulated hail; thus, growers should consider factors other than hail damage when making fungicide application decisions for corn.

Distribution and characterization of soil-borne sugar beet viruses in Iran

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Phytopathology 99:S15

The Iranian provinces where sugar beet is cultivated have been surveyed for the presence of soil-borne sugar beet viruses. *Beet necrotic yellow vein virus* (BNYVV) was detected in 288 of the 392 samples collected in Iran. A-type BNYVV was mostly detected. The p25 coding region on BNYVV RNA-3 was amplified by RT-PCR. Nine different p25 variants of the highly variable amino acid tetrad at positions 67 to 70 were identified i.e. ACHG, AHHG, AYHG, ALHG, AFHR, AFHG, AHYG, VLHG and VHHG. The first three variants were the most commonly found. In 23 out of the 303 BNYVV-positive samples we detected the P type of BNYVV. Surprisingly, none of these samples contained the fifth RNA species usually associated with P type BNYVV in other countries. The *Beet black scorch virus* was also detected in 62 of 203 samples in ten out of thirteen Iranian provinces. The BBSV satellite was found in 18 of 203 samples in nine provinces. The quasi absence of BBSV infection in the warmer provinces is possibly due to the fact that *O. brassicae* are less tolerant to warmer temperature than other *Olpidium* sp. The *Beet soil-borne virus* was detected in 101 of 203 samples in all of provinces under cultivation of sugar beet. By comparison of the nucleotide sequence of the coat protein gene, the Iranian BSBV were shown similar to other BSBV isolates from Germany, France and USA. The *Beet virus Q* was finally detected in 34 samples in five different Iranian provinces. The distribution and co-occurrence of these four viruses will be analyzed.

Prevalence and distribution of *Rhizoctonia solani* AG 2-2 ISGs in sugar beet-growing areas of Minnesota and North Dakota with different crop rotations

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Rhizoctonia solani AG 2-2, cause of Rhizoctonia crown and root rot (RCRR) of sugar beet, is further classified into the intraspecific groups (ISGs) IIIB and IV. Typically, IIIB is more aggressive than IV in causing disease on sugar beet, beans and corn. There is no information on the prevalence and distribution of these ISGs on sugar beet in the Red River Valley (RRV) of Minnesota and North Dakota (spring wheat is a primary rotation crop) and in southern Minnesota (SMN, corn and soybean are commonly rotated). Nearly 1,000 isolates of *R. solani* AG 2-2 were cultured from sugar beet with RCRR symptoms in 2005–2008 (47% from RRV, 53% from SMN). Isolates were preliminarily identified to ISG by measuring radial growth on potato dextrose agar at 25 and 35°C (ISG IIIB grows at 35°C, but IV does not). In the RRV, 27% of isolates were IIIB, 66% were IV and 7% were "intermediates" (grew sparsely at 35°C). In SMN, 60% of isolates were IIIB, 23% were IV and 17% were intermediates. The ISGs were distributed throughout both geographic regions but prevalence differed depending on the crop grown the previous year. Prevalence of the more aggressive ISG IIIB isolates was 19, 45, 57, and 59% when the previous crop was wheat, soybean, edible bean, and corn,

respectively. Prevalence of ISG appears to be influenced by crops grown before sugar beet and may be an important factor in management of RCRR.

Lack of *Pythium aphanidermatum* transmission by adult fungus gnats (*Bradysia impatiens*) and investigation of larval vectoring capacity
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Studies have provided evidence for transmission of plant pathogens by greenhouse-inhabiting fungus gnats (*Bradysia* spp.). The goal of this study was to determine if fungus gnats are vectors of *Pythium aphanidermatum*. In the first of a series of laboratory experiments, 10 adult gnats were released into small chambers containing two *P. aphanidermatum*-infected and two healthy geranium seedlings. None of the healthy plants became infected after 7 days. In a second experiment, adult gnats were dragged across *Pythium* culture plates and immersed in solidifying water agar or released into a dish with solidified agar. *Pythium* colonies developed in 11% of the plates with gnats immersed in agar, but in only 1% of plates where gnats moved freely on an agar surface. In a third experiment, 50 adult female gnats were placed in containers with potting mix and 35 either infected or healthy seedlings. After 24 hours, 20 of the 50 gnats were transferred to a new container with 35 healthy seedlings and 10 were immersed in water agar. None of the seedlings became infected, and no *Pythium* colonies formed in the agar dishes. Replication of these experiments demonstrated that adult fungus gnats are unlikely aerial vectors of *P. aphanidermatum*. Experiments are being conducted to correlate levels of inoculum pick-up by adult gnats with real-time PCR. Molecular assays are also being conducted to determine the potential of larvae to vector the pathogen directly or transtadially.

Basipetal movement of fungicides in peanut plants in the greenhouse
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Phytopathology 99:S16

Tests were conducted to quantify fungicide movement in peanut plants. Three terminal leaves on main stems of plants in the greenhouse were treated with 4 applications of prothioconazole (Proline, 0.16 kg/ha a.i.), prothioconazole + tebuconazole (Provost, 0.58 kg/ha a.i.), tebuconazole (Folicur, 0.20 kg/ha a.i.), or 2 applications of azoxystrobin (Abound, 0.88 kg/ha a.i.) or flutolanil (Moncut, 0.79 kg/ha a.i.). Portions of the whole plant were bioassayed with *Sclerotium rolfsii* 14 days after the last treatment to determine fungicide movement. Provost and Proline affected peanut growth with 1 to 2 more leaves than control plants. Provost and Folicur decreased *S. rolfsii* colonization on nontreated, cotyledonary branch leaves (60 and 80%) and pods (77 and 62%), and reduced the number of sclerotia formed on stems (73 and 88%), roots (74 and 73%), and pods (92 and 83%) compared to control plants. Abound had moderate reductions of *S. rolfsii* colonization on leaves and pods, but high reductions of sclerotial counts on stems (74%) and pods (83%) compared to the control. Moncut moderately decreased colonization of leaves (40%) and sclerotial counts on roots (40%) and pods (19%). Proline moderately reduced sclerotial counts on stems (30%), roots (45%), and pods (36%). Results indicate that these fungicides applied to foliage can reduce diseases in the lower, nontreated portions of the plant, even when no rain or irrigation has been applied to the foliage.

Poly(ADP-ribosyl)ation and host-pathogen interactions
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Phytopathology 99:S16

Poly(ADP-ribosyl)ation is a post-translational modification in which ADP-ribose polymers are attached to a protein. Activation of poly(ADP-ribosyl)ation has been linked in animals to the DNA damage response and programmed cell death. We have discovered that poly(ADP-ribosyl)ation processes influence plant responses to pathogen attack. We initially detected up-regulated expression of the poly(ADP-ribose) glycohydrolase gene *PARG2* after infection with *Pseudomonas syringae* pv. *tomato*, and see similar up-regulation in response to *Botrytis cinerea* or upon treatment with flg22 (a bacterial flagellin-derived elicitor of innate immune responses). ADP-ribose polymer levels also increase moderately upon infection, and poly(ADP-ribosyl)ation of discrete proteins occurs. Paradoxically, while *PARG1* gene expression is not significantly altered during plant defense responses, *parg1* but not *parg2* mutants display exaggerated responses to MAMPs such as flg22 or elf18. However, loss of either *PARG1* or *PARG2* results in an accelerated onset of *Botrytis*-induced disease symptoms. Pharmacological inhibitors of poly(ADP-ribose) polymerase (PARP) disrupt some but not all flg22-elicited responses. Early responses including the oxidative burst and expression of many defense-associated genes remain normal, but callose deposition and lignin deposition are significantly altered in elicited plants treated with PARP inhibitors. Our recent progress will be discussed.

Phenotypic plasticity, fitness and multilocus genotypes of *Phytophthora ramorum* populations in southern Oregon tanoak forests
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Phytopathology 99:S16

We tracked the spread and progress of *Phytophthora ramorum* infections in southern Oregon forests from 2001 through 2008. Using microsatellite markers we identified 75 novel multilocus genotypes (MGs) with 10 to 35 MGs found in each year. While the majority of MGs were present in very low numbers (< 1%) one MG was dominant in all years representing 35 to 65% of isolates. Although microsatellites are neutral markers and different genotypes are not by their nature related to fitness, the presence of one dominant type led us to investigate if the most common MG is more fit. We examined relative fitness of the most and least common MGs occurring in all years. We used *P. ramorum* isolates from 2001 to 2007 representing the full geographic range. We set up a genotype by environment interaction (G X E) experiment. We assayed 40 isolates representing eight genotypes under five temperatures on nutrient rich and nutrient poor media. We measured colony growth rate, chlamydospore production, and morphology. We found very little difference among genotypes. Variation among individuals within temperature and media type was quite high. Colony morphology was highly variable even within clones under identical conditions. A similar experiment, on tanoak tissue, is currently underway.

Trees, soils, streams and rain traps: Intensive sampling leads to recovery of multiple genotypes from hosts of *Phytophthora ramorum* in Oregon forests
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Phytopathology 99:S16

How *Phytophthora ramorum* infects and spreads through individual trees and entire forests is a topic of great interest. *P. ramorum* can infect plants through leaves, stems, or bark; is found in the xylem of oaks; and persists in streams and soils. Inoculum is produced in large doses and on many different hosts but how does this relate to successful infections? To better understand pathogen dispersal and infection in tanoak forests, we intensively sampled and genotyped isolates from infected plants and from soils, streams, and rainwater in southern Oregon forests. From 2001 to 2008, we identified about 80 multilocus genotypes (MLGTs) using microsatellite markers. We genotyped isolates from a variety of sample units including multiple lesions in single trees, soils from known infection sites over multiple years, rain water caught under infected trees, and streams from within infected sites over multiple weeks and years. We found over 50% of all repeated or multiple sampling recovered more then one MLGT, and 50% of those had more then two MLGTs. We also compared the within sample unit genotypic diversity results to the genotypic variation at the site the tree was located, and to the overall molecular diversity in the southern Oregon tanoak forest.

Development of species-specific primers for the detection of the butternut canker pathogen *Sirococcus clavigignenti-juglandacearum*
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Phytopathology 99:S16

Butternut canker, caused by the fungal pathogen *Sirococcus clavigignenti-juglandacearum* (SCJ), is present throughout the entire range of butternut (*Juglans cinerea*) and is the primary cause for its decline. A quick and reliable method for identification of SCJ would assist studies targeted at the epidemiology of this pathogen, in particular the dissemination of the pathogen by seeds of the butternut. The objective of this study was to develop and test species-specific primers which could detect SCJ in the presence of other fungal and bacterial organisms found on butternut seed. The primers were developed using multiple alignment of ITS sequences from isolates of SCJ and several closely related species. These primers were tested on *J. cinerea*, 18 isolates of SCJ recovered from diseased trees, 30 species of fungi, and 12 bacterial isolates recovered from butternut nuts using a nut wash technique. The primers amplified all isolates of SCJ, and did not amplify any of the fungal or bacterial isolates. The primers detected SCJ using DNA at a concentration as low as 0.1 ng/µl, detected the presence of SCJ using direct colony PCR, and at a concentration of 1×10^2 spore/ml using a bead beating DNA extraction technique. The primers developed in this study will be a

valuable tool for the detection of SCJ present on butternut seeds, and could be adapted as a rapid diagnostic tool for early detection of SCJ on butternut trees.

Crop management strategies and disease resistance control the severity of false smut and kernel smut of rice

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Phytopathology 99:S17

False smut and kernel smut are common diseases of rice capable of severe epidemics with dramatic yield losses. The importance of rice smuts is often overlooked in the United States, and highly susceptible varieties are now being grown on the majority of production acres in the southern rice producing states. Our objectives were to identify crop management practices that affect smut severity on susceptible rice varieties, to identify factors that may promote or reduce disease. Concurrently, a rice germplasm evaluation was undertaken for the long-term goal of identifying disease resistance. Using a long-term rice cropping systems study we evaluated the effects of tillage, crop rotation, irrigation, and fertility on smut severity. In an independent study designed to maximize disease pressure, smut severity was evaluated in rice variety-entries in a screen for resistance. As expected both diseases responded positively to increasing fertility, and the principal rice culture system (rice-soybean rotation, tilled soil, high fertilizer input) was found to promote the highest levels of both diseases. Conversely, fertility moderation, conservation tillage and continuous rice cropping all dramatically reduced false smut severity on susceptible rice varieties. Kernel smut was moderated only by reduced nitrogen fertility. All rice varieties tested were susceptible to false smut, but two rice hybrids were identified that were resistant to kernel smut.

An objective process for selecting regulatory responses to exotic pest detections

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One of the greatest challenges for plant protection agencies is the development of appropriate regulatory responses to exotic pest detections. Sub-optimal decisions can result in the ineffective use of resources, i.e. an ineffective eradication campaign. A decision support process can help program mangers select appropriate responses from the range of possibilities, including eradication, containment, slow the spread, management, or complete deregulation. We propose a conceptual process involving four steps: i) numerical characterization of the pest's biological, epidemiological and phytosanitary properties; ii) determination of a management response score based on the pest's properties; iii) validation of the management score with historical pest detection responses; and iv) creation of a decision tree or matrix based on the management score and the spatial extent of the pest outbreak. The proposed scheme will be compared with other techniques including pest clusters.

Lesion expansion of *Sclerotinia minor* and *S. sclerotiorum* on two peanut cultivars

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Phytopathology 99:S17

Inoculation of peanut stems with *Sclerotinia minor* (SM) or *S. sclerotiorum* (SS) causes Sclerotinia blight, which is characterized by the formation of tan, water-soaked lesions on infected plant parts, leading to tissue collapse and necrosis of the affected tissue. Significant losses occur in Oklahoma when Sclerotinia blight is severe, or the disease is not managed properly. Considerable information is known about the reaction of SM on peanut, but less is known about the reaction of peanut to SS. The purpose of this research was to quantify the rate of lesion expansion (RLE) on Okrun, a cultivar susceptible to SM, and Valencia C, a cultivar moderately resistant to SM. Stems of 6 to 8 week old plants were inoculated at midpoint with mycelial plugs from two-day-old *Sclerotinia* cultures (two SS isolates from peanut and pumpkin, and one SM isolate from peanut) grown on potato dextrose agar with 100 mg/L streptomycin sulfate. Plants were then placed in clear, polyethylene humidity chambers (>95% RH) for 7 days. Lesion length measurements were taken at 3, 4, 5, 6, and 7 days after inoculation. RLE (cm/24 hr) on Okrun were 2.62 for SM peanut, 2.53 for SS pumpkin and 0.56 for SS peanut with an $LSD_{0.05}$ of 0.30. RLE on Valencia C were 1.78 for SM peanut, 2.37 for SS pumpkin, and 0.11 for SS peanut with an $LSD_{0.05}$ of 0.36. These results demonstrate that SS from peanut was the least virulent, and indicate the importance of isolate selection in testing of pathogenicity.

Evaluating resistance to *Phytophthora cinnamomi* and *P. citricola* in clonal hybrids of *Juglans* species

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Phytophthora crown and root rots cause serious losses to walnut production worldwide. In California, more than 10 species of *Phytophthora* are implicated in the disease, but only *P. cinnamomi* (*Pcin*) and *P. citricola* (*Pcit*) are highly aggressive. *Pcin* causes mainly root rot (RR), and *Pcit* causes mainly crown rot (CR). Moderate resistance to *Pcit* was reported previously in walnut rootstock clones RX1 (*Juglans microcarpa* × *J. regia*) and VX211 (*J. hindsii* × *J. regia*), but resistance to *Pcin* was not examined. Here, we report on repeated evaluations of resistance to *Pcin* as well as to *Pcit* in 15 clonal hybrids under consideration as walnut rootstocks. The selections were transplanted into non-infested soil or soil infested with multiple isolates of *Pcit* or *Pcin* and subjected to repeated episodes of soil flooding to stimulate infection. Resistance, assessed according to severity of CR and RR 3 mo after transplanting, varied significantly among rootstocks for each pathogen (P = 0.03 to <0.0001). RX1 was highly resistant to *Pcin* (mean RR 13%) and, as in previous tests, moderately resistant to *Pcit* (CR 19%). The other selections, including VX211, (simple and complex hybrids among *J. californica, hindsii*, *major*, *nigra*, and *regia*) ranged from moderately resistant to highly susceptible to *Pcin* (RR 20 to 86%) and *Pcit* (CR 17 to 55%). The results suggest that the use rootstock RX1 has the potential to reduce losses caused by *Pcin* and *Pcit* in commercial walnut orchards.

Functional characterization of *Magnaporthe oryzae* effectors in the infective process of rice

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Rice is one of the three most important food crops of the world with an increasing worldwide production during the last decade. One of the major problems in rice production is rice blast disease caused by the fungus *Magnaporthe oryzae*. During the last decade, the genomic sequence of rice and this pathogen had been obtained allowing the computational identification of genes. Using bioinformatics, we have been able to predict secreted proteins that potentially act as elicitors of host defense. Using a transient protoplast assay, *M. oryzae* genes MGG00194 and MGG03356 were identified as putative effectors of host defense. We predict that the translated proteins are secreted in a specific spatiotemporal pattern during the infective process. The purpose of this work is to determine these patterns and how these effectors are involved in the pathogenic process using *in-planta* secretion and functional analysis. Differences between independent knockout mutants and native and constitutive GFP-fusion mutants will be tested for variations in phenotypic features. These include growth/sporulation in vitro, appressorium formation and pathogenicity. Pathogenicity is tested based on three different bioassays: sheath assay (fluorescence secretion patterns), drop inoculation and whole plant infection.

Genetic variability of *Bipolaris oryzae* in the Philippines

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Phytopathology 99:S17

The genetic diversity of a collection of *Bipolaris oryzae* causing brown spot of rice was estimated with a VNTR marker. Intensive sampling from a single field showed that the population was mostly clonal with almost all the isolates belonging to one VNTR haplotype. Isolates within that haplotype had a continuous range of aggressiveness when inoculated onto susceptible rice cv. IR72. Several different lesion types were observed in the field, but isolates from each lesion type produced a range of lesion types on leaves of IR72, which appeared to become more resistant with age. A collection of 325 isolates obtained from multiple locations in the Philippines could be divided into 50 VNTR haplotypes with a genetic diversity (H_T) value of 0.89. However, there were three or fewer isolates in 39 of the haplotypes, whereas 80% of the isolates belonged to only eight haplotypes with each containing 10 to 71 isolates indicating clonality. No major relationship between haplotype and geographical location or host variety was observed. These results show that rice fields may contain *B. oryzae* populations with considerable genetic diversity, but the majority of infections arise from a small subset that can increase to form large clonal populations, likely through conidia acting as secondary inoculum. The source of genetic variation of *B. oryzae* is not

known as the sexual stage, *Cochliobolus miyabeanus*, has not yet been reported in the Philippines.

Temporal shifts in trichothecene profiles of *Gibberella zeae* isolates from barley in North Dakota and Minnesota

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Gibberella zeae (Gz), a principal cause of Fusarium head blight of barley, produces several trichothecene mycotoxins including deoxynivalenol (DON), nivalenol (NIV), and its acetyl derivatives. Mycotoxin contamination in barley grain is a major problem for its various end uses of malt, human food, and animal feed. Little information is available for trichothecene profiles of Gz isolates from barley. Using *TRI* (trichothecene biosynthesis gene)-based PCR assays, 116 Gz isolates collected during 1997 to 2000 from major barley growing districts of North Dakota and Minnesota were compared with 148 Gz isolates collected in 2008 from the same regions. All the Gz isolates had DON markers. The frequencies of isolates with a 3-acetyldeoxynivalenol (3-ADON) marker among isolates collected in 2008 were approximately ten-fold higher than those among isolates collected during 1997 to 2000. Analysis of genetic structure of these populations using microsatellite markers is in progress.

Development and evaluation of canola populations with potential segregation for Sclerotinia stem rot resistance

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Phytopathology 99:S18

Sclerotinia sclerotiorum (Lib.) de Bary is the causal agent of sclerotinia stem rot (SSR) of canola (*Brassica napus* L.) and many other dicotyledonous crops. SSR is endemic to canola producing areas of North Dakota, state that plants more than 90% of the canola produced in the United States of America. The identification of new sources of resistance to SSR and identification of molecular markers (quantitative trait loci) associated with it will contribute to our understanding of the genetic basis of resistance to this important disease and could help in development of materials with improved resistance. To that effect, an F_2 population, produced by crossing two *B. napus* plant introductions (458939 and Ames 26628) previously identified as resistant to SSR was evaluated for their reaction to *S. sclerotiorum* using the petiole inoculation technique under greenhouse conditions. Of the 230 F_2 plants evaluated almost two thirds died within 23 days from inoculation and 69 survived. Seeds produced by these plants will be screened again and surviving plants will be taken to seed production. This selection cycle will be repeated two more times with intent of increasing the level of homogeneity of the surviving population. These lines will be used to develop resistant germplasm for SSR and to develop mapping populations.

Impact of anaerobic soil disinfestation on introduced inoculum of *Phytophthora capsici* and *Verticillium dahliae*

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Phytopathology 99:S18

Anaerobic soil disinfestation (ASD) combines solarization with brief periods of soil saturation and has been investigated as an alternative to soil fumigation. To optimize ASD for Florida vegetable and Coastal California strawberry production, experiments were implemented to examine the effect of ASD on key soilborne pathogens. In Florida, a complete factorial field experiment was established to evaluate three levels of applied water (10, 5, or 0 cm), two levels of poultry litter (amended or unamended), and two levels of molasses (amended or unamended) in combination with solarization. Untreated and methyl bromide (MeBr) controls were established for comparison. Strength of anaerobicity (Eh) was increased by both molasses and poultry litter amendments, however, control of *P. capsici* was equal to that of MeBr for all solarized treatments regardless of applied amendments or water. Numbers of marketable fruit harvested from the bell pepper crop planted after ASD treatments were greater than the untreated control for all solarized treatments and with few exceptions, equal to the harvest from the MeBr control. In California, a greenhouse incubation experiment examining two soil types and several tarping materials determined that the standard 1.25 mil polyethylene tarp (green or black/white) created sufficient anaerobicity without carbon amendments for suppression of *V. dahliae* when compared to an untarped control.

Abiotic and biotic risk factors associated with *Bean pod mottle virus* in Iowa

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Bean pod mottle virus (BPMV) prevalence and incidence were quantified within Iowa counties during a three-year, state-wide soybean disease survey (2005 through 2007). Both BPMV prevalence and incidence were found to occur at non-random among Iowa counties indicating that disease risk may be influenced by abiotic and biotic risk factors. Potential abiotic risk factors evaluated were: number of days the daily mean temperature was below 0°C (October through April), planting date, county centroid latitude, county centroid longitude, and elevation. Biotic factors by county evaluated include: soybean acreage, total alfalfa harvested, and number of soybean farms. The county centroid latitude had a significant linear relationship with BPMV incidence risk increasing from northern to southern latitudes in all three years (R^2 = 10.4, 57.9, and 17.4% for 2005, 2006, and 2007 respectively). Number of days with daily mean temperature <0°C explained 51.1% of the variation in BPMV incidence in 2006. County mean elevation was correlated (r = 0.49, P < 0.0001 and r = 0.20, P = 0.0049) with county BPMV incidence in 2006 and 2007, but not 2005. Date of planting (before 7 May) was highly correlated with BPMV incidence only in 2006 (r = 0.67, P < 0.0001). Number of soybean, and alfalfa acres harvested within a county were correlated with BPMV incidence in 2006 only.

An RNA virus from *Phytophthora infestans* with no apparent similarity to known viruses

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Phytopathology 99:S18

In an effort to examine extrachromosomal genetic elements in *Phytophthora infestans*, five double-stranded RNAs (dsRNA) in four patterns were identified. A large dsRNA, approximately 11.2 kb, was found in two isolates from the United States. The dsRNA in one of the isolates was completely sequenced and it was 11,170 bp in lenght. A large open reading frame was found on one strand (nt 7-11139), which could encode a protein of 3710 aa (calculated molecular weight 410.94 kDa). Both 5′ and 3′ UTRs were AU-rich, and the 3′ UTR contained a sequence similar to the canonical mRNA polyadenylation signal. Bioinformatic analysis indicated an RNA-dependent RNA polymerase (RdRp) at the C-terminus with marginal *P*-value and visual inspection identified the three conserved motifs of RdRps: D-x(4,5)-D, [S, T]-G-x3-T-x3-N and GDD. However, in BLAST searches, this dsRNA had no siginificant similarity to known viruses or other sequences. We tentatively named it *Phytophthora infestans* RNA virus 2 (PiRV-2). PiRV-2 was cured in one isolate by chemotherapy. Its potential impact on the host strain is currently being examined.

Mid-infrared and near-infrared spectroscopic properties of *Fusarium* isolates: Effects of culture conditions

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Phytopathology 99:S18

The *Fusarium* genus includes soil saprobes as well as pathogenic or toxin-producing species. Traditional classification of *Fusarium* isolates is slow and requires a high level of expertise. The objective of this project is to describe culture condition effects on mid-infrared (MidIR) and near-infrared (NIR) absorbance spectra of several Fusarium species. The ultimate goal of this research is finding diagnostic spectral regions that can be used to quickly differentiate fusaria. We cultured isolates from sections Roseum (*F. graminearum*, *F. avenaceum*) and Gibbosums (*F. equiseti*, *F. acuminatum*). *F. solani* was included as an outgroup from the Nectria clade, along with two non-Fusarium (Phoma and Bipolaris). The isolates were grown on two different growth media (potato dextrose broth or V8 broth), under light or dark conditions, and at different temperatures (20°C or 25°C). Principal components analysis of the MidIR spectra shows a strong growth medium influence. The V8 medium separated fusaria from the outgroups better than the PDA. Light and temperature conditions had little effect on the MidIR spectral properties. The multivariate analysis of the NIR separated the fusaria from the Bipolaris and Phoma isolates, and also showed a strong growth medium effect. This results show the possible diagnostic value of infrared spectroscopy to differentiate fusaria from other fungal species, as well as the possible effect of nutritional state on the separation of the taxa.

Interaction effects of two biological control organisms on resistant and susceptible weed biotypes of *Chondrilla juncea*

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Phytopathology 99:S19

Interactions between natural enemy species may modify their net effect on host plants, but little research has been done to examine how plant resistance influences species interactions in biological weed control. We performed common garden experiments with the invasive plant *Chondrilla juncea* to compare a rust-susceptible weed biotype with a rust-resistant biotype. Inoculations with two biological control organisms, the rust fungus pathogen *Puccinia chondrillina* and the eriophyid gall mite *Eriophyes chondrillae*, were applied separately and in combination to test if plant performance is modified by antagonistic or facilitative species interactions. As expected, genetic resistance of weed biotypes modified the effects of rust disease. We found no significant rust × mite interaction effects for several plant performance traits, and analysis of effect sizes and their confidence intervals supports the conclusion of truly additive interactions. Therefore the two natural enemies appear to have independent and complementary effects on plant performance. However, a competitive indirect effect was detected because rust inoculation reduced the total dry biomass of mite galls in the rust-susceptible biotype due to decreased growth of diseased shoots, but not in the rust-resistant biotype. Our results indicate that rust disease may have the potential to modify mite gall epidemiology as well as relative plant performance in mixed populations of resistant and susceptible *C. juncea* biotypes.

Accumulating candidate genes for broad-spectrum resistance to rice blast in a drought-tolerant rice cultivar

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Phytopathology 99:S19

We used the candidate gene (CG) approach to accumulate different disease QTLs from Moroberekan, a blast-resistant variety, into Vandana, a drought-tolerant variety in India, and evaluated the advanced backcross progenies for resistance to blast and tolerance to drought in India and the Philippines. We used *in-silico* analysis to determine gene organization of each CG and identify potential markers for selection. Gene-based markers were also designed to conduct a genome scan to determine introgression of Moroberekan alleles for 11 CGs into the progenies. Six CGs which co-localized with known dQTLs – chitinase, HSP90, oxalate oxidase, oxalate oxidase-like proteins, peroxidase and thaumatin and 21 SSR markers were significantly associated with resistance to blast across screening sites in India and the Philippines. The F_6 lines were evaluated for morphoagronomic traits at IRRI and exposed to less than 10cm rainfall for more than 30 days at rice reproductive stage. Several lines with resistance to rice blast were also tolerant at reproductive stage drought stress. Using molecular and *in silico* approaches, we were able to identify several promising lines showing resistance to blast and drought tolerance.

Genetic population structure of *Cochliobolus miyabeanus* on cultivated wild rice (*Zizania palustris* L.) in Minnesota

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Phytopathology 99:S19

Cochliobolus miyabeanus (*Bipolaris oryzae*) is the causal agent of fungal brown spot (FBS) in wild rice (*Zizania palustris* L.), an aquatic grass, endemic in Minnesota, Wisconsin, and parts of Canada. Grain yield losses can reach up to 74% when the disease starts at the boot stage and continues until grain maturity. In Minnesota, management of FBS in grower paddies is done mostly by sanitation and application of fungicides. Breeding for resistance is in progress. Knowledge of the amount and distribution of fungal diversity is required for an integrated management system of FBS. The fungus, an ascomycete, is believed to be genetically diverse but no data is available yet. A collection of 200 isolates was made from Polk, Clearwater, Aitkin, Beltrami, Itasca, and Lake of the Woods counties during 2007 and 2008. Analysis of amplified fragment length polymorphism (AFLP) markers from 93 isolates with 17 polymorphic markers, generated with two primer-pair combinations indicate a total average gene diversity ($\hat{H}$) of 0.34 and low, although significant, population subdivision by area of collection (F_{st} = 0.14, P = 0.0). $\hat{H}$ values within areas of collection were different. Forty-three haplotypes were present among 93 isolates. These preliminary results indicate that the population of *C. miyabeanus* in Minnesota is genetically diverse.

Biological diversity, pathogenicity and population structure of *Rhizoctonia* spp. associated with rice sheath blight in Arkansas

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Phytopathology 99:S19

Sheath Blight (SB) is one of the most important diseases of rice in the southern United States, and *Rhizoctonia solani* (Rs) AG1-IA is considered to be the primary SB pathogen. Of 310 isolates recovered from symptomatic rice tissue from fields in Arkansas from 1996 to 2008, 65% were identified as Rs AG1-IA. A subgroup of 80 isolates composed of various *Rhizoctonia* species, Rs AG1-IA and other AGs, and a binucleate Rhizoctonia-like isolate was examined. The objectives were to phenotype isolates by anastomosis grouping, nuclear number, and colony morphology on PDA; genotype isolates using ITS-RFLP analysis, ISSR, SSR and UP-PCR markers; and assess relative virulence on nine cultivars using a micro-chamber greenhouse method. ITS-RFLP showed polymorphism among different species and AGs, but not among AG1-IA isolates. The ISSR $(GACA)_4$ and $(CAC)_5$, and SSR markers produced polymorphic patterns for AG1-IA isolates. All UP-PCR markers were polymorphic among species, AGs and within AG1; markers UP-PCR45 and UP-PCR15 were the most robust. Pathogenicity tests revealed differences in virulence among AG1-IA isolates. Molecular marker results indicated that Rs AG1-IA isolates from rice in Arkansas are genetically diverse. Comparisons between virulence and genotype diversity are underway.

Occurrence of *Sclerotium rolfsii* on *Ascocentrum* and *Ascocenda* orchids in Florida

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Phytopathology 99:S19

In summer and fall of 2008, a severe outbreak of southern blight affected Vanda orchids in commercial nurseries and landscapes throughout south Florida. Several Ascocentrum and Ascocenda orchids grown in the landscape were found severely wilted at the apex, while around the base of the plants tan, soft, water-soaked lesions were present. As the lesions progressed, basal leaves began to drop, leaving the stems bare. After two days, white, flabellate mycelium was seen progressing up the stem with numerous tan to brown sclerotia present. Leaves and stems were plated on APDA and grown at 25°C. The fungus was identified as Sclerotium rolfsii and a voucher specimen deposited with the ATCC. A PCR was performed on the ITS1, 5.8S rDNA, and ITS2 and the sequence was deposited in GenBank. Pathogenicity of an isolate was tested by placing 6 mm plugs taken from APDA plates directly against the stem of 5 different Ascocentrum and Ascocenda orchids. Five Ascocentrum and Ascocenda orchids were inoculated with 6 mm plugs of plain APDA, and 5 were untreated controls. Plants were kept in a shade house under 50% shade, 60–95% humidity and temperature ranging from 75–88 F. Within 7 days, all inoculated plants developed symptoms that were identical to the symptoms observed on original plants and S. rolfsii was consistently re-isolated from symptomatic tissue. To our knowledge, this is the first report of S. rolfsii affecting Ascocentrum and Ascocenda orchids.

Occurrence of *Dickeya chrysanthemi* (*Erwinia chrysanthemi*) on *Tolumnia* orchids in Florida

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Phytopathology 99:S19

Tolumnia species represent a genus of small, epiphytic orchids cultivated by commercial producers and hobbyists throughout Florida. In the fall of 2008, approximately 100 *Tolumnia* orchids were found at two nurseries in Homestead FL showing macerated, brown, water soaked leaves. Isolated bacteria grew at 37°C, were gram negative, produced brown pigment on NGM medium, and tested positive for pectolytic activity and phosphatase. MIDI (Sherlock version TSBA 4.10; Microbial Identification (16 System, Newark, DE) (SIM 0.732–0.963) identified the bacteria as *Erwinia chrysanthemi* (*Dickeya chrysanthemi* Burkholder et al. 1953) Samson et al. 2005. A PCR was performed on the 16S rRNA gene and Subsequent DNA sequencing and GenBank search showed the isolated strain is 99% identical to that of *Dickeya chrysanthemi*. Pathogenicity was confirmed by injecting approximately 100 µl of a bacterial suspension at 1×10^8 CFU/ml into leaves of 10 *Tolumnia* orchid mericlones. Ten plants were inoculated with water as controls. Plants were placed in a greenhouse at 29°C and 60–80% RH. Within 24 h soft-rot symptoms appeared on inoculated leaves. The water control appeared normal. *Dickeya chrysanthemi* was re-isolated and identified using the above method, fulfilling Koch's postulates. To our knowledge, this is the first report of a soft-rot caused by *Dickeya chrysanthemi* on *Tolumnia* orchids.

Molecular characterization of insertional mutant 8B5 of *Fusarium graminearum*

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Phytopathology 99:S20

Fusarium graminearum is an important fungal pathogen of small grains and maize cultivated throughout the world. Losses are due to reduced yield and contamination of infected grain with mycotoxins. The fungus produces asexual spores (conidia) on the plant surface that facilitate secondary spread of the pathogen. To identify genes that impact asexual development, we initiated a forward genetics approach to identify random-insertional mutants of *F. graminearum* wild-type strain (PH-1) impaired in asexual development. A total of six developmental mutants have so far been identified from these studies, which include both loss- and gain-of-function phenotypic classes. Plasmid-rescue analysis of an aconidial mutant, designated 8B5, revealed the site of integration to be within the promoter region of locus FGSG_10780, which putatively encodes a hypothetical protein. RNA isolated from strains 8B5 and PH-1 cultured in resuspension experiments was used to compare relative levels of gene expression by qPCR analysis. Interestingly, transcript levels specific to FGSG_10780 were substantially elevated (>50X) in 8B5 compared to PH-1. We also found two additional candidate regulatory genes to be aberrantly expressed in 8B5. Future experiments will test our hypothesis that overexpression of FGSG_10780 represses asexual development in *F. graminearum*.

The mechanism of ascospore discharge and implications for control

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Phytopathology 99:S20

Forcible dispersal of ascospores is an important dispersal mechanism among the pathogenic fungi, yet the mechanism of ascus function is largely unexplored. We have used physiological, genetic and genomic approaches to understand ascus structure and function in the wheat pathogen, *Fusarium graminearum.* The turgor pressure that drives discharge relies on buildup of K+ and Cl- ions. We have identified two genes that serve to regulate ascus firing, as determined by mutational analysis. Inhibition of ascospore discharge could serve as an effective control particularly in multicyclic diseases, where ascospores are the primary inoculum. Research approaches towards this end will be presented.

Effect of different *Rhizoctonia oryzae-sativae* genotypes on disease development of aggregate sheath spot disease of rice

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Phytopathology 99:S20

Twenty-two isolates of *Rhizoctonia oryzae-sativae*, the cause of aggregate sheath spot disease of rice, were collected from eleven rice breeding lines in the same field in California. All isolates were genotyped with six single-locus microsatellite markers. There was no relationship between rice breeding lines and *R. oryzae-sativae* genotypes since many genotypes were isolated from a bleeding line and identical genotypes were shared between breeding lines. The virulence of five unique genotypes collected from different breeding lines was determined on five different rice cultivars, S-101, M-202, L-206, CM-101, and CT-202, in the greenhouse. Four pots, with five rice plants in each pot, were inoculated with each *R. oryzae-sativae* genotype. The lesion length on each rice plant was recorded weekly for three weeks to estimate disease severity. The disease incidence was evaluated by the percentage of infected tillers in a pot on the fourth week after inoculation. Based on repeated measures analysis, disease severity among cultivars and among genotypes were significantly different. The effects of cultivars and genotypes on disease incidence were also significant. None of the *R. oryzae-sativae* genotypes affected rice dry weight compared to the noninoculated control.

Efficacy of natural plant products on the control of aggregate sheath spot of rice

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Phytopathology 99:S20

Four plant extracts, ginger, pepper, basil, and garlic and four plant essential oils, neem oil, garlic oil, lemongrass oil, and cinnamon oil, were examined for antifungal activity against *Rhizoctonia oryzae-sativae,* the cause of aggregate sheath spot disease of rice. The antifungal activity of each compound was evaluated by its ability to inhibit vegetative growth of the fungus and its effect on the number of sclerotia produced by the fungus and inhibition of germination of sclerotia. Cinnamon oil, the most efficacious product in vitro, was tested in two experiments in the greenhouse for the control of the disease. There were four treatments in the first experiment, including no oil (control) and 0% (vegetable oil), 12.5%, and 87.5% cinnamon oil. All treatments were tested on two rice cultivars, M-205 and M-206, and two *R. oryzae-sativae* isolates, 3B and 13B. Vegetable oil had no effect on disease severity and rice dry weight. No concentration of cinnamon oil inhibited isolate 13B. Disease severity of isolate 3B was reduced by 87.5% cinnamon oil compared to 0% cinnamon oil. However, 87.5% cinnamon oil decreased rice dry weight. The second greenhouse experiment was conducted with four treatments, including no oil and 0%, 37.5%, and 62.5% cinnamon oil. The 62.5% cinnamon oil reduced disease severity in both cultivars. The 37.5% cinnamon oil suppressed disease severity only in M-206. Neither 37.5% nor 62.5% cinnamon oil affected rice dry weight.

Selection of molecular aptamers for identification of live cells of *Ralstonia solanacearum*: A new method in plant pathology

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Phytopathology 99:S20

Ralstonia solanacearum (Rs) has the potential to be one of the most damaging plant pathogenic bacteria in production fields of diverse crops. One subgroup of Rs, designated race 3 biovar 2 (R3bv2), is on the select agent list and is subject to the strictest biosecurity regulations in the United States. Due to its status, the need for unambiguous identification of R3bv2 is critical; however, diagnostic tools currently used by official laboratories lack the requisite sensitivity, specificity, or speed. Molecular aptamers are single-stranded oligonucleotides that can specifically bind with high affinity to a variety of molecules ranging from macromolecules to small compounds. They are generated through an *in vitro* selection process termed SELEX (system evolution of ligands by exponential enrichment) and have been used quasi-exclusively in human cancer research. Recently, a cell-SELEX protocol was developed to successfully produce aptamers that could specifically differentiate two closely-related cell lines using whole live cells. In this study, we used Rs race 3 strain UW485 (target cells) and race 1 strain Rs5 (control cells) to evaluate the potential for cell-SELEX to produce Rs race 3-specific aptamers. After 16 rounds of selection, flow-cytometry data indicated enrichment of aptamers specific to the UW485 strain, with evidence for targeting cell exopolysaccharides. Aptamers potentially could be produced and used in the future as reproducible, fast and highly-specific diagnostic tools for R3bv2.

Corresponding metabolic reactions in host and pathogen modulate opposing functions of defense and virulence

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Phytopathology 99:S20

The outcome of pathogenesis is usually governed by the potency of complementary mechanisms of host defense and pathogen virulence. We show that analogous metabolic reactions catalyzed by glycerol-3-phosphate (G3P) dehydrogenase (dh) in a plant and its fungal pathogen modulate defense and virulence in the respective organisms. Inoculation of Arabidopsis with Colletotrichum higginsianum was associated with an increase in G3P levels and a concomitant decrease in glycerol levels, in the host. Plants impaired in plastidal G3P catabolism (act1), accumulated elevated levels of pathogen-induced G3P and displayed enhanced resistance. The act1 mutation also improved resistance in hypersusceptible camalexin deficient plants. Overexpression of the host G3P generating G3Pdh (GLY1) also enhanced resistance to C. higginsianum. Correspondingly, a mutation in gly1 enhanced susceptibility to C. higginsianum. In vitro studies showed that exogenous application of G3P suppressed the transcription of fungal G3Pdh but not the host GLY1 gene. Knock-out mutations in the fungal G3Pdh dramatically reduced the virulence of C. higginsianum on wild-type plants, however G3Pdh-defective fungi remained virulent on mutant hosts defective in G3P generation. Together, these results suggest a novel and specific link between G3P metabolism in the host and pathogen during pathogenesis. Chanda et al. (2008) Plant Physiology 147:2017-2029.

Comparison of protein profiles between light- and dark-grown *Cercospora kikuchii*

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Cercospora kikuchii, the causal agent of soybean leaf blight and purple seed stain diseases, has become a serious concern in the southern United States

during recent years, and it has spread gradually to the north. Pathogenesis of *C. kikuchii* has been attributed to cercosporin, a polyketide toxin produced by the fungus during infection. Light is an important factor for both production and activation of cercosporin. Significant differences in cercosporin production have been observed between cultures grown under light and dark conditions for 8 to 10 days. In this study, the protein profiles of *C. kikuchii* grown under light and dark were compared using two-dimensional gel electrophoresis (2DGE) to identify proteins involved in the biosynthesis of cercosporin. Light- and dark- grown cultures of *C. kikuchii* were harvested after 4, 6, 8, 10, 12 and 16 days and proteins were extracted from each time point and subjected to 2DGE. Currently, the protein profiles of *C. kikuchii* cultures grown under light and dark conditions are being compared to identify up- and down-regulated proteins. The peptide sequences of these differentially expressed proteins will be determined using mass spectrometry, and their expression at transcript level also will be measured using quantitative real-time PCR. The potential importance of these differentially expressed proteins in cercosporin biosynthesis will be discussed.

First report of a new *Exserohilum* disease on bermudagrass in Texas
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Phytopathology 99:S21

Exserohilum spp. infect and cause foliar diseases on diverse gramineous plants. We first isolated an *Exserohilum* causing a foliar disease on bermudagrass in Houston, TX. In closely mowed bermudagrass fairway, disease symptoms appear as dark brownish to black spots of about 5 centimeters in diameter. Symptoms on leaves and stem include small purplish to black lesions. *Exserohilum* isolates from infected leaves produced minimal to no conidia. The conidia have a long protruding hilum and 5-10 septa, and measured 100-150 µm in length. The internal transcribed spacer (ITS) region of rDNA was sequenced and showed 90% similarity with *E. longirostratum*, 87% with *E. pedicellatum*, 86% with *E. turcicum* and *E. monocreas*, and 84% with *E. rostratum*. Koch's postulates were conducted for the *Exserohilum* disease. Ten-day-old seedlings of bermudagrass were inoculated with agar plugs from an *Exserohilum* culture, and incubated in the growth chamber at 30°C. Ten days after inoculation the symptoms of *Exserohilum* disease were visible on leaves and stems. By re-isolating *Exserohilum* from these infected plants, the pathogenicity of the *Exserohilum* was verified and further taxonomic characterization is currently underway.

Molecular detection of *Fusarium oxysporum* f. sp. *niveum*, the causing agent of watermelon Fusarium wilt disease
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Phytopathology 99:S21

Fusarium oxysporum f. sp. *niveum* (E. F. Smith) Snyder & Hansen (Fon) is the damaging pathogen causing Fusarium wilt on watermelon (*Citrullus lanatus*). Rapid and reliable disease diagnosis and pathogen detection is the foundation of Fusarium wilt control in watermelon production. In order to accelerate the detection process and to increase the sensitivity and specificity of detection strategy, we have developed a reliable method to specifically and rapidly detect Fon isolates in Taiwan. With optimized PCR parameters, the molecular method using the designed Fon-1/Fon-2 primer set could not only directly detect small quantities of Fon (as low as 10 pg DNA and 5 conidia) but also tag mild-diseased watermelon samples. Fon could be easily differentiated from other *F. oxysporum formae speciales* in Taiwan by our PCR assay. (Supported in part by BAPHIQ, Council of Agriculture, Taiwan, R. O. C. under grant numbers 93AS-1.9.2-BQ-B1 and 94AS-13.3.2-B1; by the Ministry of Education, Taiwan, R. O. C. under the ATU plan; and by National Chung Hsing University, Taiwan, R. O. C.)

Biological control of Fusarium root rot of lupin with *Trichoderma* species
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Phytopathology 99:S21

Fusarium root rot of lupin (*Lupinus angustifolius* L.) caused by *F. avenaceum* (Fa) is widespread in Alberta, Canada. Eighteen strains of *Trichoderma* spp. were evaluated for antagonism against isolates of Fa. The *Trichoderma* strains exhibited various degrees of overgrowth of Fa colonies in paired culture on potato dextrose agar. Seeds of the lupin cultivar Arabella were coated with a slurry of *Trichoderma* and grown in soil-less mix inoculated with Fa or a non-inoculated control. A thin layer of Fusarium inoculum (1 part colonized wheat grains: 4 parts sand; w/w) was added into each pot as inoculum. The inoculated treatments all developed root rot, but seed treatment with certain *Trichoderma* isolates (Bh-10c, Tf-7, Bh-2) resulted in higher emergence and biomass than treatment with the other isolates. This result indicates that certain *Trichoderma* strains may have potential for use in the management of Fusarium root rot of lupin. A study to confirm the efficacy of *Trichoderma* for control of this disease under field conditions is underway.

Molecular and biological characterization of a mechanically transmissible *Tomato leaf curl New Delhi virus* infecting oriental melon plants
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Phytopathology 99:S21

Oriental melon plants, *Cucumis melo* L. var. Silver Light, showing virus-induced symptoms of leaf curl and puckering were observed in field in 2007. A virus culture isolated from the diseased melon was established in plants of the systemic hosts, *Nicotiana benthamiana* and oriental melon, by mechanical inoculation. In sequence analyses, the cloned full-length DNA-A and DNA-B showed the highest (97.7% and 90.6% respectively) nucleotide identities to those of a cucumber isolate of *Tomato leaf curl New Delhi begomovirus* (ToLCNDV). Hence, the pathogen was identified as a mechanically transmissible isolate of ToLCNDV, designated ToLCNDV-oriental melon isolate (ToLCNDV-OM). When compared to those of the non-mechanically transmissible isolates of ToLCNDV, the mechanically transmissible isolates of ToLCNDV, including oriental melon and potato isolates, have shorter nucleus shuttle protein (NSP) genes. An agroinfection system of ToLCNDV-OM was established for confirming its pathogenicity and mechanical transmissibility. ToLCNDV-OM caused severe symptoms of mosaic, leaf curl and puckering on oriental melon, squash, loofah and cucumber, when inoculated by sap from agroinfected *N. benthamiana* plants. This information revealed that ToLCNDV-OM associated with the field-infected oriental melon was a mechanically transmissible and cucumber well-adapted begomovirus and its mechanical-transmissibility may be related to the NSP gene.

Fungicide sensitivity of *Phakopsora pachyrhizi* (soybean rust) isolates
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Phytopathology 99:S21

Phakopsora pachyrhizi Syd., the causal agent of soybean rust, is a widespread and damaging pathogen found throughout tropicaland sub-tropical regions of the world with the first reported outbreak in the continental USA in 2004. The primary means to control soybean rust is with fungicides. Several fungicide groups, based on their chemical properties and mode of action, were used at varying concentrations to determine their effects on urediniospore germination after 12 hr. There were differences in urediniospore germination between the isolates at the varying fungicide concentrations. Also, there was a twofold difference for effective dose of 50% germination rate among isolates. This suggested that isolates of *P. pachyrhizi* vary in their sensitivity to fungicides.

A survey of *Venturia inaequalis* fungicide resistance in Indiana and Michigan apple orchards
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Phytopathology 99:S21

Apple growers rely heavily on fungicides to manage *Venturia inaequalis*, the fungus that causes apple scab. Fungicide resistance has developed as a result. To quantify and assess the levels of fungicide resistance, isolates of *V. inaequalis* were collected from Indiana and Michigan orchards and fungicide resistance was evaluated. Previously published works were used to determine the baseline concentrations of fungicides and thresholds for growth. Differences were found in the levels of resistance between the two states. In Michigan, 2.3% of the isolates tested were resistant to Sovran (defined as 90% relative growth in the presence of fungicide), but 50% were shifted and less sensitive to the fungicide. 66.7% of MI isolates were resistant to Topsin M. With respect to Dodine, 13.3% were resistant (90% relative growth), but 71.1% showed a shift in resistance. 37.8% of isolates tested with Nova were resistant (80% relative growth), and resistance had shifted in 62.2% of isolates. For Indiana, there was no indication of resistance to Sovran. 77.4% of isolates tested had resistance to Topsin M. Dodine testing showed that 11.1% of isolates were resistant and 77.8% had shifted resistance. Of IN isolates tested with Nova, 33.3% were resistant and 61.9% were shifted in their resistance. On a state level this survey will provide the opportunity to educate growers on the degree of fungicide resistance present in local orchards and prevent ineffective fungicide applications.

The *so* locus plays a role in the mutualistic interaction between *Epichloë festucae* and meadow fescue (*Festuca pratensis*)

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Phytopathology 99:S22

Epichloë endophytes are intimate fungal symbionts of cool-season grasses that generally grow as sparse, unbranched hyphae in the intercellular spaces of host tissues. Anastomosis plays an important role in vegetative growth, colony establishment and sexual development of filamentous fungi. A *soft* (*so*) mutant of *Neurospora crassa* and *aso-1* mutant of *Alternaria brassicicola* have been shown to be incapable of self-fusion. The inability to form interconnected networks of self-anastomosed hyphae may prohibit a fungus from producing structures of complex organization including sexual fruiting bodies. The *so* knockout was generated by targeted gene replacement through homologous recombination. Meadow fescue infected with the *so* mutant showed an increased tiller number, severe stunting, and the infected plants eventually died. However, the *so* mutant grew as wild type on potato dextrose agar. This study suggests that the *so* gene is required to maintain the mutualistic relationship between the fungus and meadow fescue. Further work is in progress to determine the role of the *so* gene in the symbiotic interaction between these two organisms.

Occurrence of mycotoxins in corn in South Dakota in 2007

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Phytopathology 99:S22

Concerns over mycotoxins in South Dakota were raised in fall 2007 when harvested corn brought to several local elevators tested positive for aflatoxin. Reports were widespread enough to prompt a survey. Samples were obtained from local elevators and farmers and assayed for four mycotoxins, including aflatoxin, fumonosin, zearalanone and deoxynivalenol (vomitoxin). The survey included all counties with significant corn acreage in the state. Most of these counties are located in the eastern half of South Dakota. High levels (> 20 ppb) of aflatoxin were detected in samples from several counties. Fumonosin levels were above 2 ppm in several samples, but did not exceed 5 ppm (FDA limit recommended for horse feeds) in any sample. During July, many locations in southeast South Dakota experienced record or near-record dry conditions (many locations received less than 5 mm of precipitation) followed by record or near-record wetness in August (some locations received over 125 mm of precipitation). Temperatures in July were also much warmer than average. These conditions were likely a major predisposing factor to the increased levels of mycotoxins.

BioIntensive management of collar rot affecting tropical sugar beet with biopesticides NIPROT (*Trichoderma viride*) and Su-Mona (*Pseudomonas fluorescens*)

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Phytopathology 99:S22

Biopesticides Niprot (*Trichoderma viride*) and Su-Mona (*Pseudomonas fluorescens*) were evaluated for studying the dosage, extent of suppression of collar rot caused by *Sclerotium rolfsii* in sugarbeet grown in India. Apart from their application as enriched farmyard manure and seed coating biopesticide were also drenched at 30,60 and 90 days after sowing to evaluate the need of additional applications. During the crop period (October-March), the first incidences of *S. rolfsii* was observed 60 days after sowing and the disease incidence gradually increased to 17% till the crop was harvested at 120 DAS. Though the individual treatment of Niprot and Su-Mona was able to suppress the disease but their combined application gave highest plant stand and lowest disease incidence (2.48%) and yield (73.16 tons/ha). The brix content of the beet was also observed to be significantly higher (21.80) as compared to the control (17.98). The microbial profile of the field showed that the bioagents could establish themselves well in the treated plots and could bring down the population of pathogen *Sclerotium rolfsii* effectively. Comparing the cost benefit ratio, the best application strategy to apply biopesticides when the disease incidence is low (<20%) is soil preparation with enriched FYM and seed dressing (1:17.89). In tropical country like India, sugar beet was observed to gain the required average weight of 1.8 kgs within a short period of four months as compared to longer crop duration of six months in temperate regions.

Investigation of tissue tropism of curtoviruses in the plant and the beet leafhopper vector

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Tissue tropism of the curtoviruses, *Beet severe curly top virus* (BSCTV) in *Nicotiana benthamiana* plants and *Beet mild curly top virus* (BMCTV) in the beet leafhopper vector (*Circulifer tenellus*), was investigated. BSCTV induced hyperplasia and abnormal growth of infected plants and, using immunolocalization with an affinity-purified capsid protein (CP) antibody, it was localized to phloem-associated cells in leaves, stems and flowers. BSCTV was not detected in the meristem. A combination of dissection-PCR, immunofluorescence and immunolocalization methods were used to investigate the distribution of wild-type BMCTV and a non-insect transmissible capsid protein (CP) mutant, CP25-28, in *C. tenellus*. Wild-type BMCTV DNA was detected in the leafhopper digestive tract, hemocoel and the salivary glands; whereas DNA of the CP25-28 mutant was detected in the digestive tract and hemocoel but not in the salivary glands. In immunofluorescence and immunolocalization assays, wild-type BMCTV and the CP25-28 mutant were detected in the filter chamber and the anterior, mid- and posterior midgut. However, wild-type BMCTV, but not the CP25-28 mutant, was detected in the principal salivary glands. This was fully consistent with the inability to detect DNA of the CP25-28 mutant in this organ. Together, these results are consistent with acquisition of BMCTV by the leafhopper involving multiple barriers, and the movement of the CP25-28 mutant being blocked at the salivary gland barrier.

Genomic characterization of a phage in *Xylella fastidiosa* almond leaf scorch strain

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Xylella fastidiosa causes almond leaf scorch disease (ALSD) in California. The bacterium is nutritionally fastidious. Therefore, studies on the biology of *X. fastidiosa* are highly challenging. We previously reported the morphological observation of bacteriophages from *X. fastidiosa* culture. Current efforts are on genomic characterization of the bacteriophages. We have developed a procedure to enrich and partially purify the bacterial phages. Phage particles were examined through electron microscopy. In addition to the previous observation of icosahedral morphology, short tails of some particles were observed, confirming that the phages are in the Family of *Podoviridae*. Phage preparations were treated with DNase to remove contaminating chromosomal DNA from the outside of the phage particles. Phage genomic DNA fragments were then PCR amplified. Sequence analysis identified that a prophage of about 40 kb in the whole genome sequence of X. fastiosa strain M23 could be the true genome of the bacterial phage.

Biological control of rice sheath blight and blast by mixture preparation of three strains of antibiotic bacteria

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Phytopathology 99:S22

Rice sheath blight caused by *Rhizoctonia solani* and rice blast caused by *Magnaporthe grisea* are serious diseases in China. Some researches indicated biocontrol of rice disease was an eco-friendly and cost effective strategy. In our previous study, 1200 antagonistic bacteria were obtained by different screening system from different soil and microenvironments. From this pool, we choose 3 strains *Bacillus* sp. (AR156), *Bacillus* sp. (SM21) and *Serratia* sp. (XY21), and mixed them together with the ratio of 1:1:1, and named this mixture as BBS. In this study, BBS showed a high activity against mycelial growth of *R. solani* (98.31% mycelial inhibition) and *M. grisea* (99.16% mycelial inhibition). It achieved biocontrol efficacy of 81.22% towards rice sheath blight and 69.83% towards rice blast on the average, better than that of jinggangmycin in the greenhouse. In the field experiment towards rice sheath blight in 2007–2008, the BBS mixture provided biocontrol efficacy of 67.31%, and the yield increase of 61.24% compared with the blank control group which was only treated with the same amount of water. In the field experiment towards rice blast, the average biocontrol efficacy was 64.96%, and the yield increase was 48.12%. This study showed a good application future of BBS mixture. Thanks to Chinese 863 High-Tech Program (2006AA10Z431).

Analysis of MoCRZ1 downstream genes in *Magnaporthe oryzae* via mutagenesis approach

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MoCRZ1 (*Magnaporthe oryzae* Calcineurin Responsive Zinc Finger) acts as a downstream regulator in Ca^{2+}-dependent signaling for gene expression and pathogenicity. MoCRZ1 binding sites in the *M. oryzae* genome have been identified by ChIP-chip analysis and verified via microarray gene expression experiments. Taken together, these data provide strong support for genes regulated by MoCRZ1 and functioning as downstream targets in the

Ca^{2+}/calmodulin signaling pathway. Candidate MoCRZ1 regulated genes were selected for functional characterization using a mutagenesis approach. Adopting the Hygromycin B split marker system, knockout mutants were generated in *M. oryzae* strain KJ201. Infection related development, ability to cause disease, virulence and other phenotypical characteristics of mutants were assayed to model the late events of MoCRZ1 mediated calcium signaling. Here we present progress towards mutating MoCRZ1 regulated genes and dissection of the Ca^{2+} signaling pathway as it regulates to virulence.

Identification and functional analysis of a candidate parasitism gene *Gr-33E05* of the potato cyst nematode *Globodera rostochiensis*

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Phytopathology 99:S23

The potato cyst nematode, *Globodera rostochiensis*, has evolved an intimate parasitic relationship with host plants by transforming selected root cells into a specialized feeding structure called syncytium that provides essential nutrients for the feeding nematode. Secreted proteins encoded by parasitism genes expressed within the nematode's esophageal gland cells are the major molecules responsible for the formation of syncytium. We cloned a putative parasitism gene (*Gr-33E05*) from *G. rosotchiensis* and also determined its genomic structure. The genomic sequence of *Gr-33E05* consists of six introns and seven exons. Interestingly, additional cloning and sequencing analysis identified two splice variants of *Gr-33E05* that are revealed to be generated by alternative 5′ splice site selection in the third intron. Both *Gr-33E05A* and *Gr-33E05B* encode putative secreted proteins that differ by only a 22-amino acid (aa) segment with Gr-33E05A but not Gr-33E05B containing the 22-aa segment. Transcripts of *Gr-33E05* were found to be exclusively accumulated within the dorsal gland cell of both preparasitic and parasitic stages of the nematode, suggesting a role of its encoded products in nematode parasitism. Functional analyses including analyzing the effects of *Gr-33E05* overexpression in potato hairy roots and *Arabidopsis* and nematode infection assays on transgenic potato hairy roots expressing dsRNA targeting *Gr-33E05* are underway to discover the role of *Gr-33E05* in plant parasitism.

Somatic hybridization in *Puccinia striiformis* revealed by virulence patterns and microsatellite markers

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Puccinia striiformis that causes stripe rust on wheat, barley, and many grass species does not have known alternate hosts for sexual recombination. Somatic hybridization as one of the mechanisms for variations in the asexually reproduced population is hypothetical. This study was to search for evidence of somatic hybridization through characterization of isolates collected from various grasses as well as wheat and barley. A total of 62 isolates were tested on 20 wheat and 12 barley genotypes that are used to differentiate *P. striiformis* f. sp. *tritici* (*Pst*, the wheat stripe rust pathogen) and *P. striiformis* f. sp. *hordei*, (*Psh*, the barley stripe rust pathogen), respectively and tested with 24 microsatellite markers. Dendrograms were generated using the virulence and marker data. Clustering analysis showed groups related to their hosts: isolates infecting only wheat; isolates infecting only barley; isolates infecting both wheat and barley differential genotypes; and isolates infecting only wild grasses with some overlaps and exceptions. Six co-dominant markers grouped the isolates into 20 haplotypes. Haplotype 1 consisted of typical *Pst* isolates, haplotype 2 consisted of typical *Psh* isolates. Haplotype 3 appeared to be a somatic hybrid group from haplotypes 1 and 2, and was able to infect some of the wheat and barley differential genotypes. The results suggest that somatic hybridization occur in the stripe rust population.

Analysis of exudates of sclerotia of *Sclerotinia* spp. for the stimulatory effect on *Coniothyrium minitans*

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Phytopathology 99:S23

Coniothyrium minitans controls *S. sclerotiorum* but not *S. minor* in lettuce production fields of Arizona. Although, both *Sclerotinia* spp. are closely related, the differential efficacy of *C. minitans* on these two fungi requires further study. The objective of this study is to examine sclerotial exudates of these *Sclerotinia* spp. and determine if the compound(s) that act as primary stimuli for *C. minitans* germination are differentially present. Sclerotia were soaked in water for 1 hr and exudates passed through 0.2 µM filters. The exudates were fractionated with ethyl acetate and each fraction collected. The ethyl acetate fraction was dried and resuspended in water. *C. minitans* spores (30 µl at 10^5 spores/ml) were mixed with 30 µl of each fraction and incubated at 20°C for 48 hrs. Percent spore germination was calculated by counting 100 spores on cavity slides in 3 fields of view. Polar fractions from both *Sclerotinia* spp. stimulated spore germination and the percent germination was not significantly different between species. In addition, the percent germination using polar fractions from either species was similar to that using crude exudates. The compounds collected from ethyl acetate fractions did not stimulate spore germination. These results were similar to those using pure water as a control. These results indicated that only polar compounds in exudates are necessary to stimulate spore germination and these polar compounds are present in both *Sclerotinia* spp.

The inhibition by caffeic acid of the expression of the *Monilinia fructicola* cutinase gene *Mfcut1* is regulated by cellular redox

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Phytopathology 99:S23

Monilinia fructicola causes brown rot blossom blight and fruit rot in stone fruits. Infections occur in immature fruit and can remain quiescent, but then develop into rotting lesions during fruit ripening. In a previous study, we provided evidence for a role of host exocarp phenols, notably chlorogenic acid and caffeic acid (CA), in suppression of green fruit infections. CA inhibited the formation of appressoria, the production of virulence factors (cutinase, polygalacturonase), and lesion development. The inhibition of the expression of the cutinase gene, *Mfcut1,* is associated with changes in intracellular glutathione pools. In this study we used 2', 7'– dichlorofluoresce in diacetate and total glutathione measurement to demonstrate that exogenous CA can alter the intracellular redox status in *M. fructicola.* Genes involved in the GSH cycle, including glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase, were isolated and the effect of CA on enzyme activities and gene expression was determined. Addition of buthionine sulfoximine (BSO), a specific inhibitor of γ-glutamylcysteine synthetase involved in GSH synthesis, increased the expression of *Mfcut1*. Our results suggest that CA inhibits *Mfcut1* expression in part by altering the intracellular redox potential as reflected by an increase in the level of reduced GSH.

Oxytetracycline dynamics on peach leaves in relation to temperature, sunlight, and rainfall

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Phytopathology 99:S23

Oxytetracycline (OTC), a member of the tetracycline antibiotics, is used as a foliar spray to control *Xanthomonas arboricola* pv. *pruni* on peach and *Erwinia amylovora* on apple and pear. Applications need to be made at close intervals in order to be effective. We studied the dynamics of OTC on peach leaves treated with 300 ppm of an agricultural OTC in relation to temperature, natural sunlight, and simulated rainfall. We further evaluated the potential of three UV protectants (lignin, titanium oxide, and oxybenzone) and one sticker-extender (Nu Film-17) to prolong OTC longevity on the leaf surface. OTC residue was determined by HPLC (C18 reverse-phase column) with UV/Vis detector. In darkness, constant temperatures up to 40°C did not affect OTC degradation. In contrast, OTC residue decreased rapidly in natural sunlight, declining, on average, by 43.8, 77.8, and 92.1% within 1, 2, and 4 days after application, respectively; 7 days after application, OTC levels were near the detection limit. Use of shade fabric with 25 and 75% sunlight transmittance, simulating overcast sky, reduced OTC degradation significantly. UV protectants were ineffective in improving OTC persistence in outdoor conditions. Simulated rainfall at 44 mm/h drastically (by 67.2%) lowered OTC residue after 2 min, and levels were near the detection limited after 60 min. OTC is highly photosensitive and possesses a low retention capacity on the leaf surface.

Propiconazole and fludioxonil for managing postharvest fungal decays of fresh market tomato

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With the cancellation of ortho-phenylphenate for managing fruit decays of tomato, no postharvest fungicides are currently registered on this crop in the United States. Major postharvest decays of tomato are similar to stone fruit and include sour rot, gray mold, and Rhizopus rot. Currently, fludioxonil and propiconazole are used as postharvest treatments of stone fruit and thus, their use as treatments for tomato was evaluated. Propiconazole was highly toxic in

vitro to *Geotrichum candidum* with EC_{50} values of 0.01 to 0.05 mg/L, whereas fludioxonil was highly toxic to *Botrytis cinerea* and *Rhizopus stolonifer* with EC_{50} values ranging from 0.02 to 0.05 mg/L. The efficacy of the fungicides either alone or in a mixture was evaluated in studies using wound-inoculated fruit. The fungicides completely prevented decay when fruit were first treated and then inoculated (i.e., pre-infection activity). In post-infection activity studies, propiconazole prevented sour rot when applied up to 6 h after inoculation and incubation at 20°C, while fludioxonil prevented gray mold and Rhizopus rot when applied up to 12 h after inoculation. Both fungicides were stable in sodium hypochlorite solutions and commercial in-line, re-circulating drench applications may be possible. Thus, these treatments may improve food safety for tomato by preventing fungal decay that could provide entry sites for human bacterial pathogens while minimizing crop losses during storage and marketing of fruit.

Viruses identified in blackberries grown in Alabama

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Phytopathology 99:S24

A survey was conducted to study the incidence of *Tobacco ringspot virus* (TRSV), *Tomato rinspot virus* (ToRSV), *Raspberry bushy dwarf virus* (RBDV), and *Impatiens necrotic spot virus* (INSV) in commercial blackberry in Alabama. Blackberry plantings at 13 growers' sites and two research stations were tested. A total of 239 samples (symptomatic and asymptomatic) from 14 blackberry cultivars were collected. ELISA kits (Agdia, Inc.) were used for detection of each virus. Virus was detected in 188 samples. Eighty five samples tested positive for infection by a single virus, 85 for the presence of two viruses, 29 for three viruses, and two samples tested positive for all four viruses. ToRSV was detected in 107 (45%) of the samples, INSV in 84 (35%), TRSV in 75 (31%) and RBDV in 61 (26%). TRSV detection was confirmed by reverse transcription PCR amplification and agarose gel analysis. Of the 15 sites from which samples were collected, TRSV was detected at 13, ToRSV at 14 and RBDV and INSV were detected at all 15 sites. Of the180 asymptomatic tissue samples collected, 138 tested positive for at least one virus. Symptoms observed include chlorotic spots on leaves, veinal chlorosis, stunting and combinations thereof. No particular symptom type correlated with a specific virus. These data illustrate the extent of viral diseases in Alabama-grown blackberry plants. Further study is planned to determine the occurrence of other viruses known to threaten blackberry production.

A new compound class synergizes with fungicides to inhibit plant pathogenic fungi

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Phytopathology 99:S24

A proprietary fungal histone deacetylase inhibitor (HDACi), MGCD290, which is currently in human clinical trials, has demonstrated the ability to enhance the activity of azole antifungal agents against medically important fungal pathogens and to reverse fungicide resistance. The objective of this study is to determine if similar HDAC inhibitors can act in synergy with fungicides used in agriculture against a spectrum of plant pathogenic fungi. *In vitro* checkerboard assays using different concentrations of inhibitor and fungicide (Headline, Folicur, or Dividend) were set up and the amount of growth determined after 3 and 5 days. Synergistic activity was seen against *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Monilinia fructicola*, *Botrytis cinerea*, *Colletotrichum* spp., and *Alternaria* spp. Fungicide activity was enhanced from 2- to 16-fold, depending upon the combination, concentration, and fungus tested. In addition, growth of some species was directly suppressed by the HDACi alone. These results demonstrate that mixing this new class of HDACi with fungicides can reduce the amount of fungicide needed to inhibit growth of some plant pathogenic fungi. Additional *in vitro* and *in vivo* studies are underway or planned including testing additional fungi and fungicides, seed and fruit protection assays, and determining if fungicide resistance can be reversed.

Development of rapid field-based detection methods for *Synchytrium endobioticum*

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Phytopathology 99:S24

Synchytrium endobioticum is a soil-borne fungus that infects susceptible potato cultivars, rendering crops unmarketable. It infests the soil by releasing thick-walled spores which are easily transported and viable for over 40 years. The pest is endemic in the Netherlands and parts of Canada, and must be restricted from entering uninfected regions since its spread could impact the potato industry substantially. Current detection methods for *S. endobioticum* rely on microscopic identification by skilled trained personnel and take about 1 day, followed by bioassays which require weeks to obtain a result. Two field-compatible nucleic acid-based testing systems under investigation are surface plasmon resonance (SPR) and hybridization-induced gold nanoparticle aggregation, both of which provide results within 5 minutes. A custom-built SPR machine is being tested to quantify *S. endobioticum* in field settings. Thiolated probe sequences (20 µM) were covalently bound onto gold-coated sensor chips. Target DNA hybridization was detectable at concentrations as low as 20 nM, and the probe chips can be regenerated at least 10 times. Gold nanoparticle aggregation provides a rapid colorimetric assay for *S. endobioticum* detection. Nanoparticles functionalized with two separate thiolated probes (1.5 µM) were mixed and changed color from dark red to purple after the addition of target DNA down to 196 nM. Microliter probe volumes are used and the test is performed at room temperature.

Forecast and virtual weather driven plant disease risk modeling system

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Phytopathology 99:S24

We describe a system in use and development that leverages public weather station data, several spatialized weather forecast types, leaf wetness estimation, generic plant disease models, and online statistical evaluation. Convergent technological developments in all these areas allow, with funding from CSREES (several competitive grant programs) and other sources, the creation of a broad scale decision support system that can serve the US, particularly for plant biosecurity needs, and with forecasted weather, the Western US for some IPM needs. Significant developments of this system include: a) Public/private partnership in downscaling weather forecasts down to 2km resolution, out 6–7 days, at hourly time steps, b) Real-time data ingest of hundreds of weather networks and over 12,000 weather stations, c) Use of a near real-time data quality assurance system, d) Use of weighted distance-elevation regression of nearby weather for virtual weather estimation, e) Online statistical evaluation software, and f) A generic suite of 10+ plant disease risk models. Thus far mean absolute error rates for virtual weather have averaged as low as 0.9°C for hourly temperature, and 1.4°C for dew point. The system shows promise for increasing adoption for IPM in Western states, and can provide national decision support for plant biosecurity applications.

Characterization of unknown fungus associated with symptoms similar to dollar spot on warm-season turfgrass in Florida

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Phytopathology 99:S24

Dollar spot is a disease of turfgrass caused by *Sclerotinia homoeocarpa* (Bennett). The pathogen produces abundant mycelia on potato dextrose agar followed by a large plate-like irregularly shaped stroma embedded in the media. Tan foliar blight symptoms of dollar spot rarely exceed 5 cm on greens and fairways, and stroma are infrequently observed. Symptoms initially similar to those were observed in Florida on bermudagrass and seashore paspalum. The patches were larger than dollar spot rarely exceeding 9 cm in diameter, and affected turf turned nearly white with time. The most distinguishing feature of this disease was sclerotia-like structures with an average diameter of 0.2 mm were embedded in tissue of both hosts. The fungus isolated from these symptoms produced stroma of regular size (0.5 – 2.0 mm) in culture after 2 to 4 weeks. Isolates of this fungus and one *S. homoeocarpa* isolate were inoculated onto bermudagrass, seashore paspalum, and creeping bentgrass to test pathogenicity. Media type used to generate the inoculum had a significant effect on the amount of disease that occurred. Sterilized wheat seed inoculum produced the most disease. Symptoms occurred after 2 to 7 days, and the fungi were re-isolated from infected turfgrass. Inoculations were conducted three times with the same results. Future work will focus on morphology and genetic sequence of the anamorph. Initial results indicate the fungus is a previously undescribed *Poculum* species.

Risk of Rhizoctonia web blight development on container-grown azalea

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Phytopathology 99:S24

Rhizoctonia web blight, caused by binucleate *Rhizoctonia* spp., is an annual problem in the southern United States on container-grown azaleas

(*Rhododendron* spp.). Disease was assessed weekly from May to September at three locations from 2006 to 2008 by counting the number of blighted leaves on 15 randomly selected plants from 200 to 400 plants per location. Disease onset occurred from mid-July to mid-August, and disease severity peaked between late August and mid-September. Based on the relative increase in the log-transformed number of infected leaves per plant, weekly assessment periods were classified as having rapid (≥10% increase), intermediate (0 to 10%), or slow (≤0%) disease progress. Three-day moving averages (MAs) of various weather variables were calculated, and lagged values (by 5 days) of the MAs were evaluated as predictors of disease progress periods. Slow disease progress was characterized by meeting at least one of the following criteria for the lagged MAs: min., avg., or max. temperature of <20, >28, or >35°C, respectively, or an avg. vapor pressure deficit <2.5 hPa. These weather variables allowed reasonably good distinction of rapid vs. slow disease progress periods, but were poor at distinguishing either from intermediate progress. Weather variables could be useful for a negative prognosis of disease risk, and correctly predicted disease progress as 'not rapid' and 'not slow' for 87.5 and 91.2% of the weekly periods, respectively.

High-resolution mapping of the wheat *Lr46* pleiotropic rust resistance locus

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Phytopathology 99:S25

Rust diseases are globally the most important diseases of wheat, and durable sources of genetic resistance are considered the most effective means of control. The pleiotropic *Lr46* gene confers durable, race non-specific resistance to leaf rust and stripe rust but little is known about it's mechanism of action. Using a population of 3931 lines with a genetic resolution of approximately 0.01 cM, fine-scale mapping of the *Lr46* locus was carried out for both the development of molecular markers and map-based cloning of the gene. Existing markers were used to probe wheat hexaploid and tetraploid bacterial artificial chromosome (BAC) libraries and, following low-pass sequencing of selected BACs, contigs were assembled that were the source of many additional markers. However, no recombination was detected between BAC-derived markers, so synteny with the model grass genome, *Brachypodium distachyon*, was explored. Markers spanning the *Lr46* locus were colinear with a 90 kbp physical region from *Brachypodium*, which was used to identify wheat ESTs and develop new markers. Subsequently, we delimited *Lr46* to a 13 kbp physical region in *Brachypodium* and a 0.45 cM genetic interval, where the closest marker was 0.02 cM distal to the gene. This high-resolution map will form the basis of map-based cloning.

Natural and synthetic products on the protection of cocoa seedlings against *Moniliophthora perniciosa*

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Phytopathology 99:S25

Currently, the control of witch's broom disease needs the adoption of the integrated disease management, which includes the genetic, cultural, chemical and biological methods. Alternative methods such as induced resistance and natural fungicides, mainly produced from plant extracts, are promising, although research on this area is sparing. The objective in the present study was to verify the effect of natural and synthetic products on the protection of cocoa seedlings against *Moniliophthora perniciosa*. As natural products, plant extract based on coffee leaves obtained from rust susceptible and resistant coffee trees, and from the peel of cocoa fruits obtained from witch's broom susceptible and resistant cocoa trees were tested. Synthetic products were based on phosphorilated mananoligosacharide (Agro-Mos®) with two formulations (with and without Cu^{++} and Zn^{++}) and Recop® (copper oxichoride), a conventional protective fungicide were also tested. The treatments were compared with an inoculated-only control treatment. The plant extracts were not efficient on the protection of cocoa seedlings against *M. perniciosa*, however the Agro-Mos® treatment showed the same effect on the reduction of witch's broom disease incidence as observed for the treatment with Recop®, in all tested doses. The inefficiency of the Experimental Agro-Mos® is likely to be due to the absence of a copper component in the formulation.

Development and evaluation of detection-based air sampling programs for grapevine powdery mildew in eastern Washington

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Phytopathology 99:S25

Powdery mildew of winegrape (Vitis vinifera L.), caused by Erysiphe necator, is one of the most problematic diseases of grapevine worldwide. A real-time PCR assay using species-specific primers was developed for qualitative and quantitative detection of E necator in vineyard air samples collected by Rotorod sampling devices. Three methods (FastPrep DNA kit, UltraClean MoBio and FastPrep DNA kit for soil) were used to purify DNA of E. necator collected from air samples and evaluated with respect to conidia DNA yields. The DNA yields varied considerably with the extraction procedure used. The temporal concentration of E. necator propagules in the vineyard air was verified using a Burkard volumetric spore trap. Foliar disease incidence and severity was evaluated in leaves during both years. Regression analyses of the vineyard data revealed significant relationships between DNAsignal strength and aerial spore concentrations, foliar disease incidence, and foliar disease severity during both years of the study. The findings of this study describe a rapid, reliable method to assess the presence and concentration, of E. necator propagules in thevineyard air. Further elucidationof the quantitative relationship between DNA signal strength and treatment thresholds may result in an accurate means to time initial and subsequent fungicide applications, and through the incorporation of an inoculum component make grapevine powdery mildew models more precise.

Integrated management of strawberry gray mold

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Phytopathology 99:S25

Gray mold, caused by *Botrytis cinerea*, is an important strawberry disease. As gray mold control is difficult, there is a need to evaluate integrated methods to achieve disease management. The efficiency of integrating *Clonostachys rosea* sprays, fungicide sprays, and crop debris removal to manage gray mold was evaluated in field experiments conducted in 2006 and 2007. Leaf colonization by both *C. rosea* (CrC) and *B. cinerea* (BcC), gray mold incidence in both flowers (Iflower) and fruits (Ifruit), and yield were evaluated. In both years, CrC was higher in the treatments with no fungicide. BcC, Iflower and Ifruit were most reduced in the treatments that included *C. rosea* sprays, as compared to the check. Maximal reductions were achieved by combining *C. rosea* sprays, fungicide sprays and debris removal (96.62%, 86.54%, and 65.33% reductions of BcC, Iflower and Ifruit, respectively). Maximal yield (103.14% increase as compared to the check) was achieved by combining the three treatments. With just *C. rosea* sprays, BcC, Iflower, and Ifruit were reduced by 92.01%, 68.48%, and 65.33%, respectively, whereas yield was increased by 75.15%. Therefore biological control with *C. rosea* was an efficient method of managing gray mold, which when associated to chemical and cultural methods increased the efficiency of disease management under field conditions. Financial support: FAPEMIG and CNPq.

Practical implications of fungicide resistance in Northeastern US populations of the apple scab pathogen *Venturia inaequalis*

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Phytopathology 99:S25

Demethylation inhibitor (DMIs) and Quinone outside inhibitor (QoIs) fungicides are essential for managing apple scab and other early season apple diseases. Shifts toward both DMI and QoI resistance have been observed in Northeastern US populations of *Venturia inaequalis* over the past five seasons as use of these fungicides continues. In 2007 & 2008, we surveyed a minimum of 25 commercial, 4 research, and 3 baseline apple orchards for sensitivity to myclobutanil (DMI), trifloxystrobin (QoI), and dodine (guanidines) using microscopy-aided relative growth assays. All of the commercial orchards were strongly shifted above baseline sensitivity to myclobutanil and trifloxystrobin. More than 75% of the orchards had *V. inaequalis* populations with myclobutanil sensitivity reduced to the point at which we fail to achieve apple scab control in a DMI-resistant research orchard using DMI fungicides. Interestingly, several orchards displayed dodine sensitivities approaching that of *V. inaequalis* populations from baseline orchards. However, it remains to be seen if dodine resistant *V. inaequalis* populations will quickly re-emerge during a season of renewed use. Field trials using DMI and QoI fungicides in DMI-resistant and QoI-shifted research orchards suggests that newly-introduced DMI chemistries may overcome practical resistance in varieties less susceptible to apple scab, but not in highly susceptible varieties that also contribute to high levels of *V. inaequalis* inoculum.

Organic apple disease management in Vermont with alternative fungicides
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Phytopathology 99:S26

A major challenge in organic apple production in Vermont is the available fungicide options for apple scab (*Venturia inaequalis* (Cooke) Wint.) management. The objective of this study was to compare the effectiveness of potassium bicarbonate, neem oil, and *Bacillus subtilis* to a standard organic lime sulfur/sulfur fungicide program and a non-sprayed treatment for control of scab and other fungal diseases. Treatments were applied to 'Empire' trees arranged in a completely randomized design with five single-tree replications at the University of Vermont Horticultural Research Center in South Burlington, VT. Fungicides were applied with a handgun to drip, using maximum label rates. In 2007 and 2008, applications began in late April and continued on approximately a weekly schedule through the end of June and then every two weeks through mid July. The standard lime sulfur/sulfur treatment resulted in the best overall scab management in both years. The neem oil treatment showed significantly lower levels of foliar and fruit scab than the non-sprayed and the other alternatives in 2008. However, both the lime sulfur/sulfur and neem oil treatments had disadvantages, including phytotoxic burning and/or significantly more russetting on the fruit at harvest. This research shows that potassium bicarbonate, *Bacillus subtilis*, and neem oil do not offer substantial advantages over the standard lime sulfur/sulfur fungicide program in organic apple production.

Using pesticide residue photos to change behaviors of pesticide applicators
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Phytopathology 99:S26

In 2006, the pesticide education programs in Colorado and Wyoming staged and took photos mimicking pesticide residue transfer. Using the fluorescent tracer dye, Glo-Germ(R) as a substitute for pesticide residue, photos were produced that captured the proper use of Personal Protective Equipment (PPE), cleaning PPE, proper removal of PPE, and residue transfer from the applicator to everyday items like cell phones, food and drink, and automobiles. These photos have been used for three years in the training of farm workers in Colorado and Wyoming. In 2008 and 2009, these photos were incorporated into a series of lectures and trainings for homeowners, landscapers, and Master Gardeners in Fresno County, California. The photos and the lecture series have been found to be a powerful outreach tool that changes behaviors and makes people more aware of the pesticide residue that they may be transferring to their homes and families.

Paralogs of the *Trichoderma virens* elicitor SM1
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Phytopathology 99:S26

Trichoderma virens, a biocontrol agent, is known to protect plants by several mechanisms, including mycoparasitism, production of antibiotics, and elicitation of plant defense responses. Previous studies demonstrated that, *T. virens* SM1, a member of the cerato-platanin family of proteins, induces systemic resistance in both cotton *and maize.* A survey of the *T. virens* genome revealed three paralogs of *sm1* (arbitrarily designated *sm2, sm3, and sm4*). A gene phylogeny of mature cerato-platanin proteins showed that SM1 and SM3 were more closely related than the other paralogs. The proteins SM2 and SM4 were located on a separate branch from SM1 and SM3 indicating possible functional divergence. Real-time PCR confirmed that in the presence of maize roots or the pathogen *Rhizoctonia solani, sm3* was upregulated, similar to *sm1*. The potential role of *sm3* was assessed by examining the gene expression during different *T. virens* lifestages. We also generated gene-deletion and over-expression mutants to assess the induction of resistance in maize against the foliar pathogen *Colletotrichum graminicola* by measuring the expression of six-defense related genes and lesion size. Furthermore, SM1 and SM3 were expressed in *Pichia pastoris* and the proteins were compared for their ability to induce resistance in maize. The study of families of elicitor proteins of beneficial fungi is an innovative approach that will increase our understanding of plant-microbe interactions.

Influence of soils, nutrition, and water relations upon charcoal rot disease processes in Kansas
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Phytopathology 99:S26

Charcoal rot (caused by Macrophomina phaseolina) is the most important soybean disease in Kansas. Several strategies have been recommended to control this disease including crop rotation, lower planting density, biological control, plant tolerance/resistance, and fungicide application. However, these techniques have not been completely effective. Information on the effect of soil texture and the interactions with irrigation and fertilization (particularly manganese) upon charcoal rot disease severity and pathogen population is limited. To determine the effect of the aforementioned variables, a field experiment was conducted in Manhattan and Rossville, Kansas. Pathogen colonization was assessed by measuring colony forming units from root tissue at R2-R4 (post-flowering/pod) and R8 (maturity) stages. Soil populations of M. phaseolina (pre-planting and post-harvest), yield parameters, and plant characteristics were obtained. Results indicate that there are complex relationships between soil physiochemical properties (pH, nitrogen, phosphorus, exchangeable ions, organic matter) and soil texture (sand, silt, and clay composition), which may affect disease severity and pathogen levels in host tissue. Although soil populations did not increase over the course of the season, root colonization increased variably. Soil and environmental information obtained from field studies will be used to test the disease in artificial inoculation experiments under controlled environmental conditions.

Evaluation of flue-cured tobacco for resistance to TSWV in Georgia
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Phytopathology 99:S26

Tomato Spotted Wilt Virus continues to be one of the greatest tobacco production problems in Georgia. A trial was initiated in 2008 to evaluate tobacco lines and cultivars for resistance to TSWV. The trial was initiated at the University of Georgia's Bowen Farm in Tifton, Georgia. The trial was a split plot, randomized complete block design with entries having 5 or 10 replications depending on the entry. Each plot was 2 rows, one row non-treated, and the second row treated in the float house with Admire Pro and Actigard. Only H 22, H 106, and K 326-T had significantly lower TSWV than the NC 71 standard in the non-treated plots. However, all cultivars/entries, except H 50, had lower TSWV than the standard when treated with Admire Pro plus Actigard in the float house. Fifteen of the twenty entries had significantly lower levels of TSWV when treated with Actigard and Admire Pro when compared to the non-treated. Only yield of K 326-T was higher than the standard (NC 71) in the non-treated plots. No differences in yield were noted among yields of tobacco treated with Admire Pro plus Actigard in the float house. The yield of seven of the twenty entries significantly increased over the same entry that was not treated with Admire Pro plus Actigard.

Effect of copper compounds on the survival and PCR-based detection of the bacterial spot pathogen on tomato plants
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Phytopathology 99:S26

The disease bacterial spot is a persistent and serious problem for Ontario tomato producers. Disease control depends on copper-based sprays such as Kocide 2000 tank-mixed with chorothalonil or mancozeb. The effectiveness of copper sprays is limited; yet, in a survey of Ontario strains of the pathogen isolated over the past 16 years, only 3.5% (7/201) were resistant to copper. Greenhouse-based experiments indicated that Kocide reduced but did not eliminate bacterial populations on tomato plants. Although poor timing and incomplete spray coverage contribute to the ineffectiveness of sprays, copper also may be inducing the pathogen to enter into a viable but non-culturable state, as has been noted with other bacterial plant pathogens. The objective of this work was to determine the effect of exposure to various concentrations of copper (Kocide and copper sulfate) on the culturability, viability and PCR-based detection of this pathogen. Viable counts were determined using the Molecular Probes Live/Dead BacLight Bacterial Viability Kit. At a Kocide concentration of 27.5 µg/ml, no culturable cells were found yet PCR assays were positive. After a 10-day exposure to 0.01 mM or 0.1 mM copper sulfate, culturable cells could no longer be detected yet the number of viable cells remained close to that observed after a 1-day exposure, or 1% of the number added at day 0. Viable but non-culturable cells could be returned to an active growth phase by the addition of copper-complexing substances.

Development of a chitinase assay for tall fescue challenged with *Rhizoctonia solani*
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Phytopathology 99:S26

Tall Fescue is a commonly utilized turfgrass in the transition zone of the U.S. However, it is susceptible to the fungal pathogen *Rhizoctonia solani*. Chitinase activity has been correlated with resistance to *Rhizoctonia solani* in several plants. We hope to develop a protocol for quantifying chitinase levels

in tall fescue challenged with *Rhizoctonia solani*. Three tall fescue cultivars Matador, Kentucky 31, and Jaguar were inoculated with AG-2 141, Ag-2 146 strains of *Rhizoctonia solani* or non-inoculated control. Two hundred mg of shoot tissue was extracted 0 and 48 hours after inoculation from each sample. A bicinchoninic acid (BCA) colometricassay was utilized to quantify protein in the tissue extracts. Concurrently, tissue extracts were incubated with blue-dye labeled chitin and absorbance was measured. Finally, visual ratings for brown patch were taken one week after inoculation. Forty-eight hours post inoculation chitinase activity was greater in the Jaguar variety across both isolates when compared to the other two cultivars. Additionally, Jaguar showed less visible symptoms then the other cultivars. Based on these preliminary findings Jaguar expresses chitinase in an efficient manner i.e., when the plants senses the fungal pathogen (possibly senses chitin). The high expression of chitinase in Jaguar relative to other cultivars during the potential colonization period of *Rhizoctonia solani* may explain why Jaguar is more resistant then Matador or K31 to brown patch.

New biovar 3 *Dickeya* spp. strain (syn. *Erwinia chrysanthemi*) as a causative agent of blackleg in seed potato in Europe

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Blackleg caused by *Pectobacterium* and *Dickeya* species is a worldwide disease of potato. Effective disease management strategies to control blackleg are not yet available, partly because our knowledge on the ecology of blackleg pathogens is incomplete. In Europe, an increasing frequency of seed potato infections with *Dickeya* spp. is observed. Till 2000, only *D. dianthicola* (biovar 1 and 7) was associated with potato in temperate climates. However, in recent years a new unclassified *Dickeya* spp. biovar 3 clade has appeared in Finland, Poland, Israel and The Netherlands. Strains of this bacterium isolated from potatoes in the different countries are clonal according to sequence data, biochemical and REP-PCR analysis. Studies on the distribution in seed potatoes revealed that this new *Dickeya* sp. was located mainly inside tubers at the stolon end. A GFP-tagged new biovar 3 strain was able to systematically colonize potato plants after soil infestation and enter progeny tubers and stems via infected roots. Systemic colonization of plants was also found after stem inoculation, resulting in infection of roots, stolons and progeny tubers. Leaf inoculation resulted in a spreading of the inoculum to the main stem, but not to the roots. In conclusion, a new *Dickeya* spp. biovar 3 clade has been efficiently colonizing various plant tissues and seems to displace other blackleg pathogens, as since 2003 no other *Dickeya* spp. have been isolated from seed potato in The Netherlands.

Biological control of aflatoxin contamination using non-toxigenic *Aspergillus flavus*

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The use of non-toxigenic isolates of *Aspergillus flavus* to control aflatoxin contamination of seeds has been used in a number of crops. The non-toxigenic isolate is usually applied on a substrate to the soil surface where it saprophytically colonizes organic debris and out competes the indigenous toxigenic *A. flavus,* presumably lowering toxigenic inoculum potential. Other work has shown that co-inoculation of non-toxigenic and toxigenic isolates in the infection court results in amelioration of aflatoxin contamination. This is considered a parasitically based mechanism. The intra-specific inhibition of aflatoxin synthesis by non-toxigenic isolates toward toxigenic isolates growing together *in vitro* provides a partial explanation of the co-inoculation phenomenon. Our work has shown that the intra-specific aflatoxin inhibition is nutrient and vegetative compatibility group independent, and during the first 24 hrs of toxigenic isolate growth it must physically touch or come in contact with the growing non-toxigenic isolate. Furthermore, we have shown that there is specificity in the "thigmo-down regulation" of aflatoxin synthesis. Just because an isolate inhibits one toxigenic isolate doesn't insure that it will inhibit another one. The specificity of aflatoxin inhibition allows testing of the relative importance of the two contributing mechanisms to biological control: the saprophytic/epidemiologic mechanism vs. the parasitic/thigmo-down regulation mechanism.

Effect of minerals on biofilm formation by *Xylella fastidiosa*

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The bacterium *Xylella fastidiosa* (*Xf*) causes diseases in important crops such as grapes, citrus, almond, coffee and blueberries. The bacterium is limited to live in the xylem vessels of the host plant. The mechanism of infection is not fully understood, but the most plausible explanation is that the formation of biofilms inside the xylem vessels obstructs the passage of water. Nevertheless, some studies show that the amount of vessels occluded during *Xf* infection (less than 50%) is not enough to be the sole responsible for the disease. We are studying the effect of mineral ions in the infection process of *Xf*, and in particular in the biofilm formation. Once a microorganism is inside the host, it needs to acquire essential mineral and trace elements, therefore competing with the host. We hypothesize that *Xf* biofilms are efficient scavengers of mineral ions. The first step in our research was to study the effect of minerals in the biofilm formation by *Xf*. Addition to the broth media of 100 µM of iron or calcium resulted in an increase in biofilm formation. Manganese increased the biofilm formation at concentrations higher than 800 µM. Addition of copper, potassium and magnesium had no effect on biofilm formation. None of the ions added had an effect in *Xf* planktonic growth at the range of concentrations tested (100 to 2500 µM). Inductively Coupled Plasma (ICP) is being used to measure the mineral quota of *Xf* in biofilm versus planktonic cells.

In vitro transcripts of a full-length cDNA clone of *Hosta virus X* are infectious to Hosta and Nicotiana benthamiana plants

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Hosta virus X (HVX) is an easily transmitted potexvirus that causes important economic losses to the Hosta market. No significant source of resistance to this virus is known, and information regarding the virus biology is limited. The aim of this work was to build an HVX infectious clone, which can be used both as a system to study virus-host interactions and as a tool to screen for resistance among the diverse *Hosta* germplasm. *Hosta* 'Sum and Substance' and *Nicotiana benthamiana* plants were rub-inoculated with *in vitro* RNA transcripts produced with T7 RNA polymerase from pHVX, a full-length cDNA clone of the Ohio HVX isolate H37. Inoculated leaves were tested by DAS-ELISA and Immunocapture RT-PCR (IC-RT-PCR) at 18 dpi. *Hosta* and *N. benthamiana* plants were positive for HVX by DAS-ELISA, with higher OD_{405} values for the *Hosta* cultivar. IC-RT-PCR resulted in the production of an amplicon internal to the replicase open reading frame (ORF). An amplicon of expected size was also produced by RT-PCR from total RNA extracted from *in vitro* RNA transcript-inoculated plants using primers flanking the coat protein ORF.

The genome sequence of *Pantoea ananatis*

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Pantoea ananatis is a pathogen on a wide range of plants and causes opportunistic human infections. It is frequently isolated from diverse environmental sources including the plant environment and from insects. It thus has an extraordinary ability to survive in a multitude of environmental niches, under a variety of conditions and cause cross-kingdom infections. The genome of a pathogenic strain, isolated from diseased *Eucalyptus* tissue, was sequenced using 454 technology. The genome consists of a single circular chromosome 4.65 Mb in size with a GC content of 53.7%, encoding 4,140 protein coding sequences (CDS). Genome comparisons against closely related phytopathogens revealed the presence of an exopolysaccharide with high homology to stewartan, the major pathogenicity factor in the corn pathogen, *Pantoea stewartii* subsp. *stewartii* A Type III secretion system is absent. Three copies of a novel disease-associated Type VI secretion system are present on the genome. Further comparisons against all available genome sequences indicated a large number of CDS with distinct homology to bacteria occupying specific niches, particularly the plant, insect and animal niches frequented by *P. ananatis*. The genome sequence of *P. ananatis* thus gives an indication of an organism that is well adapted to survival in a wide range of environments and that is capable of causing disease symptoms in a number of hosts.

Victoriocin, a novel broad-spectrum antifungal protein secreted by virus-infected *Helminthosporium victoriae* isolates

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Victoriocin, a 10-kDa secreted protein, was purified from culture filtrates of a virus-infected isolate of the plant pathogenic fungus *Helminthosporium* (teleomorph: *Cochliobolus*) *victoriae* by a multi-step procedure involving ultra-filtration and reverse- phase high performance liquid chromatography (RP-HPLC). Amino acid sequences, obtained by automated Edman degradation sequencing of RP-HPLC-purified polypeptides, were used to

design degenerate primers for PCR amplification from fungal DNA and cDNA. An open reading frame coding for a victoriocin precursor of 183 amino acids with calculated molecular mass of 20 kDa was amplified by PCR from *H. victoriae* genomic DNA. Sequence analysis indicates that victoriocin has a sequence motif similar to that found in scorpion short toxin/charybdotoxin and a consensus sequence similar to that of defensins. Although victoriocin is encoded by the fungal host, it resembles the virally-encoded killer proteins in that it is expressed *in vivo* as a preprotoxin precursor consisting of a hydrophobic N-terminal secretion signal, followed by a pro-region and terminating in a classical Kex2p endopeptidase cleavage site that generates the N-terminus of the mature victoriocin predictably in a late Golgi compartment. Overproduction of victoriocin in a virus-free strain transformed with the *victoriocin* gene mimicked the effects of virus infection on inducing overexpression and secretion of victoriocin in cultural filtrates.

Diversity of *Fusarium oxysporum* isolates infecting cortical tissues of chickpea roots

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Fusarium oxysporum (*Fo*) comprises host-specific pathogenic populations that cause mainly Vascular wilts as a result of vascular infection of a host plant; and non-pathogenic but parasitic soil-inhabitant populations capable of colonizing the root cortex of host and non-host plants. The latter statement implies that any soil-inhabitant *Fo* isolate can potentially colonize the root cortex of any plant, but this assumption has remained largely unaddressed. Consequently, *Fo* populations resident in soil or colonizing the plant root cortex have received little attention. *Fo* f. sp. *ciceris* is a monophyletic group that causes Fusarium wilt of chickpea. In this study, we analyzed 42 putatively non-pathogenic to chickpea *Fo* isolates obtained from surface-disinfested chickpea tissues from Ethiopia, Spain, Mexico, Morocco and Syria. Based on phylogenetic analysis of the translation elongation factor 1-alpha gene, the non-pathogenic isolates displayed a certain degree of diversity. The highest diversity was found in isolates from Ethiopia, thought to be a center of diversity of chickpea. However, isolates from different areas were often closely related to previously well-characterized non-pathogenic cortex-colonizing *Fo* isolates obtained from chickpeas. Mexican isolates were the exception as they were placed in a distinct clonal lineage. These results suggest that only certain *Fo* genotypes have the ability to colonize the host root cortex and may represent a possible origin of host-specific pathogenic forms.

Current situation of citrus Huanglongbing in Guangdong, P. R. China

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Guangdong Province is an important citrus production region in China. Citrus Huanglongbing (HLB, yellow shoot disease) was observed in Guangdong probably in the late 1800's and the disease was first studied there. Since the 1990's, citrus production in Guangdong has gradually shifted from the coastal Chaoshan and Pearl River delta plains, where HLB was endemic, to the mountainous / hillock North and West areas, where citrus production was limited and HLB was little known. As citrus production expanded, reports of HLB followed. To understand the HLB situation in Guangdong, we collected symptomatic citrus samples from 16 cultivars in 12 prefecture cities in 2007. PCR with primer set OI1/OI2c was used to detect "*Candidatus* Liberibacter asiaticus" for HLB confirmation. Among the total of 359 samples collected, 241 (67.1%) were positive in "*Ca.* L. asiaticus", distributed in all 12 cities. Of particular importance is the confirmation of HLB in a mandarin cultivar "Shatangjie", which currently occupies two third of the citrus planting acreage in Guangdong. We also identified HLB in the less popular cultivars such as "Mashuijie" and "Cuntianjie" which have high potential for future citrus cultivations. Our data indicated that affected budwoods probably played a key role in the current spread of HLB. To avoid future HLB outbreaks, strict regulation of propagation materials should be exercised along with optimal orchard management.

Comparisons of plant cover estimates using APS *Assess* software and point-frame transects at Camp Guernsey, Wyoming

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The U. S. Army currently uses vegetative surveys to monitor ground cover and vehicle damage on training areas; however, methodologies for determining vegetative cover are not universal and vary among installations. These methods can be so labor-intensive and time consuming that repeated estimates per plot during the year become unrealistic. There is a need for techniques to estimate ground cover on training lands that are quantitative, accurate, inexpensive, and do not require extensive technical botanical skills. A commercially available software program, Assess (Lamari 2002), was used to analyze a series of digital images collected at Camp Guernsey, Wyoming. Three sites at Camp Guernsey were subdivided into twelve plots each. Ten random digital images were taken in each plot with a Nikon Coolpix® 4300 digital camera set on "normal" (1600 dpi resolution). Photos from Camp Guernsey were then analyzed using the Assess batch processing analysis feature. The analysis of digital photos using the *Assess* software is a very quick and accurate way to estimate ground cover. The collection and analysis of the photos took significantly less time than the point-frame method and the cover estimates were not significantly different during the spring and summer sampling periods. The results of this study show that the use of digital image analysis to determine vegetative cover can be an accurate, cost-effective way to monitor vegetative conditions on Army training lands.

A simple, reliable method for creating unmarked mutations in Gram-negative bacteria

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The ability to introduce site-specific unmarked deletions into a bacterium's genome is an important genetic tool. However, we found that creating the 'deletion allele' for the targeted gene using splice-overlap extension PCR or ligation independent cloning was problematic. As an alternative, we adapted the recently described DelsGate method, which employs a Gateway donor vector and the BP clonase reaction, for use in Gram-negative bacteria. First, we created a new vector, pDONR-SacTet, by introducing a cassette with *sacB* and a tetracycline resistance marker into pDONR201. Second, we reversed which site-specific primers normally are modified by the addition of *attB*1/*attB*2 and the very rare I-*Sce*I recognition sequences. Third, we electroporated the circular plasmid carrying the deletion allele, rather than a linearized form, into *R. solanacearum*. Typical of SacB-assisted two-step allelic replacement, we then: a) selected transformants on agar plates supplemented with tetracycline or kanamycin and b) plated colonies on a minimal medium supplemented with 5% sucrose as the sole carbon source. Sucrose-sensitive colonies were screened for antibiotic sensitivity, and small pools were tested by colony PCR to detect those with the desired deletion. This method of creating deletion alleles is simple and robust, and should work for all Gram-negative bacteria that are amenable to SacB-assisted selection.

New records for the Brazilian Cerrado of leaf pathogens on *Jatropha curcas*

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Jatropha curcas is an euphorbiaceous plant that produces seeds with high oil concentration. The species is being extensively studied by Embrapa in several research centers throughout warmer and drier areas of Brazil to explore its potential as raw material for bio-diesel production. In Planaltina, Distrito Federal, leaf spots caused by a cercosporoid hyphomycete was observed and *Cercospora jatrophae-curcas* was the agent precisely identified. The symptoms consisted of well delimited light brown irregular necrotic spots where fascicles of sympodial cicatrized conidiophores were found in large numbers yielding simple obclavate conidia with an slight tint of pale brown. The dimensions of all structures agreed with those shown in the original species description. Also depressed yellowish leaf areas containing several acervuli were present on the host leaf. Two Colletotrichum species isolated are still being identified and tested. As *J. curcas* is still a crop under domestication in Brazil, it is important to take note of its associations with pathogenic fungi that may become limiting factors to the future growth of this potential commodity.

First record of *Jatropha* rust (*Phakopsora arthuriana*) in Central Brazil

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Jatropha curcas (Euphorbiaceae) is a potential source of biodiesel with ongoing research in Mato Grosso do Sul (MGS), Brazil. From March 2007 serious outbreaks of rust (*Phakopsora arthuriana* Buriticá & Hennen,

anamorph: *Malupa jatrophicola* (Arthur) Buriticá & Hennen) were detected in the municipalities of Dourados and Eldorado. It deserves attention the fact that until 1994 the fungus was known as *P. jatrophicola* Cummins, however, according to Hennen et al. [In: "Catalogue of the species of plant rust fungi of Brazil, 2005", also on line at http://www.jbrj.gov.br] Buriticá & Hennen in 1974 published *P. arthuriana* - "because *P. jatrophicola* (Arthur) Cummins published in 1937, technically refers only to an anamorph. Cummins (1937) transferred an anamorph name, *Uredo jatrophicola* Arthur, to the teleomorph genus. He did publish a description of the telia in English, but not in Latin as required by the Code. Later, Cummins (1956) published *P. jatrophicola* Cummins as new species, but the combination *P. jatrophicola* had been preoccupied, and could not be used as a name for a new species". – Symptoms description of the disease, and detailed illustration of the fungus specimen from MGS will be shown including both teleomorphic and anamorphic phases.

Impacts of Fusarium root inoculation on soybean plants
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Fusarium is a common fungal genus that has been implicated in soybean root rot, but its impact on yield is unclear. To estimate potential yield loss caused by *Fusarium* spp., fumigated and non-fumigated microplot experiments were established near Gilbert, IA. Microplots contained a single row of 20 plants. Sorghum seeds colonized with *Fusarium* isolates from soybean roots were used as inoculum. Fumigated plots were infested with 19 isolates representing seven species (*F. oxysporum, F. solani, F. graminearum, F. acuminatum, F. semitectum, F. equiseti, F. sporotrichioides*) and non-fumigated plots were infested with one isolate each of *F. oxysporum, F. solani, F. acuminatum,* and *F. graminearum*. Plants at R1 were evaluated for root rot, root and shoot dry weight, and yield was measured. In non-fumigated plots, inoculation increased root rot severity, but this increase was significant only for the *F. oxysporum* (120L9) and *F. graminearum* isolates. Root dry weight, yield and seed moisture did not differ among isolates, but shoot dry weight differed (P = 0.0126). In fumigated plots, *Fusarium* isolates differed (P = 0.0068) in aggressiveness, with *F. oxysporum* (120L9) causing the most severe root rot and *F. oxysporum* (34T5) causing least severe symptoms. Yield and seed moisture were unaffected. Late planting possibly affected root rot severity and plant growth in fumigated plots. Pathogenicity and aggressiveness of each *Fusarium* isolate are being tested in the greenhouse.

Further characterization of the MST12 transcription factor genes in *Magnaporthe oryzae*
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In *Magnaporthe oryzae*, the *PMK1* MAP kinase is known to regulate appressorium formation and plant infection. Homologues of *PMK1* have been shown in other phytopathogenic fungi to be important for plant infection. However, there are only limited studies on transcription factors and genes regulated by this MAP kinase pathway. In *M. oryzae*, one of its downstream transcription factors is *MST12,* which is essential for infectious growth. The *mst12* mutant was non-pathogenic but still formed appressoria. In yeast two hybrid assays, a weak interaction was detected between Pmk1 and Mst12. In CoIP assays, Pmk1 was co-purified with the Mst12-3FLAG fusion protein. When overexpressed with a strong, constitutive promoter, *MST12* could partially suppress the defects of the *pmk1* mutant. On penetration assays with onion epidermal cells, a few melanized appressoria were observed and none of them were able to penetrate and form infectious hyphae. Our preliminary data of Mst12-binding site assays suggest that Mst12 has a binding site similar to that of yeast Ste12. Microarray analysis has been used to identify genes regulated by *MST12*. Promoters of genes with significantly altered expression levels in the *mst12* mutant were analyzed for common regulatory elements. Sequences similar to yeast filamentation and pheromone response elements were identified in some of these genes. A few of them have been selected for verification by qRT-PCR and further functional characterization. Data on these genes will be presented.

Response of US *Cucumis melo* Plant Introductions to *Phytophthora capsici*
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Phytophthora capsici is distributed worldwide, and is an aggressive pathogen with a broad host range infecting solanaceous, leguminaceous, and cucurbitaceous crops. Over the past two decades, increased incidence of Phytophthora blight, particularly in eastern states, has threatened production of many vegetable crops. *Cucumis melo* (honeydew and cantaloupe), while especially susceptible to fruit rot, is also susceptible to crown/root rot. Currently, little is known about host resistance to *P. capsici* in *C. melo*. To assess resistance in *C. melo* seedlings, 318 *Cucumis melo* US Plant Introductions (PIs) from diverse geographic locations and two commercial hybrid cultivars (Athena and Dinero) were grown under greenhouse conditions. At the three to four leaf stage, seedlings were inoculated with a five isolate zoospore suspension (1.0×10^4) at the crown and monitored for six weeks. All the susceptible checks ('Athena' and 'Dinero') died within seven days post inoculation. Several PIs (PI 181748, PI 182964, and PI 273438) succumbed earlier than Athena and Dinero due to crown rot. Eighty seven PIs (27%) appeared to have some degree of tolerance to *P. capsici*. The level of resistance to *P. capsici* within individual PIs was variable. The 87 PIs selected from the primary screen are currently being re-screened and the results of this study will be presented.

Cloning of putative secreted protein genes from wheat infected by *Puccinia striiformis* f. sp. *tritici*
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Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a major disease of wheat worldwide. *Pst* genes encoding secreted proteins serving as effectors to interact with plant resistance genes are hypothesized to express specifically in haustoria. The objective of this study was to isolate secreted protein genes from *Pst* haustoria. Six putative secretion protein genes with partial sequences derived from the *Pst* haustorial cDNA library were selected to obtain full-length cDNA using the 5' rapid amplification of cDNA ends. The full-length of these cDNAs ranged from 543 to 1,152 bp encoding proteins of 175 to 378 amino acids without significant similarities with any accessions protein databases. We selected four of the genes for assaying their expression patterns in urediniospore, germinated urediniospores, and infected wheat tissues using quantitative real-time PCR. These genes had different expression patterns, but all tended increase their transcript level during the infection process. Two of them had the highest transcript level in infected wheat tissues and lower transcript levels in urediniospores and germinated urediniospores. The third gene had a higher transcript level in urediniospores. The transcript level of the fourth gene in urediniospores was slightly higher than in the infected wheat tissues, but much higher than in germinated urediniospores. This study has provided putative genes for further studies to identify *Pst* avirulence genes.

Role of exopolysaccharide in the biology *Enterobacter cloacae*
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The bacterium, *Enterobacter cloacae,* is the causal agent of Enterobacter rot of onion bulbs in storage. This disease results in a discoloration of the inner scales, but no maceration of the tissue. Exopolysaccharide (EPS) production can have a role in some plant-pathogen interactions, although its role is unknown in the *E. cloacae*-onion interaction. A mini-Tn*5* mutagenesis library of *E. cloacae* strain ECWSU1R was screened using Luria Bertani (LB) media modified with congo red dye, resulting in 61 mutants. Growth on congo red media allows for the differential identification of bacteria with potential disruptions in EPS production. These mutants were screened on LB, nutrient broth yeast extract and minimal medium agars for their ability to produce EPS. The mutants were inoculated into onion bulbs to determine if they differ in their pathogenicity relative to the wildtype strain. In addition, the mutants were screened for their ability to form biofilms, as well as determine their resistance to various salts compared to the wildtype strain. The insertions will be sequenced to identify which genes are being disrupted. Since EPS can have a role in the survival of bacterial pathogens, mutants are being assayed for their ability to resist desiccation compared to the wildtype strain.

A trans-Atlantic partnership for reducing the spread and impact of new and emerging viruses in ornamental crops
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The ornamental industry is considered to be a truly global industry as it involves significant movement of plant material across time zones, countries

and continents. Significant economic losses and the inadvertent spread of new viruses through vegetative propagation necessitate periodic testing to ensure virus-free status of propagating material. Availability of rapid and sensitive virus detection methods is critical for reducing the spread of viruses in ornamental crops. An international collaborative effort to identify and characterize new and emerging viruses in ornamentals has resulted in the increased knowledge on viruses of dahlia (*Dahlia variabilis*), an important ornamental crop of trade between Europe and the USA. Three distinct caulimoviruses of dahlia have been identified and characterized at the molecular level which provided virus-specific primers for use in rapid and sensitive PCR assays for each virus. Additionally, degenerate, group-specific primers were designed and tested for broad-spectrum detection of these viruses. Surveys of dahlia cultivars and breeding material from Europe and the US were conducted which demonstrated the widespread occurrence, with differences in the frequency of incidence of each of these viruses. The tools and technologies developed are useful toward developing an effective strategy involving production and use of virus-free propagating material.

Efficacy of organic and conventional seed treatments for management of *Verticillium* in spinach seed

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Verticillium dahliae is systemic and readily seed transmitted in spinach. Strains of *V. dahliae* from spinach can be pathogenic on lettuce, raising concern about introducing *V. dahliae* on spinach seed into fields subsequently planted to lettuce. To assess inoculum potential from spinach seed, a seed lot naturally infected with *Verticillium* at 64% was planted in sterilized sand, and the leaves harvested after 35 days ('baby leaf' spinach). The roots, crown, and cotyledons remaining were crushed in buffer and plated on NP-10 agar. *Verticillium* was detected at >9,000 CFU/100 emerged seedlings. The seed lot was used to evaluate 11 organic and 9 conventional seed treatments for reducing the impact of seedborne *Verticillium*. Seven treatments reduced the incidence of *Verticillium* to <10% in a seed health assay. The most effective conventional treatments were thiophanate-methyl (0%), thiabendazole alone (0.3%) or with mefenoxam + fludioxonil + azoxystrobin (0%), and triticonazole (2.0%). The best organic treatments were Seedgard (steam treatment, 2.8%), Seed Support II (disinfectant, 3.3%), and Seed Support I (disinfectant, 7.0%). When pericarps and embryos were separated, surface-sterilized individually, and assayed, *Verticillium* was observed on 45% of the pericarps and 74% of the embryos, demonstrating the internal nature of spinach seed infection. The treatments most effective in the seed health assay are being tested for reducing seed transmission/soil infestation.

Characterization of a tailocin from *Burkholderia*

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Members of the Burkholderia cepacia complex (Bcc) are plant and human opportunistic pathogens. Essentially all Bcc isolates demonstrate in vitro broad-spectrum antibiotic resistance. In fact, many clinical isolates are resistant to all currently available antibiotics, rendering therapy ineffective. There is a substantial need to develop new antimicrobial therapies. The potential use of phage-tail-like high molecular weight bacteriocins, or "tailocins", as alternative anti-bacterial agents against Bcc is being investigated in our laboratories. We have isolated a tailocin designated Bcep0425, which exhibits broad host range activity against members of the Bcc. We have determined that Bcep0425 has a sheath protein of ~43 kD, a core protein of ~17 kD, and a tail fiber protein of ~100 kD. Adsorption studies using purified lipopolysaccharide (LPS) isolated from both resistant and sensitive isolates indicate that the putative receptor for the Bcep0425 is LPS. Ongoing studies are focused on determining which component of the LPS is specific for tailocin attachment.

Preliminary screening for resistance to Penicillium decay in *Allium* accessions

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Penicillium decay is a persistent problem in garlic (*Allium sativum*) and related species. Excepting limited partial resistance, we have been unable to locate documentation for resistance. Accessions of *A. sativum* (PI 540355, W6 1858, W6 12839, W6 12840, Wild Buff, Siberian), *A. ponticum* (W6 17232), *A. ampeloprasum* (PI 390582, W6 29777), *A. acuminatum* (Site 18, Site 24), *A. canadense* (W6 12817), *A. aflatunense* (W6 1867), *A. stipitatum* (PI 576941), *A. longicuspis* (PI 576914, PI 540357), *A. molly* (W6 1952), *A. roseum* (W6 20312), *A. senescens* (W6 17149), and *Allium* sp. (W6 1874) were screened for resistance, using aggressive strain IR 13B, and susceptible *A. sativum* Rose Du Var (positive control). In comparisons of lesion growth rates (ANOVA, LSD, two trials each of n = 2, r = 3, shallow wounds; repeated with deep wounds), inoculated cloves of PI 576941 and W6 1867 did not significantly differ from their negative controls but were less than positive controls ($P < 0.001$). In analogous trials with shallow wounds, then deep wounds (each with n = 2, r = 3), there were no significant differences between inoculated and uninoculated cloves in W6 20312, W6 1874, and W6 17149, but inoculated treatments had smaller lesions than positive controls ($P < 0.001$, 0.001, 0.014 respectively). Partial resistance (significant differences between treatments and controls, but lesions smaller than in Rose Du Var, $P < 0.007$) was evident in both accessions of *A. ampeloprasum*, elephant garlic.

Evaluation of *Mentha longifolia* for resistance to *Verticillium dahliae* isolates from various hosts

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Verticillium wilt (VW), caused by *Verticillium dahliae*, is a major constraint to mint (*Mentha*) production and resistant cultivar development is an important aspect of VW management. Four genotypes of the wild mint species *M. longifolia*, including two previously identified as resistant and susceptible to VW, were evaluated for resistance to *V. dahliae* isolates from different hosts and vegetative compatibility groups (VCG). Cuttings were subjected to root-dip inoculations and disease severity index (DSI) ratings, plant mortality, stem colonization and yield reductions recorded over three successive periods of growth and harvest in the greenhouse. Isolates of *V. dahliae* from peppermint (*M.* × *piperita*) caused significantly higher ($p \leq 0.05$) DSI, incidence of plant mortality and yield reductions than isolates from other hosts regardless of VCG, demonstrating variation in aggressiveness on mint among and within VCGs. All *M. longifolia* genotypes exhibited the ability to recover from infection by developing new growth from rhizomes. Incidence of mortality in *M. longifolia* ranged from 19 to 44% and surviving *M. longifolia* plants displayed mild to moderate symptoms upon regrowth. The pathogen was isolated from 14% and 73% of stems from resistant and susceptible *M. longifolia* genotypes, respectively. Results suggest that the restriction of pathogen movement in aboveground tissue and the ability to recover from infection may be important components of VW resistance in mint.

Immunodetection of *Beet curly top virus* complex in beans and sugar beet in Idaho

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Leafhopper-transmitted curly top virus is a serious problem in both beans and sugar beet in the semi-arid western U.S., including Idaho. Curly top is caused by a genetically diverse complex of phloem-limited viruses we are collectively calling *Beet curly top virus* (BCTV). BCTV belongs to the family *Geminiviridae*, and has small spherical or geminate particles built of single-stranded DNA and a single species of 28-kDa capsid protein (CP). Due to the phloem restriction of BCTV and the lack of a good laboratory host-vector system for BCTV propagation and purification, no immunodetection tests are available for BCTV. Routine diagnostics for curly top relies either on visual symptoms or PCR tests. Lack of an ELISA test system is one of the factors hampering development and screening of the curly top resistant germplasm in sugar beet breeding programs. To fill in this gap, we developed an ELISA based detection system for BCTV which utilizes virus-specific antibodies generated against bacterially-expressed CP of BCTV. Bacterially-expressed CP was affinity purified and used as an antigen for antibody production in three animal species. Specificity of the resulting antisera was tested in Western blots and various ELISA formats with bean and sugar beet leaf tissue. We demonstrate reliable detection of BCTV in sugar beet and beans in TAS-ELISA format, suitable for large-scale screening of germplasm in breeding programs.

Resistance to bacterial spot can be correlated with resistance to canker in transgenic citrus

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In Florida, citrus bacterial spot (CBS, *Xanthomonas citri* pv. *citrumelo*) causes minor disease problems which are restricted entirely to nurseries. In contrast, citrus canker (CC, *X. citri* ssp. *citri*) is a serious disease of citrus in Florida, and threatens the existence of the citrus industry. CBS has been deregulated as a pathogen of quarantine concern and is not under any state or federal regulations, which makes it easier to work with. CC however remains

regulated and research can be carried out only in approved facilities. Young flushes of transgenic grapefruit plants containing either the LIMA or AttacinE antimicrobial peptide gene under control of a CaMV 35s promoter were screened for resistance to CBS using an attached leaf assay test. Leaves were infiltrated with 10^6 cfu ml^{-1} bacterial cell suspension and observed after 2 weeks. Transgenic plants exhibited a range of symptoms from leaves with few lesions to leaves that were similar to non-transgenic control. For the CC assay, leaves at similar stage of growth were challenged using a detached leaf assay method with a 10^6 cfu ml^{-1} bacterial cell suspension in the laboratory. It was observed that transgenic lines which exhibited decreased lesion expansion and resistance to CBS in the greenhouse were also tolerant to CC. Screening transgenic lines for resistance to CBS provided a quick screening method to limit the number of potential transgenic lines that can be subsequently evaluated for resistance to CC.

Effects of temperature and moisture on the infection of wheat by *Puccinia striiformis* f. sp. *tritici* in an outdoor environment

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici,* has historically been a problem in the Pacific Northwest and California, but has not been a problem in the Great Plains. However, Kansas had significant losses due to stripe rust in 2001, 2003, and 2005. Recent research on *P. striiformis* isolates suggests the introduction of a novel population may have been responsible for these epidemics. Previous work determined the effect of temperature and leaf wetness duration on infection prior to the population shift. The objective of this research is to determine conditions that are favorable for the infection of *P. striiformis* f. sp. *tritici* isolates from the newly emerging population. Two week old potted seedlings were inoculated with an isolate of *P. striiformis* and placed outside over night (16 hours). Weather variables were recorded. During the exposure, some plants were misted with distilled water and then covered with a plastic bag to retain moisture, and others received no additional moisture except during rain events. Plants were then incubated at 12°C and evaluated for disease severity after 14 days. Infections occurred at a wide range of temperatures (4°C–22°C), and were most frequent when more than 6 hours of leaf wetness occurred during exposure. Future research will use these results to determine weather patterns that influence the probability of stripe rust epidemics in Kansas and to facilitate the development of fungicide application recommendations.

Biologically active transcripts of *Oat blue dwarf virus* (OBDV) – the first infectious clone of a *Marafivirus*

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Marafiviruses are a small group of phloem-limited, leafhopper-borne viruses closely related to those in the *Tymovirus* genus. We now report the development of infectious cDNA clones of OBDV – the first such clones of any marafivirus. Prior to clone construction, the reported sequence of the 5' and 3' ends was confirmed using 5' RACE, primer extension, and ligation-anchored PCR. Primers designed to published terminal sequences and internal regions were used to generate 5' and 3' amplicons subsequently fused into full-length clones. The 5' primer incorporated the T7 RNA polymerase promoter sequence to facilitate production of RNA transcripts. The 3' primer incorporated 28 adenosine residues and an Spe I restriction site to allow clone linearization prior to transcription. Using vascular puncture of maize seeds with capped transcripts, multiple clones were shown to be infectious at an average rate of 24.3% (range 14–36%), as determined by ELISA. Proteins and RNAs consistent in size with those expected in OBDV infection were detected in young leaves via western and northern blotting, respectively. Aster leafhoppers successfully transmitted OBDV to oats and barley after feeding on detached, infected maize leaves. Infectious clones of these viruses will be valuable in further studies of marafivirus functional genomics, the interaction of the virus with its insect and plant hosts, and the relationships between marafiviruses and others within the family *Tymoviridae*.

Para-retroviral sequences in wild *Dahlia* spp. in natural habitats from the Mexican mountain ranges

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Three distinct caulimoviruses, *Dahlia mosaic virus* (DMV), DMV-D10, and Dahlia common mosaic virus (DCMV), were found to be associated with cultivated dahlia (*D. variabilis*) in the US and other countries. Genus *Dahlia* was first described in 1791 and the mountain ranges of central Mexico are home to more than 35 wild spp of *Dahlia.* To better understand the incidence of these plant pararetroviruses, leaf samples from selected wild dahlia species in their natural wild habitats from the mountain ranges of central Mexico were collected and tested for the three caulimoviruses. Results showed that the wild dahlia contained DMV-D10 and no evidence of presence of the other caulmoviruses. Viral sequences were found in 89% of the samples (n = 56) representing four different wild species. Studying the genome organization and characterization of DMV-D10 from wild species and compare it to DMV-D10 from cultivated species would help in understanding the possible emergence, co-existence and co-evolution of pararetroviruses and their host plants. The complete ca. 7 kb dsDNA genome of DMV-D10 from the wild *Dahlia* species, *D. coccinea,* was characterized. The DMV-D10 genome from *D. coccinea* had the structure and organization typical of a *Caulimovirus* species and shared 89.3 to 96.6% amino acid sequence identity among various ORFs when compared to those of DMV-10 from *D. variabilis.*

Targeting genes involved in ochratoxin A biosynthesis in *Aspergillus ochraceus*

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Ochratoxin A (OTA) is a neurotoxic, immunotoxic, mutagenic and teratogenic mycotoxin produced by many *Aspergilli* and *Penicillia*. *Aspergillus ochraceus* is an important producer of OTA, and it contaminates a wide range of food commodities such as grapes, coffee beans, nuts and others. To tag and characterize genes that are responsible for OTA biosynthesis in this fungus, we used a restriction enzyme mediated integration approach (REMI). REMI yielded 500 transformants that were screened on coconut agar for their ability to produce OTA. Five of these transformants were unable to produce OTA when tested by TLC and HPLC. Further molecular analysis performed on one of these mutants, REMI5.1, revealed that the gene disrupted by REMI shares 80% sequence similarity with a gene that codes for the mitochondrial division protein (MDV1); the identified gene was named *AoMDV1*. To confirm the role of this gene in OTA biosynthesis, a split marker transformation approach was performed to disrupt *AoMDV1* in a wild type strain of *A. ochraceus* (NRRL5175). Resulting transformants were screened for gene disruption by PCR and for OTA production by TLC. In one transformant, sequencing of the resulting PCR product showed that disruption had occurred within the *AoMDV1* locus and was accompanied with a loss of OTA production. This is a first report of the potential involvement of *AoMDV1* in OTA biosynthesis in *A. ochraceus*.

A profile of ochratoxin A producing fungi occuring on wine grapes from Southern Illinois

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Several Penicillia and Aspergilli cause fruit rot on grapes and result in contamination with ochratoxin A (OTA), a secondary metabolite classified as a possible human carcinogen. As commercial wine grape production in the Midwest has grown over the past years, it has become more of a priority to learn about the grape mycobiota that prevail in this region of the USA, as well as to assess the occurrence and distribution of OTA producing fungi on these grapes. For that purpose, berries were collected from twelve grape varieties at different veraison seasons: early season, mid-season and late season. The berries were homogenized in a blender, and decimal serial dilutions were made from the homogenate under sterile conditions. These dilutions were then used to inoculate malt extract in petri dishes. The cultures were then incubated at 28°C for 7 days without light. After incubation, the number of forming fungal colonies was evaluated. Preliminary results showed that fungal contamination rates depend on the variety and on the veraison season, varieties collected at late season were more contaminated than those collected earlier in the season. Within each season, red grape varieties showed a higher rate of contamination compared to the white grape varieties. Fungal species were isolated from the different grape varieties and identified through macroscopic observation, microscopic observation, and by sequence analysis of the mitochondrial large subunit (mtLSU) rDNA of the isolated strains.

Characterization of aster yellows phytoplasma strains in leafy green crops in Ohio

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Aster yellows, caused by the aster yellows phytoplasma (AYP) is an important disease of lettuce and other vegetable crops in Ohio and other vegetable

growing areas. It is transmitted primarily by the aster leafhopper, *Macrosteles quadrilineatus*. Seven strains of AYP (AY-WB, AY-S, AY-BW, AY-SS, AY-BD2, AY-BD3, and AY-SG) were identified previously in Ohio lettuce. The objective of this research was to determine the current diversity and distribution of AYP strains among leafy green vegetables in northwest Ohio. Two AYP strain-specific multiplex PCR assays (CPA1 and CPA2) were developed using primers previously designed to differentiate between two aster yellows (16SrI) subgroups. The CPA1 assay distinguishes AY-WB and AY-S while the CPA2 distinguishes AY-BD2 and AY-WB. In addition, either assays detect a strain or strains not yet characterized that may be novel. The multiplex PCR assays were used to characterize 584 infected leafhoppers collected from two different sites in northwest Ohio. AY-BD2 infected leafhoppers were the most abundant (55%) followed by AY-WB (36%). Only one leafhopper infected with AY-S was detected and AYP strains infecting the remaining leafhoppers (9%) were not identified using these assays. AY-BD2 infected leafhoppers were predominant in red lettuce, whereas AY-WB infected leafhoppers were predominant in romaine lettuce. There was an interaction between host, AY-strain and site ($p = 0.00$).

A soybean leucine-rich repeat receptor-like kinase regulates the response to infection with *Phytophothora sojae*

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Phytophthora sojae is a host specific pathogen that causes root and stem rot of soybean, a serious and economically important soybean disease. A set of resistance genes (Rps) was found to confer resistance to the pathogen. However, due to selection pressure, virulent races continue to evolve. Virus-induced gene silencing (VIGS) provides an efficient tool for studying major genes pertinent to resistance pathways. Using the novel bean pod mottle virus-based VIGS vector, we silenced three leucine-rich repeat receptor-like kinase (RLK) genes in soybean. Because members of the RLK family are known to play roles in plant defense against pathogens, we examined the potential role of a resistance-like RLK gene in soybean resistance to *P. sojae* races R1 and R3. For this purpose, the cultivar Williams 82, which is resistant to both races, was used. RLK-silenced Williams 82 seedlings were found to retain resistance to both races following inoculation using the hypocotyl method. When the same experiment was repeated using the cultivar Harosoy 63, which is resistant to race 1 but exhibits a compatible reaction to race R3, the silenced plants, surprisingly, showed a resistant response (wound healing) to race 3 as it did to race 1. Our results suggest that the cultivar-specific R gene-mediated plant defense is controlled by a reaction triangle that includes RLK gene expression with the set of Rps genes present in the relevant cultivar and the virulence genes of the pathogenic race.

Bacterial soft rot in *Daphne laureola* (Thymelaceae): A histopathological investigation

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The invasive shrub *Daphne laureola* is a serious threat to certain native forest ecosystems particularly Garry oak woodlands and dry Arbutus/Douglas fir forests of coastal British Columbia. In a study examining the host range of the exotic plant pathogen *Phytophthora ramorum*, unusual symptoms were noticed on *D. laureola* foliage. Instead of typical *P. ramorum* lesions, lesions on *D. laureola* were water soaked and often involved the entire leaf. The bacterium *Pseudomonas fluorescens* was isolated from symptomatic *D. laureola* leaves. The 16S rDNA was amplified and sequenced for the bacterium. Blasting the sequence against the GenBank database showed that the bacterium shared 98–99% homology with *P. fluorescens*. Histopathological examinations of necrotic leaves under light and scanning electron microscope (SEM) suggest that these bacteria may play a role in symptom development. Several other organisms were tested to determine if they would induce bacterial soft rot in *D. laureola*. Some of the largest lesions were on leaves colonized by *Trichoderma* spp., which are non-pathogenic fungi commonly used as biocontrol agents. Leaf wounding was necessary for symptom development. It is hypothesized that *P. fluorescens* is endophytic in *D. laureola* foliage and causes bacterial soft rot after the leaf is colonized by an organism such as *Trichoderma* spp. or *P. ramorum* that breaks down plant cell walls, releasing nutrients that allow the bacteria to proliferate and cause symptoms.

***Phytophthora ramorum* - pathogenic fitness of the three clonal lineages**

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Phytopathology 99:S32

The Oomycete pathogen *Phytophthora ramorum* causes sudden oak death on oak and ramorum blight on ornamentals causing economic losses to the nursery industry. The US population of *P. ramorum* consists of three distinct clonal lineages referred to as NA1, NA2, and EU1. Differences in pathogenic fitness among the lineages were tested for through the infection of detached leaves and whole plants in wounded and un-wounded inoculations of *Rhododendron*. In independent experiments the fitness of isolates within lineages was determined using the fitness components lesion area (LA), sporulation capacity (SC), incubation period (IP_w) and the area under the lesion expansion curve (AULEC). LA demonstrated significant differences among lineages in two out of three wounded detached leaf experiments; however, SC, IP_w and AULEC showed no consistent significant differences among lineages. There was also no significant cultivar by lineage interaction among the wound inoculated experiments. The non-wounded whole plant inoculations showed a trend towards a difference between the NA1 lineage and EU1 and NA2 ($0.1 > p > 0.05$) but variability among isolates within lineages means that these slight differences are not statistically significant. Petri plate experiments also found significant differences between lineages in radial growth of isolates at 10°C ($p < 0.001$). This indicates that there are minor differences in fitness components among *P. ramorum* clonal lineages but shows that within lineage variability is great.

Comparative epidemiology of *Phytophthora ramorum* and other *Phytophthora* species

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Phytophthora ramorum causes sudden oak death on oak and ramorum blight on a wide range of ornamental plants, with severe economic losses to the nursery industry. The US population of *P. ramorum* consists of three distinct clonal lineages referred to as NA1, NA2, and EU1. Many other *Phytophthora* spp. are also problematic to the nursery industry causing foliar blight, root rot or both. *P. ramorum*, *P. foliorum*, *P. lateralis*, *P. kernoviae*, *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. nicotianae*, and *P. syringae* were studied in a containment growth chamber on *Rhododendrons* using detached leaves and whole plants and all but the first four species were taken to field experiments. Experiments using plated isolates, detached leaves, and whole plants compared the fitness components lesion area, sporulation capacity, incubation period and the area under the lesion expansion curve, among isolates within the three clonal lineages of *P. ramorum*. There were small differences between lineages, but high variation within lineages meant that these differences were not always significant. Experiments on all eleven *Phytophthora* species showed significant differences between the species in terms of lesion area. The four quarantine pathogens tended to be more aggressive at 18°C in the containment chamber. In the field there were also significant differences between the species, in terms of lesion development and within plant spread, which varied with the seasonal changes in climate conditions.

Assessing the diversity of *Pythium* species and fungicide efficacy in agronomic production fields in Ohio

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Seedling diseases in soybean fields in Ohio have increased over the past decade. There are at least 22 possible species of *Pythium* that are pathogenic to soybean. During the spring of 2008, field plots were planted at five different locations across the soybean production regions in Ohio, in a randomized block design with eight different fungicide seed treatments and four replications. The fungicide seed treatments included; mefenoxam, fludioxonil, azoxystrobin, mefenoxam + fludioxonil, mefenoxam + azoxystrobin, fludioxonil + azoxystrobin, and mefenoxam + fludioxonil + azoxystrobin. Stand counts at R1 and yield were recorded. *Pythium* species were isolated from symptomatic plants at these locations and the isolates were then identified to species using single strand conformation polymorphism. There were no significant interactions between location and seed treatment for stand count. Stands from seed treated with azoxystrobin, mefenoxam +

fludioxonil, fludioxonil + azoxystrobin, and mefenoxam + fludioxonil + azoxystrobin treatments were significantly higher than the untreated check for stand count, but not for yield. A total of 13 *Pythium* species were recovered from symptomatic plants from the five locations. *Pythium irregulare*, *P. dissotocum*, and *P. torulosum* were the most frequently recovered species from all five locations. The results from this study will be used for the development of disease management strategies for seedling pathogens in Ohio.

Occurrence of *Neonectria radicicola* as a root pathogen of avocado in California

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Neonectria radicicola is known as a causal agent of root rot in grapevine, raspberry, ginseng and forest tree nurseries worldwide. Recently, *N. radicicola* has been reported to cause wilting of young avocado trees in nurseries in Israel. During a survey of major avocado growing areas of California in 2008, *N. radicicola* was isolated from roots of approximately 50% of trees showing symptoms of reduced vigor, dying shoots, and necrotic roots. Vascular elements of avocado feeder and secondary roots (1–3 mm diam.) were isolated onto potato dextrose agar amended with tetracycline (0.01%) and incubated at room temperature. Identification of the species was confirmed by colony and spore morphology and analysis of the internal transcribed spacer (ITS) regions ofrDNA. Pathogenicity of three different isolates of *N. radicicola* was tested by dipping roots of cv. Hass seedlings in a 10^5 conidia per ml suspension for five min. The plants were then potted in soil and allowed to grow under greenhouse conditions. Leaves and shoots of inoculated plants developed wilting symptoms within 2 weeks and *N. radicicola* was consistently reisolated from necrotic roots. These results suggest that this fungus may have been overlooked as a root pathogen in CAand warrants further investigation.

Life Cycle of *Puccinia crupinae,* a candidate fungal biological control agent for *Crupina vulgaris*

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Crupina vulgaris (Common Crupina, Asteraceae) is an introduced weed pest in the western United States. An isolate of the rust fungus *Puccinia crupinae* from the Greece is currently under evaluation as a candidate for biological control of *C. vulgaris* in a Biosafety Level 3 (BL-3) containment greenhouse facility. The life cycle of *P. crupinae* has been completed in greenhouse studies, demonstrating that it is macrocyclic and autoecious on Common Crupina. Plant inoculations were made with spores from each stage in the fungus life cycle, demonstrating their viability and role in the life cycle. Data will be included in a risk assessment of *P. crupoinae* for biological control of *C. vulgaris*.

Three-dimensional spatial patterns of brown rot symptoms within sour cherry tree canopies in Hungary

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Spatial patterns of disease can provide important insights into epidemiological processes such as inoculum source and mode of spore dissemination. For brown rot of pome and stone fruits, the spatial arrangement of infected orchard trees has been examined previously, but spatial patterns of and relationships among symptom types within individual tree canopies have not been analyzed. To evaluate these patterns, a magnetic digitizer (FASTRAK 3Space System) was used to intensively map eight infected sour cherry trees in an organically cultivated orchard in Hungary. Each tree canopy had an average of 775 data points, about half of which were asymptomatic fruit and the remainder were symptomatic elements (blossom blight, shoot and fruit blight, and twig cankers) caused by *Monilinia laxa*; the most abundant symptom at the time of assessment (June 2008) was blossom blight. A typical medium-sized tree had 499 asymptomatic fruit and 127 symptomatic elements, with more than half of the symptomatic elements from previous year's infections. Nearest-neighbor distance analysis showed current infections were closest to previous year's blighted blossoms and previous year's twig cankers, whereas greater distances were found to previous year's shoot and fruit blight symptoms. The coordinates of symptomatic and asymptomatic tree elements will be used to test for patterns of aggregation and association using three-dimensional implementations of the F, G, and K functions.

PCR detection of *Pseudoperonospora humuli* in air samples from hop yards

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Pseudoperonospora humuli is the casual agent of hop downy mildew, which can negatively impact hop yield and quality. The disease is managed largely with routine fungicide applications beginning in early spring. A PCR-based air sampling technique was developed to detect airborne inoculum of *P. humuli* in hop yards to aid in timing the commencement of fungicide applications. Primer and PCR specificity was verified by testing 34 peronosporale species or organisms that commonly occur on hop. The pathogen was detected in 70% of PCR assays when DNA was extracted from a single sporangium, and was detected consistently when DNA was extracted from 10 or more sporangia. Impaction spore traps were deployed over a period of three years in experimental plots, as well as six commercial hop yards in Oregon and Washington. Inoculum was detected on average 1.3 days (range –5 to +1 days) after sporangia were trapped in a volumetric spore sampler, and on average 4.5 days (range –8 to +14 days) before the first appearance of downy mildew in commercial hop yards. In four of the six commercial yards, fewer fungicide applications or improved disease control was achieved when the initial fungicide application was timed based on PCR detection of the pathogen versus the growers' standard practices. Basing management decisions on detection of airborne inoculum of *P. humuli* has the potential to reduce inputs, and air sampling could be a component of a regional disease warning system for hop downy mildew.

Multiple fluorescent markers for *Xylella fastidiosa* subspecies

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Phytopathology 99:S33

Xylella fastidiosa is the causative agent of Pierce's disease of grape, citrus variegated chlorosis, and numerous leaf scorch diseases. Although multiple *X. fastidiosa* subspecies may be found within the same plant or insect, studying the interaction among subspecies within the same host is difficult due to highly similar bacterial morphology and genetics. The objective of this project is to develop unique markers that will provide visual and genetic labels for different *X. fastidiosa* subspecies and strains. In order to unambiguously identify and study multiple *X. fastidiosa* subspecies in the same host, different autofluorescent proteins were inserted into the *X. fastidiosa* genome. A suite of plasmids that provide a fluorescent color palette for distinguishing *X. fastidiosa* subspecies has been developed. When incorporated into *X. fastidiosa* cells, the markers allow unambiguous visual and genetic discrimination of closely related strains and subspecies co-infecting the same host.

Effect of pH, concentration and dialysis on antifungal activity and phytotoxicity of β 1-4 linked polymer of glucosamine (chitosan)

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Chitosan is a natural antifungal compound derived from the outer shell of crustaceans. It acts as a potent elicitor to enhance plant resistance against pathogens. There are several factors, both intrinsic and extrinsic, that affect the antimicrobial activity of chitosan. The in-vitro toxicity of chitosan to three *Colletotrichum* spp. was tested over a range of concentrations and at pH's ranging from pH 3 to 7. The effect of dialysis was also tested. All three species of *Colletotrichum* were sensitive to chitosan at concentrations ranging from 12.5 to 100 ppm. Non-dialyzed chitosan inhibited spore germination, mycelial growth, and appressorium formation of *C. orbiculare* and *C. coccodes* at 12.5 ppm and of *C. acutatum* at 50 ppm. However, dialysis reduced the antifungal activity of chitosan more than 50-fold in all three species. At 12.5 ppm, mycelial growth of *C. orbiculare* was completed inhibited at pH 3 and 4, but using the same concentration at pH 5, 6 and 7, inhibition decreased to 55%, 32% and 25%, respectively. Chitosan was also evaluated for phytotoxicity to cucumber plants. Plants treated with non-dialyzed chitosan by foliar application and soil amendment at pH 3 and 4 at 25, 50 and 100 ppm exhibited no symptoms of phytotoxicity. However, root dips into chitosan solutions caused severe chlorosis, wilting and death of plants. Using chitosan to control fungal foliar pathogens depends not only on sensitivity of the target organism but also the manner in which it is applied, including pH and dialysis.

International mail as a pathway for the movement of exotic plant pests into and within the Greater Caribbean Region

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Public and private postal services are an often overlooked pathway through which plants and plant pests may move into and within the Greater Caribbean Region (GCR). To evaluate the risk of pest movement associated with the mail pathway we examined the types of quarantine materials transported by mail. Of packages sent to the United States by private mail from worldwide and GCR origins, 0.13% and 1.6%, respectively, contained plant quarantine materials. Of packages sent by public mail, 1.1% from worldwide and 0.8% from GCR origins contained plant quarantine materials. High-risk items found in mail included: propagative plant materials (1/3 of the intercepted materials), soil, and wood items. Fresh fruits, vegetables, and other fresh plant parts, were also intercepted. We estimated that the GCR (excluding the United States) may annually receive between 13,876 and 14,943 mail packages containing plant materials or plant pests, with up to 4,000 of these being propagative materials. International mail may be the pathway of choice for intentional smuggling of high-risk items.

Tuber apoplastic hydrophobic proteins differentially expressed in *Solanum tuberosum* cultivars with different susceptibility to *Phytophthora infestans*

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During plant pathogen interaction, oomycetes secrete effectors into the plant apoplast where they interact with host resistance proteins, which are accumulated after wounding or infection. Expression profile of pathogenesis related proteins is proportional to the resistance of different cultivars towards *P. infestans* infection. The aim of this work was to analyse the pattern of expression of apoplastic hydrophobic proteins (AHPs), after wounding or infection, in tubers from two potato cultivars with different resistance to *P. infestans*. Intercellular fluid was extracted from tubers (IFT) and chromatographed into a PepRPC™ HR5-5 column in FPLC eluted with a linear gradient of 75% acetonitrile. Then, AHPs were analyzed by SDS-PAGE and identified by MALDI TOF. Results obtained shown that in the resistant cultivar (*Innovator*), the AHPs concentration was higher than in the susceptible cultivar (*Spunta*), in all times and conditions assayed. Quantitative and qualitative changes were observed in hydrophobic proteins in both cultivars after infection. In *Spunta* cultivar patatin precursors and a protein of 9 KDa were induced after infection. In *Innovator* cultivar, the levels of protease inhibitors, patatin precursors and a protein of 16 KDa were increased. The results obtained here suggest that changes in the AHPs levels could be necessary to protect the plant against pathogens. Furthermore, those basal levels of AHPs would be related with the degree resistant to pathogens.

Changes in protease inhibitors hydrophobicity could be associated with apoplastic potato defence response

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Phytopathology 99:S34

The plant apoplast has a high content of proteases and protease inhibitors (PI), either with or without antimicrobial activity, which are important components of the response towards different stresses. However, the way by which PI interact with pathogen effectors is unknown. On the other hand, apoplastic hydrophobic proteins and peptides (AHPs) have been associated with plant defence response. We have analyzed if stress induced changes in the hydrophobicity of apoplastic PI (API), in two potato cultivars with different degree of resistance to *P. infestans*. Intercellular fluid was extracted from tubers (IFT) and chromatographed into a PepRPC™ HR 5-5 column in FPLC eluted with a linear gradient of 0 to 75% acetonitrile. Then, AHPs were analyzed by SDS-PAGE and identified by MS-MS and MALDI-TOF. In both cultivars, API hydrophobicity was increased. After 24 h of wounding or infection, *Spunta* cv (susceptible), showed that cysteine (19–23 KDa) and Kunitz-type PI were mostly hydrophobic. In *Innovator* cv (resistant) both treatments induced an increase in PI hydrophobicity; however this effect was higher after 24 h. of wounding. The results obtained here suggest that changes in protein hidrophobicity are neccesary to protect the tuber under biotic and abiotic stress, regardless of cultivar resistance degree. However, modifications in the exposure of hydrophobic sites could be related to changes in protease-inhibitor interactions participating in the plant defence response.

Fungicide efficacy for control of cavity spot of carrots in California

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Cavity spot of carrots is one of the most important diseases of fresh-market carrots in California. A field trial was conducted to evaluate fungicides for control of cavity spot. The field location was a disease nursery that had been repeated inoculated with *Pythium violae*, *P. sulcatum*, and *P. ultimum,* the primary causes of cavity spot in California. Treatments included programs with azoxystrobin (Quadris), EC and SL formulations of mefanoxam (Ridomil Gold) and an untreated control. Mefanoxam was recently changed from an EC to an SL formulation in response to California air quality issues related to release of volatile organic compounds. Carrots (cultivar 'Choctaw') were sown in six lines into beds on 1.5 m centers in September 2007. In March 2008, carrots were hand-harvested, washed and rated for cavity spot incidence and total number of lesions per 100 roots. Treatments with low rates of the EC or SL formulations of mefanoxam were not significantly different than the untreated control. Treatments which included azoxystrobin or higher rates of the SL formulation of mefanoxam had significantly lower incidence and total number of lesions compared to the untreated control.

A diagnostic real-time PCR assay for the detection and quantification of *Xanthomonas axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *phaseoli* var. *fuscans*

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Phytopathology 99:S34

Common bacterial blight of bean is caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (Xapf). These seedborne, quarantined pathogens can cause up to 40% yield loss in susceptible cultivars and also reduce seed quality, especially if seed is produced under humid conditions. Seed health testing is one of the most significant control steps to protect against contamination of seeds for domestic planting and international seed trade. Based on sequence information from RAPD fragments generated by Xap-specific primers, we developed a real-time PCR for detection and quantification of Xap and Xapf. Primer and probe specificity was tested against DNA of several *Xanthomonas* species and pathovars of *X. axonopodis*. Several close related *Xanthomonas* strains could not be amplified using this PCR assay. The detection limit of the TaqMan assay for purified DNA and cells was 20 fg and 20 CFU per 25 µl PCR reaction mixture, respectively. This assay may be useful as a rapid, highly sensitive and specific detection method to ensure seed quality control and meet phytosanitary regulations. This is also the first real-time PCR assay developed for Xap and Xapf.

Real-time polymerase chain reaction for detection and quantitation of *Phomopsis longicolla*

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Phytopathology 99:S34

The Diaporthe-Phomopsis complex of fungi causes pod and stem blight and Phomopsis seed decay of soybean, with *Phomopsis longicolla* being the primary cause of disease in the Midwest U.S. *P. longicolla* can cause severe seed quality issues and reduction of germination in seed. Detection of Phomopsis-infected seed lots can give growers more options in managing the disease before it affects field stands. Currently, detection of the pathogen requires seedling grow-outs and blotter assays, which are time-consuming and require knowledge of pathogen morphology. To improve the accuracy and efficiency of testing soybean seed for this pathogen, a new TaqMan real-time PCR was developed for specific detection of *P. longicolla* based on a 175 bp internal transcribed spacer 1 between the 18S-5.8S rRNA genes. The probe was species-specific when tested against DNA extracted from 12 *P. longicolla* isolates collected from seven Iowa counties. The *P. longicolla*-probe did not react with any of the 52 *Fusarium* isolates recovered from soybean. *Fusarium* species are commonly associated with soybeans and it was important to ensure that they did not trigger a cross-reaction in the assay. The detection limit of the PCR assay for purified DNA was 200 fg/25 µl PCR reaction mixtures. This PCR assay was highly specific and sensitive and provides a simple, rapid alternative to other methods for identification and quantitative detection of *Phomopsis longicolla* isolated from soybean seed.

Epidemiology of *Phytophthora kernoviae* in UK woodlands and heathland and risk to North American forests

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Phytophthora kernoviae is a recently introduced pathogen in UK woodlands and heathland. Because the invasive plant, *Rhododendron ponticum* supports sporulation in woodlands, *R. ponticum* removal may protect *Fagus sylvatica* from infection. In infested heathland, *P. kernoviae* rapidly defoliates *Vaccinium myrtillus*. In both ecosystems, the mechanisms of pathogen persistence and long-term survival are not well understood. The objectives of this work were to i) assess roots of *F. sylvatica* and roots and rhizomes of *V. myrtillus* for natural infection, ii) determine potential for oospore production on inoculated foliage and roots *R. ponticum*, and iii) assess susceptibility and sporulation of *P. kernoviae* on several North American plants. *Phytophthora kernoviae* was baited from asymptomatic, surface sterilized *F. sylvatica* roots, but only from trees exhibiting trunk cankers. *Phytophthora kernoviae* was recovered from asymptomatic roots and rhizomes of heathland *V. myrtillus*, and from symptomatic stems and foliage. Oospore production was observed in inoculated roots and foliage of *R. ponticum.* The North American native plants *Rhododendron macrophyllum*, *Rhododendron occidentale*, and *Umbellularia californica* were all susceptible to *P. kernoviae* and supported sporulation. Root infectivity and *in planta* oospore production suggest mechanisms of *P. kernoviae* survival in managed woodlands and invaded heathland. This work also underscores the risk associated with introduction of *P. kernoviae* to North American forests.

Effect of low doses of disinfectants on the growth of *Pythium aphanidermatum* and *Rhizoctonia solani* in vitro

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Phytopathology 99:S35

Although the toxicological phenomenon known as hormesis (low dose stimulation/high dose inhibition) has been described in several biological systems, little is known about the effect of low-dose chemical stimulation on the growth of plant pathogens and on their ability to cause disease. Previous research demonstrated the hormetic effect of low doses of the fungicide mefenoxam on *Pythium aphanidermatum* in vitro and in planta. The aim of this research was to determine whether low doses of chemicals (sodium hypochlorite and 75% ethanol) used for routine disinfection of surfaces, equipment, and water tanks had stimulant effects on two important soilborne plant pathogens, *Pythium aphanidermatum* and *Rhizoctonia solani*. These chemicals often lose their potency over time. Twelve doses of each chemical were tested in vitro on both organisms. Each treatment was evaluated on three plates of amended corn meal agar, with three repetitions over time. Plates were inoculated using 0.5 cm mycelium disks and incubated at 23°C for 24 hours. Isolate growth was measured on two ratios in a 90 degree angle. Twelve treatments were tested on each microorganism. The EC_{50}, NOAEL and hormetic curves were determined for each pathogen/chemical combination. Our results highlight the importance of using fresh solutions of disinfectants to sanitize working surfaces in the laboratory as well as in greenhouses and nurseries. Future research will evaluate the effect of hormesis on the pathogenicity of plant pathogens.

First report in North America of *Paratrichodorus renifer*, a nematode parasite of highbush blueberry

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Phytopathology 99:S35

Between 2001 and 2008, 187 blueberry fields were sampled for plant-parasitic nematodes in British Columbia (55), Nova Scotia (7), Washington (49) and Oregon (76). Stubby-root nematodes (*Paratrichodorus* spp.) were recovered from a high percentage of fields, especially in BC and northern WA where 59 and 57% of sampled fields were positive for *Paratrichodorus*, respectively. Morphological evaluation indicated that the populations were comprised of *P. renifer*. Distinguishing features of *P. renifer* observed include: didelphic reproductive system with vulva at 55 to 60% of body length; transverse to pore-like vaginal opening; kidney-shaped 'reniform' vaginal sclerotized pieces that are not separated in lateral view; excretory pore near esophago-intestinal junction; non-overlapping esophageal bulb; absence of body pores; absence of males. The ITS1 region of rDNA was amplified and sequenced from 10 nematodes from populations from BC, WA, OR and NS. The ITS1 region was 895 bp long; the WA population differed from the BC and NS populations at two loci, and all North American populations differed from a Belgian population (NCBI GenBank #EU827611) at 4 loci. The ITS1 region of *P. renifer* is between 12 and 50% longer than *P. macrostylus, P. pachydermus*, *P. allius* and *P. teres,* the other *Paratrichodorus* species for which complete ITS1 data are available. A BC population increased 70X over 14 months on 'Chippewa' blueberry in field microplots and a WA population increased four-fold over 6 months on 'Duke' blueberry in greenhouse pots.

Effect of rice-wheat system on disease scenario in Mazandaran

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Cultivation of wheat after rice is expanded tremendously in some regions of Mazandaran like Behshahar, Neka and Sari. This system has been associated with several changes in agro ecological and pathogen dynamics. In this system there is a phenomenal change in the disease scenario of wheat crop. Survey of wheat crop in Mazandaran Province of Iran were carried out during 2002–2004. Hitherto disease like *Alternaria* leaf spot, grain discoloration and fusarial head blight became the emerging problem of the region, whereas some foot rot diseases like Take-all was not seen due to flooding of rice fields during the summer.

Tan spot as new scenario of wheat diseases in Mazandaran

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Wheat is the third crop after rice and citrus in Mazandaran province. Yellow rust, Fusarium head blight (FHB), Powdery mildew are used to be the major diseases of wheat in Mazndaran. Tan spot which was reported in 1995 has emerged as new challenges on new cultivars of wheat (e.g. N-80-19) which is resistance to yellow rust in recent years. Without chemical control, tan spot will cause noticeable damage to wheat.

Fungi associated with corn seedlings in thinning patches of corn fields in Dashtnaz of Mazandaran

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Phytopathology 99:S35

Based on a visit from Dashtnaz Agricultural Organization (Sari-Mazandaran), which done in early May 2008, some thinning patches were observed in corn fields. Samples of weak seedlings were collected from the aforementioned patches. The samples were cultured on PDA and MA media which made at Plant Pathology Lab. of Plant Protection Department of Agricultural & Natural Resources Research Center of Mazandaran. *Fusarium moniliform*, *F. oxysporum*, *F. graminearum*, *Pythium* spp., *Trichoderma harzianum*, *T. viride* and *Rhizoctonia solani* have been found associated with the syndrome.

QoI resistance in *Fusicladosporium carpophilum* populations from almond in California

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Phytopathology 99:S35

Almond leaf and fruit scab caused by *Fusicladosporium carpophilum* (*Venturia carpophila*) is a common and widespread disease in California growing regions and can cause economic losses from premature tree defoliation. Resistance of the pathogen to benzimidazole fungicides is found in many areas. Before the introduction of the QoI fungicides azoxystrobin, trifloxystrobin, and pyraclostrobin, multi-site mode of action fungicides such as captan, chlorothalonil, ziram, maneb, or sulfur have been commonly applied prior to spring rains to manage the disease. Due to their superior efficacy and low usage rates, QoI fungicides were used extensively since 1999 and for several years, scab was considered a minor disease in California almond production. Following reports in 2006 that applications with QoI fungicides did not manage the disease, isolates of the pathogen were obtained from four locations in Northern California. Approximately 90% of the isolates were found to be resistant to QoI fungicides with EC_{50} values >40 mg/L (baseline isolates had EC_{50} values <0.05 mg/L). In 2007, resistance was found to be widespread in orchards in all growing regions in California. New fungicides and treatment timings for the management of almond scab are currently being developed. To maintain a high level of disease control, dormant treatments that reduce sporulation of overwintering twig lesions and strict rotations of fungicides will need to be done in integrated programs.

Dormant treatments as a component of integrated management of almond scab caused by *Fusicladosporium carpophilum*

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Almond scab has gained increased importance in California almond production in recent years due to QoI resistance in populations of the pathogen *Fusicladosporium carpophilum* (*Venturia carpophila*). The perfect state is unknown in California and thus, the fungus overwinters in lesions on <1 yr-old twigs. These lesions develop from infections in the previous season and provide primary inoculum in the spring when the fungus produces abundant wind- and rain-borne conidia that infect young leaves and developing fruit. Severe outbreaks may lead to premature tree defoliation. Management strategies for scab include planting designs and cultural practices that minimize favorable environments for disease development as well as chemical treatments that delay the sporulation of twig lesions or prevent new infections in the spring. Field trials were conducted to evaluate the efficacy of dormant treatments on the production of primary inoculum. Liquid lime sulfur and copper-agricultural oil mixtures applied in December or late-January delayed and reduced sporulation of twig lesions until April and May, respectively, whereas lesions of non-treated, wettable sulfur, and agricultural oil treatments sporulated in March with leaf emergence. The pathogen was not eradicated, however, subsequent protective treatments with captan, chlorothalonil, maneb, or ziram applied during leaf emergence and expansion were more effective.

Difenoconazole - A new fungicide for controlling postharvest decays of pome fruit and a mix partner for fungicide resistance management

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Phytopathology 99:S36

Postharvest decay management of pome fruit has been challenged by the presence of widespread resistance in populations of *Botrytis cinerea* and *Penicillium expansum* against benzimidazoles including thiabendazole, one of the most commonly used fungicides in the past. This has restricted the fungicide's use for the management of bull's eye rot caused by *Neofabraea* spp., another decay that can be a problem in some production areas in the United States. The newly registered pyrimethanil and fludioxonil are both highly effective against Penicillium decays and gray mold; whereas only pyrimethanil is highly effective against bull's eye rot. Still, a mix partner would increase the effectiveness of the contact fungicide fludioxonil against bull's eye rot and would decrease the potential for selection of resistance to either fungicide in pathogen populations. The efficacy of difenoconazole (FRAC 3 - triazoles) was evaluated in studies using wound-inoculated fruit and was found highly effective against Penicillium decay (*P. expansum*) and bull's eye rot (all four species) at all inoculum levels evaluated, and moderately effective against gray mold under low inoculum levels. Air-samplings in commercial packinghouses showed a low potential for resistance development in *P. expansum* populations with the absence of detecting resistant isolates.

Suppression of Fusarium wilt development on banana by silicon

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Phytopathology 99:S36

This study aimed to determine the potential of silicon (Si) to decrease the symptoms of Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), on banana. Banana plants from cultivars Grand Naine (resistant) and "Maçã" (susceptible), obtained from tissue culture, were grown for 35 days in plastic pots filled with 4.5 kg of a Si-deficient soil amended with either calcium silicate (AgroSilício®, Excell Minerais e Fertilizantes Ltda) (+Si) or lime (-Si). Plants were removed from the pots and their root system was dipped into a suspension of conidia of *Foc* (2×10^6 conidia ml^{-1}) for 30 min and thereafter transplanted to the same pots that contained the +Si and –Si treatments. Root system of plants dipped into sterile water served as a control treatment. After 35 days after inoculation, Si content on roots and shoots, the relative lesion extension (RLE) on the pseudostem, and plant dry weight were evaluated. Silicon content on roots and shoots from both cultivars significantly increased for the +Si treatment regardless of whether plants were inoculated or not with *Foc*. The RLE for cultivar "Maçã" was reduced by 39% when comparing -Si to +Si treatment. Regarding cultivar Grand Naine, there was no significative difference for RLE between the Si treatments. Plant dry weight was not affected by Si, but there was significative difference between cultivars. Supplying Si to banana plants can potentially reduce the symptoms of Fusarium wilt, especially for susceptible cultivars.

Fluopyram – a new active ingredient from Bayer CropScience

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Fluopyram (tested under the code USF 2015) is a novel broad-spectrum fungicide belonging to the new chemical class, pyridylethylamides, discovered and developed worldwide by Bayer CropScience. The mode of action for this Group 7 fungicide is inhibition of succinate dehydrogenase at protein complex II in the mitochondrial respiration chain. Disease control results have been excellent, especially for ascomycete pathogens of horticultural crops. A description of the product chemistry, mode of action, biological profile, bioavailability, global MRLs, and U.S. registration timeline will be presented.

Detection of *Gaeumannomyces graminis* varieties, the causal agents of take-all diseases, by real-time PCR assay

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Phytopathology 99:S36

Gaeumannomyces graminis causes take-all disease on cultivated cereal grasses. Diagnosis of take-all disease is generally based on visual symptoms, host identification, and the presence of darkly pigmented, ectotrophic runner hyphae on plant roots and/or crowns in the absence of mature perithecia. Morphological characteristics of the teleomorphic, anamorphic and mycelial states were used to classify *Gaeumannomyces* species and varieties. Those methods are laborious and time consuming. In this work, we are developing real-time PCR assays for detection and quantification of *G. graminis* varieties (*tritici*, *avenae*, and *graminis*) using specific primers targeting the 18S rDNA region. These primers were carefully designed to amplify only one variety of *G. graminis* per primer pair. The sensitivity of real time PCR allowed detection of 1 pg (0.001 ng) of purified *G. graminis* genomic DNA both *tritici,* and *avenae* varieties. The melting temperature (T_m) values of primers targeting *G. graminis* varieties were in the range 83 to 85. Molecular detection of different varieties of *G. graminis* from various resources of infected tissues is being conducted. This technique will be of great value to identify and differentiate the different varieties of *G. graminis*, in addition to facilitate identification of the organisms and diagnosis of take-all disease in infected plants that lack visible symptoms.

HR-like resistance of kumquat (*Fortunella* spp.) to citrus canker caused by *Xanthomonas citri* sbsp. *citri*

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Genetic resistance is the most desirable method for control of citrus canker, caused by *Xanthomonas citri* sbsp. *citri* (Xcc). A comparative study of grapefruit (*C. paradisi*) cv. Duncan, a very susceptible host, and two resistant cultivars of kumquat (*Fortunella* spp.), 'Meiwa' and 'Nagami', was conducted to evaluate the mechanisms involved in the resistance of kumquat to citrus canker. Xcc inoculum densities of 10^4 to 10^8 cfu/ml were infiltrated into immature leaves in the greenhouse (*in planta*) and into detached leaves incubated on water agar plates (*in vitro*). At higher bacterial inoculum density, kumquat cultivars developed a hypersensitive (HR)-like reaction in the infiltrated area, within a period of 72 h *in vitro*, and 96–168 h *in planta*. No symptoms or a few small necrotic spots developed in kumquats at the lower inoculum density. Susceptible grapefruit infiltrated with the same inoculum densities produced no visible tissue alterations at 72 h after inoculation and required 120 h or longer to develop water-soaking, hypertrophy and hyperplasia typical of canker lesions in compatible hosts. Phenotype of the lesions, bacterial population growth, anatomical changes in the infiltrated tissue and early expression of genes related to programmed cell death are indicative of HR that reduces growth of Xcc in the inoculation site and the further development of disease.

Determination of prevalence of *Potato Yellow Vein Virus* (PYVV) in crops of *Solanum phureja* in three states of Colombia by symptom detection and RT-PCR

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S. phureja is a diploid potato species important for Colombian economy. PYVV is a quarantenary virus *Crinivirus*/*Closteroviridae,* from the Andean countries that causes leaf yellowing, and reduction of size and number of

tubers. It is transmitted by *Trialeurodes vaporariorum* (white fly) and tubers. The objective was to study the prevalence of PYVV in crops of *S. phureja* in three Colombian states by symptom observation of 3 months old plants and molecular detection. In 2008, 8 municipalities of Cundinamarca, 5 of Antioquia and 6 of Nariño were visited and symptom data of 2 or 3 plots were taken (400 plants/plot), for a total of 20295 plants. RT-PCR tests were applied to 1075 samples. According to symptoms, the prevalence of PYVV in Antioquia was 7%, in Nariño 5% and in Cundinamarca 1%. ANOVA of the percent of symptomatic plants (previously transformed data) showed significant differences between evaluated states ($p < 0.01$) and municipalities of each state ($p < 0.01$). Interestingly, PYVV was detected by RT-PCR in asymptomatic plants (27% Antioquia, 53% Nariño, 10% Cundinamarca), suggesting that the proportion of asymptomatic plants that are infected varies between 2 to 10 fold in different states. Prevalence of PYVV 1–7% is not very high, suggesting a mild effect of PYVV in this crop. Explanation for latency in some asymptomatic plants is unknown, but it could be due to the presence of defective dsRNAs. Consequences for epidemiology and crop yield are being studied at the moment.

Evidence of latency of PYVV in tubers and plants of *Solanum phureja*
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S. phureja is a diploid potato species important for Colombian economy. PYVV is a quarantenary RNA(+) virus, Crinivirus/Closteroviridae, from the Andean countries, that causes yellowing and reduction of size and number of tubers. It is transmitted by *Trialeurodes vaporariorum* (white fly) and tubers. The objective was to determine the percent of symptomatic S and asymptomatic A plants that originate from tubers of S and A plants. Parental plants were labeled in the field as pS or pA according to existence of symptoms. The presence of virus was evaluated by RT-PCR in leaf samples of 7 pS plants and 10 pA and it was detected in all S and 6/10 pA. From these, 4 negative pA and 4 positive pS plants were selected and 10 tubers of each were grown in insect-free greenhouse conditions. In the next generation, all daughter plants from pA were asymptomatic and only 33% of the plants derived from S plants had symptoms. Additional tests on 15 tubers of pA plants (corresponding to 40 buds) and 13 of pS (16 buds) showed that 53% and 82% of the buds respectively were positive by RT-PCR. Results show that not all the plants derived from S plants develop yellowing, that a large proportion of daughter tubers from pA have PYVV but are asymptomatic and not all buds of a tuber have the virus, suggesting that PYVV shows latency and heterogeneous distribution in tubers. The causes and consequences of latency and uneven distribution for yield and epidemiology are being studied.

Mutations in the target of DMI fungicides (CYP51) in *Mycosphaerella graminicola* and their impact on DMI sensitivity
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Sensitivity of *M. graminicola* towards DMIs was investigated in recent years in European monitoring studies and a shift in the population to slightly reduced sensitivities was determined. Recently, this shift has stabilized. As one reason for this shift, mutations in the target protein of DMI (CYP51) have been studied. Particularly the impact of the amino acid exchanges V136A, A379G, I381V, and mutations or deletions in the YGYG region (at positions 459-462) on the DMI-sensitivity have been discussed. A survey with > 600 isolates from European countries provided an overview on the distribution of the frequency of different CYP51-haplotypes and indicated a heterogeneous population within Europe, different regions, and even in a single field. Analysis of these isolates showed that the influence of the CYP51-haplotypes on sensitivity towards the triazole epoxiconazole is limited and that other mechanisms may be involved. No correlation between CYP51 haplotype pattern and field performance of epoxiconazole could be established in numerous field trials. Equally high levels of efficacy were achieved at sites where I381V or A379G were dominant or less frequent. In a 3-year field trial study on the selection of different mutations by DMI treatments, epoxiconazole did not select for isolates with V136A, A379G and I381V. Field studies over 4 years with more than 100 trials showed no correlation of the sensitivity pattern of the field population and epoxiconazole performance.

The *Geosmithia* causing thousand cankers disease of walnut is a new species
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Phytopathology 99:S37

Thousand cankers disease of black walnut (*Juglans nigra*) is caused by an unnamed species of *Geosmithia* that is vectored by the walnut twig beetle (*Pityophthorus juglandis*). *Geosmithia* isolates collected from walnut cankers differ from previously described *Geosmithia* species and operational taxonomic units (OTU's) isolated from bark beetles associated with conifers and hardwoods. Differences include growth habit and color of isolates in culture and morphological characteristics of the conidiophores and conidia. We sequenced the rDNA ITS region of 37 walnut isolates collected from *Juglans* species in Arizona, California, Colorado, Idaho, Oregon, Utah and Washington. Sequences from these isolates were distinct from other described *Geosmithia* species and OTU's based on parsimony and Bayesian analyses and supported morphological observations indicating this is a new species. Nevertheless, at least seven different ITS sequences have been identified among the walnut isolates. This variability was not correlated with geographic sites or hosts from which isolates were collected. Thus the walnut *Geosmithia* population in the western United States appears to be diverse and complex.

Excessive summer rains trigger outbreaks of two fungal leaf spot diseases "new" to pistachio in New Mexico
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Phytopathology 99:S37

Two fungal leaf diseases of pistachio, Septoria leaf spot (*Septoria pistaciarum*) and Alternaria late blight (*Alternaria* sp.), were confirmed for the first time in New Mexico in the fall of 2008. A survey of pistachio orchards revealed that trees in Otero, Hidalgo, Luna, Sierra and Doña Ana Counties were infected. Results indicated a high incidence of both diseases; 89% of the surveyed orchards were infested with *Septoria* and 100% were infested with *Alternaria*. The widespread occurrence of these diseases suggests that the pathogens have been present in NM orchards for several years. We believe that drier than average conditions in previous years limited the severity of these diseases. Average total rainfall in Otero County, the primary pistachio growing county in NM, in July and August is 8.64 cm (3.4"; 94 year average). In July and August of 2003–2005 the total average rainfall was only 6.83 cm (2.69"). These drought conditions were followed by above average rainfall in 2006–2008, when the total average rainfall during July and August was 18.06 cm (7.11"). This excessive moisture provided excellent conditions for widespread infection and disease development. The high level of inoculum currently present in NM orchards presents a concern that these diseases may become a recurring problem for NM pistachio producers. Further research is planned to investigate the effect of these diseases on yield and tree vigor, and to develop management strategies.

The occurrence of at least four haplotypes of *Phytophthora capsici* in Texas from isolates recovered and characterized in 2006–2008
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Phytopathology 99:S37

In many production areas of the United States, Phytophthora blight of pumpkin, squash, watermelon, and pepper (*Capsicum* spp.) has increased in importance. This disease, caused by *Phytophthora capsici*, has been reported in Texas for over 60 years. During the past three years, the disease was observed on chile pepper, pumpkin, and winter squash in the Texas High Plains (NW Texas) and on watermelon in the Lower Rio Grande Valley (South Texas). A preliminary characterization was done on isolates obtained from NW Texas and isolated from diseased chile pepper (9), pumpkin (1), and winter squash (1). Three isolates obtained from S. Texas, isolated from chile pepper (1) and watermelon (2), were also characterized. All isolates tested were heterothallic, pathogenic on chile pepper, and sensitive to mefenoxam at 5 ppm and 100 ppm. At both 25°C and 30°C, several isolates from chile pepper grew at a much faster rate on clarified 20% V8 juice agar plates than the isolates from pumpkin or squash. These chile pepper isolates were from both locations. The ITS1-5.8S-ITS2 complete region of the rDNA was amplified, sequenced, and compared to sequences reported for *P. capsici*. Based on phylogenetic analysis, at least four haplotypes of *P. capsici* were present in Texas. Further characterization of *P. capsici* in Texas will allow for a better implementation of plant disease management strategies.

Deciphering the interaction between SCN and *Fusarium virguliforme*
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Phytopathology 99:S37

Soybean Cyst Nematode (SCN) and *F. virguliforme* are two of the most important pathogens on soybean. The mechanisms governing the interaction

between SCN and *F. virguliforme*, the causal agent of sudden death syndrome on soybean (SDS), are unknown. We used green fluorescing protein (GFP) producing strains of *F. virguliforme,* to shed light on this interaction. The fluorescence of these strains allows us to visually assess the ability of the fungus to infect and colonize roots, and provides a means to assess the extent of root colonization. Moreover, in addition to its ability to fluoresce, one of these GFP-producing strains has impaired ability to infect and/or colonize roots. These fungal strains allow us to determine how SCN is assisting the fungus in causing SDS. Greenhouse experiments were conducted using soybean cultivars with varying levels of resistance to SCN, root knot nematode and SDS. Each cultivar was challenged with a GFP-expressing aggressive fungal strain or a GFP-expressing non aggressive fungal strain. Fungal strains were also co-inoculated with root knot nematode or SCN. The use of root knot nematode helped determine whether the *F. virguliforme*/SCN interaction is unique in enhancing SDS, or if other nematode pathogens could facilitate this interaction. The importance of the role of either nematode in facilitating fungal infection and colonization of roots were assessed further by using a fungal strain that is impaired in its ability to penetrate soybean roots.

Using real-time PCR to quantify aster yellows phytoplasma in its insect vector; relationship of infectivity to transmissibility in the aster leafhopper

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The aster yellows (AY) index is used to prescribe insecticide sprays that target the vector of the aster yellows phytoplasma (AYp) *Macrosteles quadrilineatus*, or aster leafhopper (ALH). The AY index is the product of the proportion of infective ALHs and the relative ALH population size at a location. Recently, polymerase chain reaction (PCR) has become used to determine the proportion of potentially infectious AYp-infected ALHs, but limited information exists to determine the proportion of PCR-positive inoculative individuals. As a persistent and propagative pathogen, our current hypothesis predicts a relationship between the population size of AYp in individual ALHs and the frequency at which individuals transmit AYp. To address this hypothesis, we have developed a quantitative, real-time PCR protocol to quantify AYp DNA in ALHs. The elongation factor TU gene was used as a target for AYp and the ALH CP6 wingless gene was used as a target for insect genomic DNA. Target sequences were chosen because they are likely present as single copies in their respective genomes and can be related in a 1:1 ratio. The ratio of AYp DNA to insect DNA is useful to avoid variation due to the DNA extraction procedure. In the insects examined to date, the ratio of AYp genomes to insect genomes range from 0.832 to 0.006. This new methodology will improve the accuracy of the AY index by providing a tool for accurate determination of infective individuals generated following acquisition and inoculation bioassays.

Refining the aster yellows index in Wisconsin: Developing sustainable control tactics for susceptible vegetable crops

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Phytopathology 99:S38

The population magnitude and infectivity associated with migratory *Macrosteles quadrilineatus,* or aster leafhopper (ALH), in early spring influences aster yellows epidemics in Wisconsin carrot. In years when the numbers of spring migrants are noted as trivial, native ALH populations can drive disease cycles by acquiring aster yellows phytoplasma (AYp) from local plant sources and subsequently transmitting it into carrot. The focus of this project is to evaluate the relative epidemiological importance of inoculum sources present in field edges compared with the estimated inoculum in-bound, in migratory ALHs. In 2007 and 2008, total DNA was extracted from ALHs collected on their spring migratory path and ALHs collected in 10 geographically distinct carrot fields. Total DNA was also extracted from symptomatic carrot plants from each of the locations. PCR analyses were used to identify AYp strain types present in the infected ALHs and the carrots. To date, the relative abundance of AYp strain types extracted from carrot and ALHs varied by location. The AYp genotypes present in fields were associated with specific disease symptoms. By combining AYp strain data with vector phenology and infectivity data, we will improve our understanding of where ALHs acquire and when they spread AYp to carrot. This integrated approach will help to refine our current management tactics ultimately contributing to the development of a comprehensive control strategy for Wisconsin carrot growers.

GmRAR1 and GmSGT1-2 participate in various modes of soybean immunity against microbial pathogens

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Phytopathology 99:S38

RAR1 and SGT1 are important components of defense signaling pathways induced by structurally diverse resistance (R) proteins. RAR1 and SGT1 are thought to modulate R protein stability serving as co-chaperones of HSP90. Similar to their counterparts from several other plants, soybean RAR1 and SGT1 proteins interact with each other and two related HSP90 proteins. However, resistance induced by two different soybean R proteins requires RAR1 and SGT1, but not HSP90. *Rsv1*-mediated extreme resistance to Soybean Mosaic Virus and *Rpg-1b*-mediated resistance to *Pseudomonas syringae* were compromised in plants silenced for *GmRAR1* and *GmSGT1-2*, but not *GmHSP90*. This suggests that RAR1-/SGT1-dependant signaling may not always be associated with a dependence on HSP90. Unlike in Arabidopsis, SGT1 in soybean is also required for resistance signaling against the bacterial pathogen *P. syringae*. Plants silenced for *GmHSP90s* or *GmRAR1* show altered morphology suggesting that these proteins also contribute to developmental processes. Silencing *GmRAR1*and *GmSGT1-2* impaired resistance to virulent strains of *P. syringae* and systemic acquired resistance (SAR) in soybean as well. Since the Arabidopsis *rar1* mutant also showed a defect in SAR, we conclude that RAR1 and SGT1 serve as a point of convergence for basal, *R* gene-mediated and systemic immunity in diverse plants.

Detection of chromosome rearrangements in *Gibberella zeae*

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Phytopathology 99:S38

Chromosome rearrangements between fungal strains may reduce fertility in sexual crosses and result in progeny that are inviable or have reduced fitness. Such rearrangements act as a post-zygotic barrier to gene flow and may contribute to speciation. *Gibberella zeae* (anamorph: *Fusarium graminearum sensu lato*) is a homothallic species that can reproduce sexually by self-fertilization or outcrossing. *G. zeae* is composed of multiple phylogenetic lineages that are morphologically, pathologically, and toxicologically similar, but contain DNA sequence polymorphisms whose distribution suggests reproductive isolation between the lineages. Genetic and cytological methods for detecting chromosome rearrangements are difficult and/or laborious in *G. zeae*. For that reason, we investigated counts of viable ascospores per ascus in *G. zeae* outcrosses as an indicator of chromosomal rearrangements. Ascospores can be observed in rosettes of asci extruded from crushed perithecia or by observing unordered tetrads that are ejected from mature perithecia. Individual asci can be assigned to the following four classes: 8, 6, 4, and 2 ascospores. Crosses between different male strains and two female tester strains often produced significant frequencies of asci with 6, 4, or 2 ascospores per ascus, which indicates the presence of one or more rearrangements. These results suggest that this method will be useful to survey populations of *G. zeae* for chromosome rearrangements.

Two separate phage genomes appear associated with citrus greening (Huanglongbin)

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Citrus Huanglongbin (HLB), also known as citrus “greening” is a lethal disease of citrus that is now widespread in Florida. HLB is caused by *Candidatus* Liberibacter asiaticus (Las), which has not been cultured; consequently Koch’s postulates have not been confirmed. The recent availability of a draft Las genome derived from infected psyllids (GenBank accession NZ_ABQW00000000) reveals that prophage DNA appears in some of the available contigs, but phage have not been previously been associated with HLB. Using dodder transmission, we have continuously curated HLB from a single infected Florida citrus tree for three years. We developed a DNA extraction protocol from dodder that enriched for Las and greatly reduced chloroplast and mitochondrial DNA contamination (as determined by PCR) and used multiple displacement amplification (MDA) to obtain sufficient DNA to create a fosmid library with an average insert size >32 kb. This fosmid library was found to be surprisingly biased towards phage DNA inserts; the phage DNA was confirmed associated with HLB. Based on shotgun library assembly, fosmid walking, and direct PCR cloning and sequencing, two related, but distinct phage partial genomes were assembled.

Both were confirmed to be associated with HLB on citrus. The idea that phage DNA may be over-represented in infected plants but not in psyllids raises the possibilities that Las is a lysogenic host and that phage may contribute to HLB disease.

Viability of *Phytophthora nicotianae* oospores in North Carolina tobacco populations

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Phytopathology 99:S39

Phytophthora nicotianae, the causal agent of black shank of tobacco, occurs in all tobacco-producing areas of North Carolina. A recent state-wide survey revealed multiple fields in which both the A1 and A2 mating types were present. Very little is known about the contribution of sexual sporulation to pathogen variability and black shank epidemiology. A1 and A2 isolates that originated from the same fields were paired on soft carrot agar amended with 10 ppm cholesterol to identify the potential for sexual compatibility and reproduction. A subset of compatible isolates from within fields was further examined to assess the percentage of oospores from pairings that were viable. After 4-wks incubation, oospores were extracted from the agar and stained with tetrazolium bromide. Viable oospores were identified microscopically based on a specific color reaction as described by Sutherland and Cohen in 1983. Viability of the within-field pairings ranged between 20 and 48%. Germination of oospore progeny is being investigated to determine if sexual reproduction serves as a source of pathogen variability. Understanding the biology of sexual sporulation in *P. nicotianae* will help elucidate how variability develops within populations and how it influences short and long term durability of resistance genes in tobacco.

Search of a "DNA barcode" for identification of species of the genus *Fusarium*

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Phytopathology 99:S39

"DNA barcoding" is a tool that aims to become a taxonomic method using a short standard genomic sequence, present in all the taxons of interest and showing sequence variation, enough to discriminate among species. The members of genus *Fusarium* are recognized as important plant pathogens, human pathogens and saprophytes and its routine taxonomic identification relies on macro and microscopical characteristics and molecular methods. However, identification can be difficult due to the lack of some structures in culture or to the lack of enough polymorphism in ribosomal sequences. Barcoding could provide an easy and reliable method to overcome these problems. This study evaluated sequences of cox (mitochondrial cytochrome oxidase subunit 1) and aox (alternative oxydase) as potential DNAs barcodes for identification of *Fusarium* species. DNA was extracted from 12 *Fusarium* isolates previously identified by traditional methods, including *F. solani, F. oxysporum, F. proliferatum*, and *F. moniliforme*. For the amplification of aox, primers were designed by our group showing amplification of a region of 800 bp approximately in all *Fusarium* species but not in *Alternaria* sp. or *Pestalotia* sp. Amplicons were sequenced and analyzed using MUSCLE alignment software. So far, our results suggest that the combination of these two genes may provide a method for identification of the species tried in this work. Future work will include a higher number of species and isolates from each species.

GFP expression from a biologically active minireplicon of Sonchus yellow net virus

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Phytopathology 99:S39

Sonchus yellow net virus (SYNV) is the most extensively characterized plant rhabdovirus that replicates in the nucleus. The minimum infectious unit of SYNV is a ribonucleoprotein core complex, which consists of the nucleocapsid (N) protein, the phosphoprotein (P) and the polymerase (L) protein complexed with the negative-strand genomic RNA. We have constructed a SYNV minireplicon (MR) that functions in planta to permit reverse genetics studies of the core N, P and L proteins, and the trailer and leader regions of SYNV RNA. The MR presents a new approach to express the core complex in plants via agroinfiltration into Nicotiana benthamiana leaves. The MR has been engineered to generate a transiently expressed antigenomic (ag) RNA that is processed by ribozymes flanking the leader and trailer regions. The transcript consists of the SYNV leader sequence, the 5'UTR of the N gene, a GFP reporter gene, a gene junction sequence, a DsRed or CAT reporter gene, the 3'UTR of the L gene and the SYNV trailer sequence. To assess the biological activity of the MR, leaves were agroinfiltrated with plasmids expressing the agRNA, the N, P and L proteins and suppressors of gene silencing. At about 5 days after infiltration, numerous GFP fluorescent foci that persisted for up to three weeks were observed within the infiltrated leaves. Mutagenesis results reveal that the N, P and L genes, and elements of the leader and trailer sequences are required for MR replication.

An intact cuticle in distal tissues is essential for the induction of systemic acquired resistance in plants

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Phytopathology 99:S39

Systemic acquired resistance (SAR) is initiated upon recognition of specific microbial effectors by cognate plant resistance proteins and immunizes distal tissues of plants against secondary infections. SAR involves the generation of a mobile signal at the site of primary infection, which then translocates to and activates defense responses in the distal tissues via unknown mechanism(s). We have recently shown that an acyl carrier protein, ACP4, is required for the processing of the mobile SAR signal in distal tissues of Arabidopsis. Although acp4 plants generated the mobile signal, they were unable to respond to this signal to induce systemic immunity. The defective SAR in acp4 plants was not due to impairment in salicylic acid (SA)-, methyl SA-, or jasmonic acid-mediated pathways but was associated with the impaired cuticle of acp4 leaves. Other genetic mutations impairing the cuticle or physical removal of the cuticle from wild-type plants also compromised SAR. This cuticular requirement was only relevant during the time of mobile signal generation and translocation to the distal tissues. Together, these results demonstrate a novel role for the plant cuticle as the site for SAR-related molecular signaling.

Biological control of Sclerotinia stem rot with an endophytic *Bacillus* sp. strain on oilseed rape

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Phytopathology 99:S39

Stem rot, caused by *Sclerotinia sclerotiorum*, is one of the most important diseases of oilseed rape in China. The antagonistic activities of sixty-three endophytic bacteria strains, which were isolated from leaves, stems and roots of winter wheat, were tested against *S. sclerotiorum in vitro*. The study showed that fourteen trains had a remarkable inhibitory effect to the growth of mycelia, production and germination of sclerotia. The evaluation of the antagonistic strains against stem rot of oilseed rape indicated that the strain Em7 reduced disease incidence. In greenhouse experiments, Em7 had 97% control effect against stem rot of oilseed rape when it was sprayed to leaves before 24 h of inoculation *S. sclerotiorum*. Em7 was primarily identified as *Bacillus* based on its morphology and physiology. Microscopic studies showed that *Bacillus* sp. Em7 induced diverse morphological alterations in pathogens hyphae. Hyphal morphological alterations include cytoplasm exosmosis, deformation, swelling of apex.

First approach to the characterization of *de novo* pyrimidine biosyntheis pathway in *Phytophthora infestans* as a target for pathogen control

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Phytopathology 99:S39

The oomycete *Phytophthora infestans* is the causal agent of the tomato and potato late blight, causing high economic losses worldwide. Current control strategies are far from being adequate and an interesting and unexplored alternative for control could be based on the inhibition of the *de novo* pyrimidine biosynthetic pathway. Indeed, inhibitors of some of the enzymes in this pathway have been proposed as therapeutic agents for a wide range of human parasites and some plant pathogens. Bioinformatic analyses of the enzymes in the *P. infestans* pathway were performed, in order to select the best targets for enzymatic inhibition. Based on the similarity to host enzymes, different predicted subcellular localization, architecture, predicted 3D structure and phylogenetic relations, the last two enzymes of the pathway, orotate phosphoribosyltransferase and orotidine-5-monophosphate decarboxylase, were selected as the most promising targets. Nevertheless, enzymes 3 and 4 dihydroorotase and dihydroorotase dehydrogenase cannot be ruled out. Key aspects of their metabolic inhibition were also determined for future virtual screening of a compound library using molecular docking. These enzymes are being cloned, expressed and purified in their recombinant form. This will allow their future biochemical characterization. To our knowledge, this is the first study of the pyrimidine biosynthesis in oomycetes.

Systemic infection of sugarcane plants in the Rio Grande Valley of Texas by non-native B and G aflatoxin-producing *Aspergillus* section *Flavi* fungi

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Phytopathology 99:S40

Aflatoxin contamination of cottonseed and maize is an economic concern to farmers in the Rio Grande Valley of Texas (RGV) where the causative fungi form dynamic and diverse communities. Sugarcane is rotated with both cotton and maize crops. Soils from fields with histories of sugarcane cultivation yield B and G aflatoxin-producing fungi distinct from those resident in cotton or maize fields. These distinct fungi are found neither outside RGV fields in Texas, nor in RGV fields without sugarcane cropping history. Phylogenetic and vegetative compatibility analyses suggest these fungi are part of a group of sugarcane-associated fungi also found in Japan and Florida and named FP-1 for similarities to both *A. flavus* and *A. parasiticus*. In the RGV, FP-1 fungi dominate communities of section *Flavi* isolated from both billets (cane pieces used for planting) and freshly harvested canes. All but 1 of 22 RGV sugarcane plants from 8 locations were infected with at least one FP-1 isolate and approximately 40% of isolations from mature stem pieces yield FP-1 fungi. Field observations and laboratory assays show FP-1 fungi are capable of systemic infection and lesion development. FP-1 fungi are globally transported with sugarcane and are apparently dependent upon the perennial cropping of sugarcane for persistence and overwintering in RGV fields.

Yield effect and control of yellow leaf disease under tropical conditions of Ecuador

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Yellow leaf caused by Sugarcane yellow leaf virus (ScYLV) was recently detected in sugarcane in Ecuador. ScYLV incidence and distribution was studied at three sugarmills. Yield losses were evaluated using disease-free seedcane obtained by meristem tissue culture. Effects of planting disease-free seedcane, application of systemic acquired resistance (SAR) products, and vector control were studied under natural infection. ScYLV and ratoon stunting disease (RSD) free plots of B76-78 variety had higher tons cane/ha (TCH) and tons sucrose/ha (TSH) than plots with ScYLV and/or RSD. In plant cane, healthy plots had 20% more TCH, 15% more sugar/TC and 36% TSH. In first ratoon, healthy plots had 24.8% more TCH and 24.6% more TSH. Tissue culture plants had lower ScYLV incidence. Application of Imidacloprid insecticide with salicylic acid or the SAR kit, Relief, reduced ScYLV infection from 33.3 to 8.3% and increased TCH and TSH. Plots treated with SAR products showed an increase of 12% in sugar/TC and 23% in TCH. Results suggested the epidemiology of yellow leaf in Ecuador is suitable for rapid disease increase resulting in significant economic loss. Yellow leaf can be best managed by the use of disease-free seedcane obtained through meristem tissue culture with prevention of early re-infection in nurseries with applications with SAR products and systemic insecticides.

Potential use of qPCR for evaluating resistance to leaf scald in sugarcane

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Leaf scald, caused by *Xanthomonas albilineans* (Xa), is one of the most widespread diseases of sugarcane. Xa is a xylem-limited bacterium that produces a toxin, albicidin, which affects proplastid synthesis. Previous work, in which tissue was blotted on selective media, showed a significant correlation between pathogen populations in the shoot apex and disease resistance. The purpose of this study was to relate bacterial populations, using qPCR, in different tissues with infection incidence and disease severity. The minimum level of bacteria detected was seven colony forming units per reaction. The infected varieties showed a single peak of amplification associated with the specific target Xa DNA sequence. Results showed that varieties CP89-846 and HoCP85-845 had high incidence and severe symptoms and a low CT value ($1.2–1.9 \times 10^6$ CFU/ml). The resistant varieties LCP85-384 and Ho55-988 had low incidence and severity and a high CT value ($3–8 \times 10^2$ CFU/ml). The information provided by qPCR about Xa populations in systemically infected plants of genotypes with varied levels of resistance could be used with genomic or proteomic approaches to elucidate the molecular basis of resistance. Use of qPCR for varietal screening for resistance is another possible application.

Isolation and characterization of *Fusarium oxysporum* causing potato dry rot in *Solanum tuberosum* in Colombia

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The anamorphic fungus *Fusarium oxysporum* has a worldwide distribution and is responsible for severe vascular wilt or root rot in many plants. Strains are classified into *formae speciales* based on their high degree of host specificity. So far, several molecular markers have been studied as a means to differentiate between *formae speciales*, but none has proven to be completely satisfactory. Two different *F. oxysporum* isolates (monosporic cultures), showing both macroscopic and microscopic differences in morphology, were obtained from potato (*Solanum tuberosum*) tubers with symptoms of dry rot. Koch's postulates were conducted on tubers inoculated with each isolate and incubating at 25°C with high relative humidity for 30 days. Both isolates induced moderate dry rot of the tubers, with one of the isolated being more aggressive than the other. Ribosomal internal transcribed spacer (ITS) and intergenic spacer region (IGS) sequences were PCR amplified and sequenced. No differences were detected in the obtained sequences from each isolate. A maximum parsimony phylogeny was built using all the *F. oxysporum* IGS sequences available in the non-redundant GenBank database (nr), which does not include f. sp. *tuberosi*. The two isolates were most closely related to two non-pathogenic *F. oxysorum* isolates from bird's-foot trefoil (*Lotus corniculatus*) and the *S. lycopersicum* rhizosphere (99% identity), and to a pathogenic isolate from red clover (*Trifolium pratense*).

Evaluation of gibberellin production by the basidiomycete *Moniliophthora perniciosa*, the causal agent of Witches' Broom Disease in cacao

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The basidiomycete *Moniliophthora perniciosa* is a hemibiotrophic fungus and the causal agent of Witches' Broom Disease (WBD) of cacao. The typical symptoms of WBD are hyperplasia and hypertrophy of infected cacao tissues which could be correlated to a hormonal imbalance. The *M. perniciosa* Genome Project has led to the identification of putative genes homologues to all genes involved in the gibberellin (GA) biosynthesis pathway of *Gibberella fujikuroi* suggesting that *M. perniciosa* could produce gibberellin. Thus, the present work aimed to verify this hypothesis through biochemical and genetic analyses. The bifunctional Copalil diphosphate synthase/ent-kaurene synthase (CPS-KS) gene expression profile was analyzed throughout the life cycle of the fungus by real-time RT-PCR suggesting that the enzyme is more expressed in the infective phase of this fungus. Moreover, using a combination of Thin-Layer Chromatography and Mass Spectrometry techniques fungal spores extract was analyzed and a substance with the same molecular weight of GA3 has been already identified, thus suggesting that this fungus may indeed produce gibberellins. This is the first report of the existence of a putative CPS-KS gene that codes for the first specific enzyme of the GA biosynthesis pathway in a basidiomycete. We conclude that *M. perniciosa* is likely to produce gibberellin as a pathogenic factor, and that this hormone may play a key role in the development of WBD.

Identification of *Botrytis cinerea* Pers.: Fr. isolated from *Rosa* spp. and assessment of its sensitivity to fungicides

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The southern part of the state of Mexico has the largest agricultural area of production of ornamentals of the country. More than 500 ha of roses (*Rosa* sp.) are cultivated under greenhouses. This crop has been affected by the fungus *Botrytis* spp. in pre- and post-harvest, damaging mostly the flower and floral bud. Control is based on chemicals. This study was conducted 1) to determine the species of *Botrytis* sp. and 2) to evaluate the *in vitro* response of the fungus to fungicides. Fifteen strains of *Botrytis* spp. were isolated, each from a different greenhouse. These were identified at the species level by their morphological characteristics. All of the strains were subjected to *in vitro* sensitivity tests with the fungicides that are most used in the region for control: prochlorazine 2.5 mL L^{-1}, thiabendazole 1.0 g L^{-1}, iprodione 1.5 g L^{-1}, metalaxyl-m 3.75 mL L^{-1} and chlorothalonil 3.25 mL L^{-1}. Growth of mycelium diameter was measured every 12 h for six days. The data were subjected to an analysis of variance and the Tukey test ($\alpha = 0.05$) of comparison of means with the *Statgraphics* version 5.1 statistics software. The

results indicated that *Botrytis cinerea* Pers.:Fr. is the fungus that causes blight on roses produced in the southern part of the state of Mexico. Regarding sensitivity to fungicides, the 15 strains have lost sensitivity to thiabendazole; 5 to iprodione, prochlorazine and chlorothalonil; only one strain was not sensitive to metalaxyl-m.

Screening for resistance in local and global wheat germplasm against *Fusarium culmorum* and *F. pseudograminearum*, causal agents of crown rot in Tunisia

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Crown rot caused by *Fusarium* species is a major constraint to cereal production in many rainfed regions of the world including North Africa. *F. culmorum* and *F. pseudograminearum* are the dominant casual agents in Tunisia and have been found to cause up to 40% of yield reduction in wheat. Screening for genetic resistance, which constitutes one the best methods to control this disease is difficult and requires many repeats to ensure useful data. Collaboration with ICWIP-CIMMYT started three years ago to adopt a screening method under greenhouse conditions. Advanced lines of the national wheat breeding program and specific Crown Rot International CIMMYT nursery were screened using a modified CIMMYT protocol. Many of the CIMMYT lines have shown repeatable valuable results for resistance for one or both species of *Fusarium*. Ten lines with effective sources of resistance already confirmed around the world, have been validated against the local Tunisian *Fusarium* isolates. In addition a few lines from the national wheat breeding program showed promising results in one year data screening. Further work is needed to validate these sources in the field. The most useful source of resistance should serve as a valuable source of resistance for breeding programs.

A new mechanisms of action of an antagonistic strain of *Fusarium oxysporum*

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Fusarium oxysporum MSA 35 is an antagonistic *Fusarium*, isolated from a *Fusarium*-suppressive soil, that lives in association with a consortium of bacteria belonging to the genera *Serratia, Achromobacter, Bacillus* and *Stenotrophomonas*. Typing experiments and virulence tests proved that the *F. oxysporum* isolate when cured of the bacterial symbionts is pathogenic, causing on lettuce wilt symptoms similar to those caused by *F. oxysporum* f. sp. *lactucae*. The antagonistic effect of MSA 35 is due to the interaction with the ectosymbiotic bacteria. Expression analysis showed that genes involved in *F. oxysporum* pathogenicity are not expressed in the wild type strain whereas they are expressed in the cured fungus. Small volatile organic compounds (VOCs) belonging to sesquiterpenes, mainly caryophyllene, emitted from the wild type strain negatively influence the mycelial growth of different formae speciales of *F. oxysporum*. Moreover, these VOCs repress gene expression of the two putative virulence genes in *F. lactucae*. This new potential direct long-distance mechanism for antagonism in *F. oxysporum* is discussed, taking into account the practical implications.

Suppression of *Phytophthora capsici* and *Pythium ultimum* by the fungal-feeding nematode *Aphelenchus avenae*

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The grazing of pathogenic fungi by fungal-feeding nematodes is a biocontrol mechanism of interest for the suppression of soilborne fungal pathogens. We evaluated the roles of the nematode *Aphelenchus avenae* in regulating the population dynamics and pathogenic activity of *Phytophthora capsici* and *Pythium ultimum*. Two model host-pathogen systems were studied; bell pepper (*Capsicum annum*) attacked by *P. capsici* in 3 soils and tomato (*Lycopersicon esculentum* L.) attacked by *P. ultimum* in 3 container media. Soils were defaunated and then inoculated with *P. capsici* and *A. avenae*, with pepper seeds added 1 week later. The seedling mortality was examined weekly and the pathogen density and nematode population were assessed 21 days after seeding. Nematodes significantly reduced disease incidence on pepper by 50–85% and soil type significantly affected the efficacy of faunal suppression ($p = 0.05$). Container media of peat moss amended with 4, 8, and 16% of compost were inoculated with *P. ultimum* and *A. avenae*, and tomato seeds were added 2 weeks later. The seedlings were examined for emergence rates and rated for disease severity; relative activities of pathogens were calculated at 5 weeks and results are being evaluated. These results suggest that the nematode *Aphelenchus avenae* may be used for suppression of seedling diseases caused by soilborne fungal pathogens.

Effects of 3-ADON and 15-ADON chemotypes of *Fusarium graminearum* on spring wheat

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Phytopathology 99:S41

Fusarium graminearum, causal agent of Fusarium Head Blight (FHB), results in yield losses and decreased grain quality in wheat due to deoxynivalenol (DON). Research shows that the higher DON producer, 3-ADON is quickly displacing the 15-ADON chemotype populations. The objectives of this study were to compare the effects of the isolates for incidence, severity, FHB index, Fusarium damaged kernels (FDK), DON accumulation, and yield of three wheat genotypes that differed in resistance. A split plot design, where isolate was the main plot effect and wheat genotype was the subplot effect, was used and replicated three times. The plots were inoculated twice with 13 3-ADON and 12 15-ADON single spore isolates. Ratings were read every three days from the onset of symptoms to natural senescence of the controls. Plots were harvested for grain yield, FDK and DON levels. Results showed that there were significant differences among isolates, genotypes and isolate-genotype interactions. Partitioning of isolate effects showed that there were no significant differences within the 3-ADON isolates but there were within the 15-ADON isolates. Results were also significant for chemotypes and genotypes separately. Further analyses will be done to determine if there is a relationship between percentage FDK and DON levels, and results of this will be presented.

Infection severity of *Colletotrichum coccodes* in Russet Burbank potatoes with respect to environmental potassium

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Black Dot (*Colletotrichum coccodes*) is a soil borne fungal disease that ruins potatoes before and after harvest. Infections initiate on plant material below ground, and control is dependent upon systemic delivery of fungicides. Potassium is an essential mineral that has been shown to be important in physiological processes such as plant defense. Our objective was to assess the affect of potassium concentrations in the plant's environment with disease severity. Russet Burbank potatoes were grown hydroponically and artificially infected with spores of *C. coccodes* in solutions of varying levels of Potassium (0, 10, 80, 160 mg K L-1). Infection was assessed by plating roots on modified potato dextrose agar (mPDA) and through quantitative RT-PCR. Using specific primers, standard curves were generated from fungal and potato DNA dilutions, and these in turn were used to generate infection coefficients (IC) for total tissue DNA. The IC from different treatments were compared. *C. coccodes* was present in all of the samples. Symptoms were visible on the tips of roots, on root hairs and on the lowest stem portions. Greatest infection occurred in treatments of 0 and 10 mg, pathogen was visibly reduced in the 160 Mg K treatment; although, at this concentration leaves appeared slightly chloritic, suggesting a possible loss of fitness. This study demonstrated that the amount of available soil K can affect plant health through disease susceptibility.

Controlling apple scab and powdery mildew with the new fungicide DPX-LEM17

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Phytopathology 99:S41

DPX-LEM17 is a new fungicide currently being developed by DuPont in the U. S., Canada and worldwide for control of a wide range of fungal diseases of cereals and specialty crops. DPX-LEM17 offers a biochemical site of action different from most commercially available fungicides, broad-spectrum control of both foliar and soilborne diseases, strong pre-infection as well as post-infection activity, a good balance of systemic uptake and persistence on the leaf surface and ultimately improved crop quality and yield. For U. S. apple growers, DPX-LEM17 controls both apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*). The high level of fungicidal activity from DPX-LEM17 against these diseases provides reliable disease control under heavy pressure while allowing flexibility in application timing and frequency. When used in programs with other fungicide classes like contacts (EBDC's, sulfur), QoI's, and DMI's, DPX-LEM17 is an effective tool for managing the risk of resistance development for all fungicide classes.

Control of gray mold and powdery mildew of grapes with the new fungicide DPX-LEM17

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Phytopathology 99:S42

DPX-LEM17, a new fungicide from DuPont, currently is being developed in the U. S., Canada and worldwide for control of a wide range of fungal diseases of cereals and specialty crops. DPX-LEM17 belongs to the new SDHI class of fungicides with a biochemical site of action different from most commercial fungicides. DPX-LEM17 provides broad-spectrum control of many fungal diseases, strong preventive as well as curative activity, a good balance of residual activity with uptake into the plant and ultimately improved crop quality and yield. For U. S. grape growers, DPX-LEM17 controls gray mold (*Botrytis cinerea*) attacking both blossoms and fruit as well as powdery mildew (*Uncinula necator*). DPX-LEM17 also controls black rot (*Guignardia bidwellii*), a serious foliar disease in some areas. When used in programs with other fungicides (sulfur, QoI's, and DMI's), DPX-LEM17 will be an effective tool for managing resistance risks and ensuring continued utility of all fungicide classes.

Controlling foliar and soilborne diseases of peanuts with the new fungicide DPX-LEM17

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Phytopathology 99:S42

Growers in the U. S. have a difficult challenge to effectively control both the numerous foliar as well as soilborne fungal diseases that affect peanuts. DPX-LEM17, a new fungicide from DuPont, currently is being developed for use on peanuts, apples, grapes, stonefruit and many other crops. DPX-LEM17 offers a biochemical site of action different from most commercially available fungicides, broad-spectrum control of both foliar and soilborne diseases, strong preventive as well as curative activity and ultimately improved peanut quality and yield. In peanuts, foliar applications of DPX-LEM17 to control soilborne diseases like *Sclerotium rolfsii* and *Rhizoctonia solani* also control leafspot complex and other important foliar diseases like peanut rust. DPX-LEM17 used in programs with other fungicides like chlorothalonil, azoxystrobin, and tebuconazole reduces the risk of resistance development for all fungicide classes as well as providing growers with flexibility in efficacy and costs of their disease management programs.

Forecasting and management of hop downy mildew

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Downy mildew (*Pseudoperonospora humuli*) is one of the most destructive diseases of hop. A degree-day model was developed to predict emergence of shoots systemically infected with the pathogen in western Oregon, and quadratic discriminant function models were developed to classify 24-h or 48-h periods as favorable for disease development. In three years of validation, fungicide applications timed by a degree-day model significantly enhance control of downy mildew as compared to routine fungicide applications. Cut-points associated with the discriminant models that minimized the average costs associated with disease control and crop loss due to classification errors were determined using estimates of economic damage during vegetative development and on cones near harvest. Use of the discriminant models was estimated to reduce average management costs during vegetative development when disease prevalence was less than 0.1 to 0.3, depending on the model and the economic impact of a false negative prediction. The value of these models in management decision making appears to be greatest when disease prevalence is relatively low during vegetative development, which generally corresponds to the normally drier period from late spring to mid-summer in the Pacific Northwestern U.S.

Comparative genomics of *Aspergillus flavus* and *A. oryzae* revealed nearly identical genomes but differences in gene expression

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Comparative analysis of *Aspergillus oryzae* and *A. flavus* genomes revealed striking similarity between the genomes of these two species. To further analyze the genomes of these two species, we used array based comparative genome hybridization to compare the genomes of three strains for each species. In this analysis we found only 43 and 129 genes unique to *A. flavus* and *A. oryzae*, respectively. Further, only 709 genes were identified as uniquely polymorphic between the two species. We hypothesized that while these two species have very similar genomes, the expression of the genes differs because they occupy different ecological niches. *Aspergillus flavus* is an important mycotoxin producing plant pathogen whereas *A. oryzae* is used in food fermentation and generally regarded as safe. Expression was compared using wheat bran solid state cultures, prepared similar to *A. oryzae* koji propagation, as well as field-inoculated developing maize kernels, representing the *A. flavus* lifestyle as an opportunistic plant pathogen. Our results showed that despite the high-level of genome similarity there is a relatively large set of genes (15–20% of the predicted genes) differentially expressed on either substrate. In addition, we observed that *A. flavus* is highly enriched for expression of secondary metabolism genes, including mycotoxins such as aflatoxin, regardless of substrate.

Cisgenic approach to disease resistance in Apple

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Phytopathology 99:S42

Apple fruits should respond to the requirement of health enhancing aspect being uncontaminated from undesirable chemicals. However apple is grown as intensive monoculture (vegetative propagation) and therefore vulnerable to a range of diseases which have to be controlled by intensive application of fungicides contrasting with the health and environmental friendly image. Classical breeding has introgressed the scab (*Venturia inaequalis*) resistance gene Vf from a wild *Malus* source and created a relevant number of scab resistant apple cultivars (Topaz, Ariwa, Florina). The limited commercial success of such cultivars is due to the fact that through breeding, always new cultivars are created with organoleptic different characteristics not familiar to the consumer. The DNA-recombinant technology allows introducing traits without changing cv. characteristics. Incorporation of genes from not crossable donors (bacteria fungi insects other plant species) is highly controversial. We cloned and introduced the *HcrVf2* gene from *M. floribunda* in popular commercial cultivars (Gala, Elstar) with its own promoter and the selectable marker *nptII*. In second step the selectable marker gene is eliminated. The final product is a plant carry only *Malus* genes and is defined as cisgenic and not transgenic. A further advantage is that the selection system can be routinely reused to introduce rapidly further genes.

Study on the mixed infection of potato viral agents in Eastern Azerbaijan province of Iran

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Phytopathology 99:S42

Combination and distribution of viral agents in potato growing plantations are largely depend on the seed origin and population of viral vectors. The aim of this study was to determining the combination of important potato viruses in mixed infection of potato plants particularly newly emerging PVA and PVM viruses. Toward this aim during the year 2007 to 2008, a total of 294 symptomatic potato leaf samples showing virus like symptoms were collected from the potato field of Eastern Azerbaijan province of Iran. Samples were tested by DAS-ELISA using polyclonal antibodies for testing the presence of most important potato infecting viruses A, Y, X, S and M. In total, 250 samples (85%) were identified as infected in which 91 samples (36.4%) were infected to PVA, 55 samples (22%) to PVM, 45 samples (18%) to PVS, 67 samples (26.8%) to PVX, and 79 samples (31.6%) to PVY. Out of these infected samples from which 67.6% were infected by single virus and 32.4% showed multiple viruses, 79 samples (31.6%) were infected with two viruses and 0.8% with tree or more viruses. PVA and PVY were detected mostly in multiple infection. In 43.9% of the cases PVA was found as single infections, 53.8% as double infections and 2.19% as multiple infections. For PVM it was 56.3% as single infections, 40% as double infections and 3.63% as multiple infections respectively. Results of this study indicate to the necessary of

monitoring the mixed infection of viral agents in potato fields considering to the newly emerging viruses.

Study of genetic variation of different *Cauliflower mosaic virus* isolates infecting canola plant in Iran

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Cauliflower mosaic virus (CaMV) is a type member of the genus *Caulimovirus*, from *Caulimoviridae* family. This virus is one of the most destructive viral diseases. The goal of this study was to detect the CaMV from canola plants. To do so, a number of 560 leaf samples showing various virus-like symptoms was collected from Varamin (Tehran province), Ghazvin and Takestan (Ghazvin province), Shahrekord (Chaharmahal-e-Bakhtiari province), Sari (Mazandaran province), Moghan (Ardabil province), Naghadeh (Azarbayejan-e-gharbi province) and Shiraz (Fars province). Using DAS-ELISA method, samples were tested by polyclonal antisera (DSMZ. Inc.) for the presence of CaMV. According to the results obtained by DAS-ELISA method, it is revealed that the above mentioned provinces are infected with the CaMV 38.4%, 36.8%, 43.3%, 31.8%, 66.6%, 52.3% and 38.2%, respectively. Using PCR molecular method and specific primers designed for the initial sequence of the ORF V of the viral genome sequence, presence of the CaMV was confirmed for the ELISA positive-tested canola plants. All infected tested samples amplified a 840-bp fragment in PCR reaction. The amplified fragments of four isolates have first been sequenced and then aligned with the corresponding data available for other CaMV isolates in NCBI. Phylogenetic analysis revealed that all these Iranian isolates together with two NCBI isolates (Cabb-D/H, Xingjiang) were categorized in one cluster while all other CaMV isolates from NCBI were categorized in a separate cluster.

Assessment of the root colonizing fungus *Sebacina vermifera* for drought tolerance in switchgrass (*Panicum virgatum* L.)

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Drought is one of the most important abiotic constraints of crop production worldwide. Symbiotic microbes play an important role in plant response to various biotic and abiotic stresses. *Sebacina vermifera* is a root colonizing fungus reported to have growth enhancement effects on barley (*Hordium vulgare*), coyote tobacco (*Nicotiana attenuata*) and switchgrass (*Panicum virgatum*). Newly germinated seedlings of switchgrass cultivar Alamo were either co-cultivated with *S. vermifera* or mock treated for three weeks in modified PNM culture medium. Seedlings were subjected to a mild drought treatment for two weeks. Preliminary results demonstrated that co-cultivated seedlings established earlier and grew faster than seedlings receiving mock inoculations. At the end of two weeks, all mock inoculated seedlings withered and died whereas co-cultivated seedlings were growing normally. A large scale experiment to examine the role of *S. vermifera* on drought tolerance in switchgrass is underway. Effect of switchgrass and *S. vermifera* co-cultivation on fresh weight, chlorophyll content, photosynthetic efficiency and drought stress related genes expression will be presented and discussed.

Distribution of *Aphanomyces euteiches* race 1 and race 2 affecting alfalfa in Wisconsin and southeast Minnesota soils

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Aphanomyces euteiches is a major yield-limiting root rot pathogen of alfalfa in Wisconsin (WI) and Minnesota (MN). Two races of the pathogen (R1 and R2) have been identified based on performance of alfalfa cultivars with varying resistance to the pathogen. The objective of this research was to determine the prevalence and distribution of *A. euteiches* R1 and R2 in WI and southeast MN. A race-specific bioassay was performed on soils (n = 255) collected from alfalfa-producing counties in WI (n = 227) and MN (n = 28). Geographic Information System (GIS) was used to map the results in relation to soil type and rainfall information. Spatial statistical analyses were performed to investigate patterns of pathogen distribution. One-third (n = 75) of WI soils were infested with *A. euteiches*, of which 80% were positive for R2 and 20% for R1. Soils positive for *A. euteiches* in MN (n = 17) were primarily infested with R2 (~95%) as opposed to R1 (5%). Spatial analyses showed significant geographic clustering of R1 and R2. The predominance of R2 in WI and MN indicates a shift in the population from R1 to R2 compared to previous surveys. Approximately 14% of the soils assayed showed significant differences in the performance of two different R2-resistant cultivars. This may indicate the presence of additional races of *A. euteiches* in WI and MN, and identify the cause of recently observed failures in previously effective *A. euteiches* resistant cultivars.

The Doctor of Plant Health: A new interdisciplinary program for plant health practitioners

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Individuals with integrated knowledge and management skills are needed to deal with the complex and frequently interacting challenges to plant health. To meet this demand for plant professionals, the Doctor of Plant Health (DPH) program has been developed at the University of Nebraska-Lincoln, Institute of Agriculture and Natural Resources. The program is the second of its kind and designed similar to the Doctor of Plant Medicine program at the University of Florida. This degree is for students interested in a successful career as a plant health practitioner to address these complex needs. Plant health practitioners have a broad interest in plant sciences and the microbes, arthropods, and environmental conditions that affect the growth and production of healthy plants. Emphasis is on the prevention, diagnosis and management of both biotic and abiotic plant health challenges. The curriculum is broad-based, but students may emphasize crop or plant areas such as field crops, ornamentals, specialty crops, turfgrass, landscapes, or other professional interest areas, including regulatory or business management. Students completing the program will have career opportunities in industry, crop consulting, government, extension, and other private practice. Industry and government, both local and national, have indicated a desire to hire graduates with this type of training.

Occurrence of *Phytophthora rubi* and *Pratylenchus penetrans* in northwestern Washington red raspberry fields

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The Pacific Northwest of the US encompasses 90% of processed raspberry acreage nationwide. The duration of harvestable plantings has declined from >10 to approximately 5 yrs. Root damage by *Phytophthora rubi* (Pr) and *Pratylenchus penetrans* (Pp) has been associated with this decline, but soil characteristics that promote these pathogens are not well understood. Ten fields with root rot symptoms were sampled (10 sites per field; 10 soil cores (15-cm deep) per site) within Skagit and Whatcom Counties in October 2008. Soil samples were sieved (2-mm diam.) and root fragments were collected. Root sections (1-cm) were surface-sterilized and cultured on P_5ARP agar medium to recover Pr. A subsample of root sections was also evaluated by ELISA (10 sites/field) and PCR (3 sites/field) for presence of *Phytophthora* spp. and Pr, respectively. Population densities of Pp were determined from root and soil samples. A composite soil sample from each field was sent to a commercial laboratory for chemical analysis. All fields had sites that were positive for *Phytophthora* spp. (30–100% of sites per field) and seven fields were found to have excessively high (>1,000 g/root) levels of Pp. Sampled fields had a wide range in pH (4.2 to 6.8), organic matter (3.3–8.7%) and available nitrate (2–145 ppm). Further analyses are in process to confirm the presence of Pr and understand the contribution of these pathogens to raspberry root rot and decline.

Incidence of *Leveillula taurica* on onion and expansion of its host range to native plant species in the Treasure Valley region of Idaho and Oregon

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Leveillula taurica (anamorph =Oidiopsis sicula), a polyphagous powdery mildew fungus with a host range of 75 plant families has been known to occur in the Pacific Northwest since the 1980's. Onion powdery mildew [OPM] was observed from 2006 to 2008 in Treasure Valley [southwestern Idaho and eastern Oregon] on commercial onion cultivars. Each year, OPM occurred on few onion cultivars, at a very low disease incidence, a few weeks prior to harvest. The teleomorph was not observed. Search for alternative sources of inoculum in the vicinity revealed the following new hosts: *Cleome hassleriana, C. lutea, C. serrulata, Sphaeralcea coccinea, S. grossulariifolia, S. parvifolia, Astragalus filipes, and Penstemon speciosus.* Each species supported abundant epiphytic hyphal growth, conidial production and

formation of chasmothecia. ITS sequence analysis confirmed the hypothesis that the same strain of *L. taurica* attacked onion and these additional hosts. Temporal patterns of occurrence of symptoms and signs suggested that the fungus jumped from senescing hosts of one species to other susceptible species through the growing season. The present study suggests that *L. taurica* expanded its host range to these new hosts and likely will become an endemic pathogen in the region. The role of overwintering chasmothecia on native plants in establishing infections on onions and other crops warrants further study.

The Erysiphales Database
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The order Erysiphales (powdery mildew fungi) is comprised of about 650 species, including many destructive plant pathogens. They remain poorly studied in many parts of the world including North and South America, Africa, and parts of Asia. The International Powdery Mildew Project promotes the ongoing work of cataloging the world's species. The Erysiphales Database (www.erysiphales.wsu.edu), a part of the Project, provides users with tools to identify species using morphological features, hosts, geographical distributions, and DNA sequences. It currently includes 644 species. It is a relational database, written in SQL, in which queries are made using drop-down menus to specify search criteria. It includes an implementation of BLAST, enabling users to search against DNA sequences. Rather than duplicating all Erysiphales DNA sequences in GenBank or other repositories, the Database includes exemplar sequences from species for which such data are available. Reports provide lists of species matching search criteria, summaries of characteristics, and links to online taxonomic publications viewed within seconds or minutes of beginning a database query. Streamlined data entry accelerates adding new information, and involvement of international taxonomic authorities facilitates curating the database. Combined with simplicity of use, these features offer compelling advantages over traditional taxonomic literature.

***Fusarium verticillioides* genes conferring xenobiotic detoxification**
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Phytopathology 99:S44

Phytochemicals, microbial metabolites, and agrochemicals can individually or collectively impact the diversity and frequency of microbial species occurring in agricultural field environments. Resistance to such chemicals by plant pathogenic fungi is common and potentially devastating to crop yield and value because those fungi may ultimately dominate the overall fungal community. The mycotoxigenic *Fusarium verticillioides* (*Gibberella moniliformis*) is such a fungus commonly associated with corn worldwide, causing ear rot and contaminating corn kernels with the fumonisin mycotoxins. The dominance of *F. verticillioides* in corn field environments may be due in part to its ability to metabolize phytoprotectants produced by corn. The benzoxazinoids and benzoxazolinones are broad spectrum allelopathic, antimicrobial, and anti-herbivory compounds, yet *F. verticillioides* can rapidly biotransform these phytochemicals into non-toxic malonamic acid metabolites. To better understand the genetics and chemistry of this metabolic process, genomic tools were utilized to identify genes essential for the biotransformation activity. Genes within two clusters conferred metabolic tolerance to 2-benzoxazolinone (BOA), one of the corn phytoprotectants. Gene deletion analyses are underway and may provide insight into novel metabolic resistance mechanisms against a range of azole and arylamine compounds, including fungicides.

Spread, genetic variation and methods for the detection of *Puccinia kuehnii*, the causal agent of sugarcane orange rust
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Phytopathology 99:S44

Sugarcane is susceptible to infection by two rust pathogens, *Puccinia melanocephala* and *P. kuehnii*, causing brown and orange rust, respectively. Orange rust of sugarcane was first reported in the Western hemisphere in Florida in July 2007. The pathogen was found to be distributed widely throughout the sugarcane growing area of Florida and subsequently has been found to be widespread among Central American and Caribbean sugarcane growing areas. The identity of *P. kuehnii* was confirmed using morphological characters and sequence analysis of portions of rDNA. The rDNA sequences from isolates from regions of South East Asia and Australia, where the pathogen has been prevalent historically contained a single nucleotide polymorphism with two alleles. Only one of these alleles was detected in isolates from the newly reported areas in the Western hemisphere suggesting a single introduction to the region. Real-time PCR methods for the detection and quantification of *P. kuehnii* DNA and the discrimination of the two alleles and from the closely related sugarcane rust pathogen *P. melanocephala* are presented. These rapid methods for identifying the pathogen will allow monitoring of the spread of *P. kuehnii* to parts of the Western Hemisphere under threat from infection.

Control of common bunt of wheat under field conditions by seed and in furrow treatments with the biofumigant fungus *Muscodor albus*
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Recent in vitro studies demonstrated that volatile organic compounds produced by *Muscodor albus* completely inhibited the germination of *Tilletia tritici* teliospores, the cause of common bunt (CB) of wheat. Field experiments were conducted to evaluate the potential of biofumigation with *M. albus* for control of CB as a pre-plant seed treatment and an in furrow soil treatment. As a seed treatment, desiccated rye grain culture of *M. albus* was ground into powder, moistened, and applied at a rate of 125 mg per g of teliospore-infested wheat seed. The seed was held 4 d and then planted in test plots. For furrow application, the *M. albus* culture was cracked into particles and applied in furrow at the rate of 4 g/m with infested wheat seed at planting. Treatments were evaluated in four replicate 2 m rows on two planting dates during two seasons. In the first year, treatments in the first seeding date reduced CB from 44% diseased spikes in untreated controls to 12% and 9% in seed and in furrow treatments, respectively, while in the second seeding date, CB was 6% in controls and 0% in both treatments. In the second year CB in the first seeding date was reduced from 8% in controls to 0.5% and 0.25% for seed and in furrow treatments, respectively, and from 0.75% in controls to 0% in both treatments in the second seeding date. *M. albus* has potential for CB control in organic wheat production where options for managing this disease are very limited.

Grapevine viruses detected in wild grapes (*Vitis californica*)
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Phytopathology 99:S44

Wild grapevines (*V. californica* and *Vitis* spp hybrids) and 19 other plant species in the natural environment near vineyards in Napa Valley, California were tested by RT-PCR and Taqman PCR for 13 viruses and phytoplasmas. *Grapevine leafroll-associated virus-2* (GLRaV-2), *Grapevine leafroll-associated virus-3* (GLRaV-3), *Grapevine virus A (GVA), Grapevine virus B (GVB)* and *Grapevine rupestris stem pitting-associated virus* (*GRSPaV*) were detected in wild grapevines. GLRaV-2 and GVB were detected in *V. californica* 'Roger's Red', an ornamental grapevine popular for its red leaf color in the fall. This is the first report of these viruses in wild grapevines. DNA fingerprinting is in progress for further grapevine species identification. These findings have implications for the control and spread of leafroll viruses.

Rapid spread of leafroll disease in Cabernet Sauvignon grapevines in Napa Valley, California
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Leafroll disease symptoms were visually assessed and mapped in a 2.9 hectare Cabernet Sauvignon vineyard in Napa Valley, California for five years. Vines were individually rated; a subsample of 75 vines with and without symptoms was ELISA tested for *Grapevine leafroll associated virus* (GLRaV)-1, -2, -3, and -4. Results of the ELISA testing found only GLRaV-3 in the samples from symptomatic vines. The visual symptom ratings were very accurate, although not in perfect agreement with the ELISA testing. Percent of vines rated positive for leafroll symptoms was 23.3%, 41.2%, 45.8%, 49.8, and 66.1% from year 1 to year 5 and spread was mainly in the direction of the rows. In the year that 45.8% of the vineyard was diseased, grapes from non-symptomatic vines were harvested several weeks earlier than grapes from diseased vines and used for reserve-quality wine. The fruit from the diseased vines did not meet that standard. Grape mealybug (*Pseudococcus maritimus*), known to be a leafroll virus vector, was observed in this and surrounding vineyards for many years, but usually at low populations that were not considered to be of economic importance. The owner is now faced with the need to replant this block after only 15 years due to the high incidence of leafroll. This is the first documentation of significant and rapid field spread of leafroll disease in a California vineyard.

Effect of soil temperature and plant age on root rot and foliar symptoms of soybean sudden death syndrome

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SDS is favored by planting in cool soils, but occasionally epidemics can be severe even when delayed planting occurs in warmer soils. We previously reported that foliar symptoms depend on plant age at time of infection and on xylem colonization needed to translocate pathogen toxins to the leaves. In this study we determined the effect of soil temperature and plant age on root rot and foliar symptoms. Soybeans were grown in mini-rhizotrons immersed in water baths at 17, 23 and 29°C. Plant subsets were inoculated at 0, 3, 7 and 13 days after planting (DAP) by soil drenching with a conidial suspension. Root growth, root rot and foliar severity were evaluated for 50 days after inoculation. All inoculated plants developed root rot, but severity was greater at cooler temperature and on plants that were younger when inoculated. Inoculation at planting resulted in severe foliar symptoms at all three temperatures. However, plants inoculated at 3, 7 and 13 DAP developed less severe foliar symptoms at higher temperatures. This suggests that accelerated root growth in warm soils reduces the window of opportunity for xylem colonization needed for foliar symptoms. These results may explain why delayed planting can reduce risk of SDS, but may not prevent severe epidemics if infection occurs shortly after planting.

Occurrence and relative incidence of viruses infecting *Capsicum annuum* in Chihuahua, Mexico

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Phytopathology 99:S45

A survey was conducted to determine the incidence of *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TbMV), *Potato virus Y* (PVY), *Pepper mild mottle virus* (PMMoV), *Pepper mottle virus* (PepMV), *Tobacco etch virus* (TbEV), and *Tomato bushy stunt virus* (TBSV) on *Capsicum annuum* of the Central South region of Chihuahua State. A total of 129 leaf samples were collected in four zones of the Central South region of Chihuahua, Mexico and tested by the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using specific polyclonal antibodies. The highest virus incidence among the surveyed zones was recorded in Meoqui-Estación Consuelo, followed by Meoqui-Lomas del Consuelo, Delicias-Naica, and Delicias-Presa Francisco I Madero. Incidence of viruses in decreasing order was CMV (50.4%), followed by TMV (25.6%), AMV (11.6%), TBSV (8.5%), TEV (8.5%), PMMoV (7.8%), PVY (4.6%), and PepMV (2.3%). CMV, AMV, TMV and PVY were detected in the four zones tested but CMV was predominant. PMMoV and TBSV were detected in three zones, while TEV and PepMV were found only in two and one respectively. Although predominantly infected plants with one virus (38%) were detected, multiple viruses were also found by plant. This is the first report of viral occurrence on pepper plants in Chihuahua State, Mexico.

Variation within the NA1 clonal lineage of *Phytophthora ramorum* from US nurseries reveals migration pathways

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Populations of recently introduced plant pathogens that are limited to clonal reproduction may contain little genetic diversity, yet rapidly mutating genetic markers such as microsatellites can exhibit variation within these populations and be used to infer evolutionary processes and migration patterns. The sudden oak death pathogen, *Phytophthora ramorum*, is clonally reproducing and exotic to North America and Europe, where it has been found to infect a wide range of species including popular ornamentals. In US nurseries, the pathogen has moved from West Coast states eastward via shipments between nurseries. We genotyped variable microsatellite loci in *P. ramorum* isolates collected between 2004 and 2007 from nurseries in 19 states. For the NA1 lineage, which was present in all 19 states, we found that a dominant genotype (23% of the sample) was present in 14 of the states. Furthermore, NA1 isolates clustered into two groups, one containing isolates from Connecticut, Oregon, and Washington and the other isolates from California and the remaining states. We inferred from this pattern a major migration pathway originating from California and distinct migration events from the Pacific Northwest. This is consistent with the findings of USDA APHIS trace-forward and trace-backward investigations.

Manipulating seeding date to minimize clubroot (*Plasmodiophora brassicae*) damage in canola and vegetable brassicas

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Phytopathology 99:S45

Clubroot of crucifers caused by *Plasmodiophora brassicae* (Woronin) is endemic on vegetable Brassicas in many parts of eastern Canada, and threatens canola (*Brassica napus* L. and *B. rapa* L.) production across large areas of the Canadian prairies. Options for control, such as durable sources of resistance, are limited. A recent study on short-season Brassica vegetables indicated that timing plantings to avoid warm conditions in the 10 days before harvest reduced symptom severity. Studies were initiated to examine the impact of seeding date on clubroot incidence and severity. Trials on *B. napus* were seeded in early, mid and late May in 2007 and 2008 near Edmonton, Alberta Canada. Also, a field trial on Shanghai pak choy (*B. rapa* subsp. *chinensis* var. *communis*) was seeded near Bradford, Ontario, Canada in May, June, July, August and September of 2007 and 2008. Early seeding reduced symptom severity on canola by 10–50% and increased yield by 30–58%. Plantings of pak choy in June and July had 64–87% clubroot incidence. Seeding in May, August or September resulted in harvests during cool conditions and little or no clubroot (0–15% incidence). Clubroot severity showed a similar pattern of response. Additional trials are underway to confirm that clubroot damage might be reduced by selecting appropriate seeding dates.

***Rhizoctonia solani* AG-5 is associated with root rot of field pea in North Dakota**

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Phytopathology 99:S45

Rhizoctonia solani Kühn is known to cause stem and root rot of field peas (*Pisum sativum* L.) and the anastomosis group commonly related to this condition in peas is AG-4. However, disease surveys conducted during the summers of 2007 and 2008 in major pea growing counties in North Dakota led to the isolation of *R. solani* AG-5 isolates in addition to AG-4. These were obtained from the brown discolored lesions on the roots and lower base of the stems of the pea samples. AG groups of the isolates were confirmed using anastomosis pairing with testers and by comparisons of ITS sequences of these isolates with those from members of all common *R. solani* AG groups. Three AG-5 isolates were tested in the greenhouse for pathogenicity on two pea cvs, yellow 'Admiral' and green 'Striker', soybean (cv. Barnes) and dry bean (cv. Montcalm) using inoculated wheat grains for infection. Non-inoculated plants and those inoculated with wheat grains infected with an AG-4 tester isolate served as negative and positive controls respectively for all the hosts. When the roots were evaluated for disease severity, distinct reddish brown lesions were observed on the hypocotyls and roots in the infested pots for all three isolates and three crops. Koch's postulates were established and initial results of pathogenicity tests suggest that these AG-5 isolates are comparable to AG-4 in their aggressiveness on field peas. To the best of our knowledge this is the first report of AG-5 on field pea.

Relative gene expression of *Citrus tristeza virus* isolate FS627 and its aphid transmitted subisolates by multiplex real time PCR

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Phytopathology 99:S45

A relative quantitative multiplex PCR assay was developed for *Citrus tristeza virus* (CTV) isolate FS627, a mixture of T30, T36 and VT genotypes. The method also was used to determine the expression of the various genotypes in aphid transmitted (AT) subisolates. CTV isolate FS627 causes decline of sweet orange on sour orange rootstock, causes moderate vein clearing in Mexican lime, no stem pitting in either sweet orange or grapefruit, and no seedling yellows. AT subisolates were produced using the brown citrus aphid *Toxoptera citricida*. The available molecular genotypic markers can identify the genotype of the isolates but cannot quantify each in mixtures. We developed a relative gene expression assay in a multiplex format in real-time PCR using specific primers and probes for all three genotypes. Gene expression was carried out using the coat protein region of isolate T36 and the 5′ end, polymerase regions and inter-domain regions of all three genotypes listed above. The multiplex real-time PCR efficiency was tested for all primers and probe sets. Regression analysis showed that the assays are highly efficient and values falls between $R^2 = 0.995$ to 0.999. The $2^{-\Delta\Delta Ct}$ method was used to analyze the relative changes in gene expression. Among the three genotypes, in CTV isolate FS627 and its AT subisolates the T30 genotype was

expressed higher compared to the T36 and VT genotypes. The relative changes in gene expression of T36 and VT isolates are discussed.

Use of online narrated presentations to provide advanced training in plant pathology to Master Gardener volunteers
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Master Gardener volunteers are often the first university trained individuals consulted by home gardeners experiencing plant disease problems in their landscape. These volunteers are required to take a course on basic horticulture including a single lecture on plant pathology. Few have advanced training or experience in plant pathology. As a result gardeners may or may not have their questions answered or be directed to the appropriate university resources. Time and travel costs are often limiting factors in providing advanced training in plant pathology to Master Gardeners. In this study, six online voice recorded power point presentations on advanced plant pathology topics were created using Adobe Presenter. A group of 62 Master Gardeners participated in the training and completed a survey about their learning experience. Questions were included to evaluate the effectiveness of features available in the Adobe Presenter software. Overall the Master Gardeners rated the trainings very highly; 35% reported learning more from the online training than from a live speaker and 59% reported learning equally well from the online training as from a live speaker. All features used to focus the learner's attention were well rated. In particular 90% of volunteers reported that the voice recorded narration improved their learning and 97.5% reported that quiz questions embedded in the presentations increased their understanding and confidence of the subject matter.

Soil drenches of imidacloprid, thiamethoxam and acibenzolar-S-methyl for induction of SAR to control citrus canker in young citrus trees
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Phytopathology 99:S46

Soil application of the systemic insecticide imidacloprid produces season-long control of citrus canker caused by *Xanthomonas citri* sbsp. *citri*. Imidacloprid, a neonicotinoid, breaks down in planta into 6-chloronicotinic acid, and induces systemic acquired resistance (SAR). Soil drenches of imidacloprid (Admire, Bayer Crop Science), the neonicotinoid thiamethoxam (Platinum, Syngenta Crop Protection), and the SAR inducers acibenzolar-S-methyl (Actigard, ASM) and isonicotinic acid, were compared with copper hydroxide (CH, Kocide 3000) as a foliar spray at 21 da interval for canker control on foliage of 3-yr old 'Ray Ruby' grapefruit trees. Each set of foliar flushes was rated for the percentage of the leaves with canker lesions. Despite above average rainfall and a tropical storm event, all treatments significantly reduced incidence of foliar canker. Spray of CH was the most effective treatment, but as a group, soil drenches of SAR inducers also reduced disease incidence to varying degree depending on rate and frequency of application. Among the treatments, 4 applications of ASM at 2 oz/A at 60 da interval or a single application of thiamethoxam at 11 oz/A at the beginning of the season equaled the control activity of imidacloprid. Although the level of SAR control did not match that of the 21 interval CH treatments, activity persisted season long.

Dispersal and movement mechanisms of *Phytophthora capsici* sporangia
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Phytopathology 99:S46

Understanding the mechanisms of *Phytophthora capsici* sporangia dissemination is paramount to understanding epidemic initiation and development. Direct laboratory observations showed *P. capsici* sporangial dispersal occurred in water with capillary force, but did not occur due to a reduction in relative humidity or wind. Atmospheric sporangial concentrations were monitored under field conditions using a volumetric spore sampler in a commercial cucurbit field and in a setting where copious sporangia were continuously available. Dispersal was infrequent (0.7% of total h monitored) during sampling in a commercial field, and 14 sporangia were detected during a 7.5-week sampling period. When sporangia were continuously available, dispersal occurred in 4.6% of the h sampled, and 438 sporangia were impacted onto tapes during a 7-week sampling period. Significant positive correlations ($p < 0.0001$ and $p = 0.0010$) between rainfall and atmospheric sporangial concentrations were found at both sites. Sporangial concentrations were negatively associated with vapor pressure deficit ($p = 0.028$) and positively associated with average wind speed ($p < 0.0001$) in the setting where sporangia were continuously available. Wind speed was not measured in the commercial field. Hence, both direct laboratory observations and volumetric spore sampling indicate that dispersal of sporangia via wind currents is infrequent, and sporangia are unlikely to be naturally dispersed among fields by wind.

Identification and activity of silicon transporters from horsetail (*Equisetum arvense*)
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Phytopathology 99:S46

The exogenous supply of silicon (Si) to plants is reported to confer beneficial effects against biotic and abiotic stresses including a wide number of fungal diseases. However, plants vary greatly in their Si content, and there appears to be a direct correlation between Si absorption and benefits. Recently, Si transporters were identified in rice, maize and barley, three species known to accumulate high concentrations of Si. The presence or absence of these transporters may explain the disparity in the Si content of plants and thus their ability to benefit from Si supply. Horsetail is a valuable model for Si absorption, because it has one of the highest Si concentration in the plant kingdom. Our objectives were to identify horsetail Si-transporters and to compare their activity with transporters from other plant species. Horsetail Si transport genes were identified by PCR using primers designed from conserved sequences identified in other species. Complementary RNAs coding for rice, wheat and horsetail Si transporters were injected in *Xenopus laevis* oocytes, an heterologous expression system. Oocytes were incubated in a Si-enriched medium, and Si content was quantified by atomic absorption spectrometry. These results provide valuable information about the biochemical basis explaining the differential affinity of plants to absorb Si. In turn, this information could be exploited to optimize the use of this element in agriculture for natural and prophylactic control of plant diseases.

Effect of post-harvest residue on ratoon crops of sugarcane infected with *Sugarcane yellow leaf virus*
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Phytopathology 99:S46

Sugarcane yellow leaf virus (SCYLV) is a luteovirus that causes yellow leaf of sugarcane. Previous studies in Louisiana focusing on the effect of post-harvest residue found that retention of the residue often reduces yield of subsequent ratoon crops. A field experiment to determine the potential interaction of SCYLV and residue retention was arranged in a split-plot design. Main (treatment) plots had either plants infected with or free of SCYLV, and the subplots were residue removal or retention. The residue treatments were applied following the harvest of the plant-cane crop (the first annual harvest) and the first-ratoon crop (second annual harvest) in plots of sugarcane cultivar LCP 85-384. Cane yield (Mg/ha) of SCYLV-infected plants was 10.7% less than the yield of the SCYLV-free plants. Among infected plants where the residue was retained, the yields were 18.1% less than the yield of the control plants; while among plants where the residue was removed, the yields of SCYLV-infected plants were 5.8% less than that of the control plants. Yields of cultivar LCP 85-384 in this study did not differ between SCYLV-free plants where residue was removed and those where the residue was retained. The results, however, indicates that yield losses associated with SCYLV is compounded when post-harvest residue is retained, thus removal of residue is essential for optimal yields when cane is infected by SCYLV.

Selection of plant-defense peptides from phage-display libraries directed towards *Gibberella zeae* for control of head blight of wheat
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A priority among wheat farmers is to protect their crop against head blight caused by the fungus, *Gibberella zeae*. Robust host resistance is limited and the most common management strategy is the application of fungicides. Defense peptides, derived from phage–display libraries could provide an alternative means of control. In past work, we discovered peptides that inhibit the development of oomycetous and basidiomycete plant pathogens. These peptides do not kill the inoculum, but rather, delay their development sufficiently to slow pathogenesis. We are now studying the potential for developing peptides that will protect wheat from blight by inhibiting *G. zeae* development. Our initial experiments have focused on selecting peptides from a phage-display library with high binding affinity for germinating *G. zeae* ascospores (germlings) that serve as primary inoculum. A phage-display library with a population of 10^{13} clones, representing 2.7×10^9 random peptides, was incubated with *G. zeae* germlings. Phage-peptide clones recovered after three rounds of screening were evaluated for inhibition of germling development. The most promising candidate defense peptides are

being further characterized for their inhibitory potential in the absence of the phage particle. At that point, we will assess the best peptides for their ability to protect flowering wheat plants from infection by *G. zeae*. Identified peptides will also be used as a tool to study the surface organization of the germling hyphal tip.

Evaluation of the effects of swathing versus straight combining on FHB DON in barley at Fargo, ND 2007
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Phytopathology 99:S47

Fusarium head blight (FHB) has frequently reduced the quality of barley grown in the Midwest for the last decade, due to fungus infected kernels and the occurrence of deoxynivalenol (DON) mycotoxin. Barley is susceptible to *Fusarium graminearum* infection and DON formation from head emergence until harvest, as the fungus may colonize the outside of the barley kernel if favorable dew periods for fungal growth occur. Because barley often matures unevenly in a field, many feed and malt barley producers use swathing (windrowing) to accelerate crop maturity and drying. However, barley in a swath may be exposed to additional high humidities if rainfall occurs, possibly favoring further fungal growth or DON production. To test if swathing affects DON production, additional misting irrigation was applied after Feekes 11.3 to swathed plots and to plots left for straight cutting. Plots were arranged in a factorial combination and treatments were applied to two rowed (Conlon) and six rowed (Robust) barley cultivars. The swathing treatments or the straight cutting had no effect on DON, but straight-cut plots had significantly higher yields than swathed plots. Swathed and straight-cut plots that had added mist irrigation had significantly higher DON than those without mist added. Results indicate that post-dough (Feekes11.3) occurrence of rainfall may have a bigger influence on DON accumulation than swathing or straight-combing practice.

Integrated pest and disease management: Reducing current season spread of Potato virus Y in potato
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Phytopathology 99:S47

In recent years, Potato virus Y (PVY) has re-emerged as serious disease problems in potato production areas in many areas of the United States and eastern Canada. Asymptomatic cultivars which express mild or no symptoms when infected with PVY combined with an increase in recombinant strains of this virus prevent accurate field identification and rouging of infected plants. There is a lack of effective strategies to reduce the incidence of PVY infected plants and tubers, and there is a need to improve cost-effective methods of determining PVY levels in seed lots and further understanding the impact of current season virus infection on tuber storage and quality attributes. Limited information currently exists to document the optimal oil application conditions to limit infection of PVY during the current season. In the first year of preliminary research, we have documented significant reductions in PVY incidence using different foliar oil protectants at varying concentrations and application frequencies. This area of investigation seems extremely important towards limiting continued losses associated with asymptomatic potato cultivars in which PVY remains a challenge.

Effects of copper-based fungicides on leaf bronzing, foliar gas exchange, and fruit quality of tart cherry
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Phytopathology 99:S47

Leaf spot disease of tart cherry is caused by *Blumeriella jaapii*. Copper-based fungicides are effective for managing *B. jaapii* but their application is often associated with bronze-colored lesions on leaves. The effects of dew formation on leaf bronzing severity and the consequences for foliar gas exchange and fruit quality of tart cherry were examined. In growth-chamber studies, leaf dew formation was associated with increased bronzing severity ($P < 0.003$) after foliar applications of copper-sulfate. In 2007 and 2008, net CO_2 assimilation (A) and stomatal conductance (g_s) were measured for leaves, and soluble solids concentration (SSC) and fresh weight (W) were measured for fruits collected from mature orchard trees which had been sprayed with copper-based fungicides in addition to synthetic fungicides. Prior to fruit harvest, as bronzing severity increased, g_s decreased ($P < 0.001$) in both years, but the effects on A were inconsistent ($P < 0.01$ in 2007 and $P = 0.08$ in 2008). After fruit harvest, leaf bronzing severity had little impact ($P > 0.07$) on A or g_s in studies conducted over 4 years (2005–2008). In addition, increased bronzing severity was not related ($P > 0.05$) to large reductions in SSC or W of mature fruit in 2007 and 2008. These results indicate that copper-associated leaf bronzing is detrimental to cherry leaf g_s and possibly A prior to fruit harvest, but these reductions do not appear to negatively affect fruit quality.

Parasexual recombination and migration maintain genotypic diversity in the aflatoxin-producing fungal plant pathogen *Aspergillus flavus*
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Phytopathology 99:S47

Aspergillus flavus, fungal pathogen of animals, wild plants and crops, is most recognized for producing aflatoxin, a cancer-causing secondary metabolite, which contaminates food and animal feed globally. *A. flavus* has a vegetative incompatibility system that limits hyphal fusion and subsequent gene flow between individuals belonging to different vegetative compatibility groups (VCG). Despite the economic and human health importance of developing strategies to decrease aflatoxin levels in crops using biocontrol methods, reproductive mode, dispersal patterns and genetic diversity within VCG are unknown. We analyzed 221 clone-corrected samples from three common VCG in Arizona and Texas on cotton using 24 SSR loci. Our results support the hypothesis that these *A. flavus* VCG are genetically isolated. We found high levels of genetic differentiation and no evidence of recombination between VCG, including VCG of opposite mating-type. Divergence time between pairs of VCG is estimated to range from 34,000 to 49,000 and 140,000 to189,000 years before present. This is the first study of any asexual *Aspergillus* species to find within VCG: 1) high genotypic diversity, 2) evidence of recombination, 3) indirect evidence for a parasexual cycle in natural populations, and 4) migration over large distances (>1500 km). We conclude these *A. flavus* VCG are genetically isolated, ancient, clonal lineages in which recombination and migration are important for maintaining genetic variation.

***Phytophthora*-ID.org: A web- and sequence based *Phytophthora* identification tool**
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Phytopathology 99:S47

Traditionally identification of species is accomplished by PCR amplification of the Internal Transcribed Spacer (ITS) region followed by either restriction analysis or direct sequencing and sequence alignment (BLAST search) against GenBank or other databases. Generally, identification of a species is only as good as the underlying database used. Retrieval of sequence alignments can be problematic as databases accumulate sequences that are poorly annotated. We created a web-based tool for the identification of *Phytophthora* species based on sequence data: *Phytophthora*-ID.org (URL: http://phytophthora-id.org/).*Phytophthora*-ID maintains a database of sequences that is curated to be selective for sequence accessions that come from a trusted source including published, peer-reviewed studies wherever possible. Recently described species that are not yet named in a species paper are only included as *Phytophthora* taxon "X" to indicate that these might or might not be new species. Our objective was to create a simple, yet robust site that is user-friendly.

Genetic analysis of the genes involved in the production of occidiofungin in *Burkholderia contaminans* strain MS14
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Phytopathology 99:S47

Burkholderia contaminans strain MS14 exhibits significant antifungal activities to a broad range of plant soilborne pathogens, and produces an antifungal cyclic oligopeptide, occidiofungin. The 56-kb occidiofungin synthetase gene cluster was sequenced and characterized, which revealed the presence of 17 open-reading frames (ORFs) in the gene cluster. Sequence and mutagenesis analysis indicated that occidiofungin was synthesized via a nonribosomal peptide synthetase (NRPS) mechanism. Five NRPS genes were identified in the gene cluster, designated *occA*, *occB*, *occC*, *occD* and *occE*. The gene products encoded the modules responsible for the incorporation of the eight amino acids of the occidiofungin oligopeptide. Quantitative real-time PCR analysis indicated that transcription of *occA*, *occB* and *occC* were significantly reduced when *occD* was mutated, suggesting that these four genes are organized as an operon. The *ambR1* and *ambR2* genes, which are located at the left border of the gene cluster, showed high similarity to LuxR-type regulatory genes and were involved in the production of occidiofungin. Transcription of all the ORFs identified in the region except ORF1 was regulated by both *ambR1* and *ambR2*. The functional *ambR1* gene was essential for transcription of *ambR2*, and constitutive expression of *ambR2* did not restore the phenotype of the *ambR1* mutant MS14GG44. This work has provided valuable insights into understanding biosynthesis of occidiofungin.

Pathogen of apple ring rot and its relation to the pathogen of *Botryospharia* canker of apple and pear

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Phytopathology 99:S48

Apple ring rot occurs through out the apple growing areas of China. The disease is characterized by ring rot on fruit and the wart symptom on bark. In Chinese literature, the causal pathogen was listed as *Botryosphaeria beregeriana* f. sp. *piricola*. The *Botryosphaeria* canker, which cause fruit rot and branch or shoot blight on infected trees without the symptom of wart, also occur in apple orchards in China. The pathogen of this disease was referred as *Botryosphaeria ribis* or *Botryosphaeria dothidea* in different reference. Moreover, ring rot and *Botryosphaeria* canker also occurs on pear. In this study, the pathogens of these diseases were investigated on the morphology, pathogenicity and the sequence of rDNA in the ITS region, and genes of beta-tublin and Actine. Thirty isolates collected from the tissue with symptom of above diseases respectively were used. The morphological characters of these isolates are similar and closely resemble to *B. dothidea*. Phylogenetic trees from these three genes are similar. All the isolates were clustered together with *B. dothidea* but separated from *B. ribis* and other close related species in this genus. When these isolates were inoculated on apple branches *in vivo*, wart symptom formed after 50 days. Results of this study suggest that *B. dothidea* is the pathogen of apple ring rot in China, and this pathogen can also cause the pear ring rot and *Botryosphaeria* canker of apple and pear in China.

Genetic structure of *Phytophthora infestans* populations in China indicates multiple migration events

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Phytophthora infestans causes late blight and is the most devastating disease of potato in China. Isolates (n = 100) collected from ten provinces between 1998 and 2004 were characterized. Twelve herbarium samples collected in China (1938–1982) were typed for mtDNA haplotype. The Ia mtDNA haplotype was found in initial outbreaks in 1938 and 1940 on potato and tomato. The Ib mtDNA haplotype was found later (1952,1956) on both potato and tomato and in 1982 on *S. lyratum*. Four allozyme genotypes were found. Most isolates were the A1 mating type (3-A2). A genotype from Siberia called SIB-1 (Gpi 100/100, Pep 100/100, IIa mtDNA haplotype) was identified among 72% of the isolates and was widely distributed in China in Gansu, Hebei, Heilongjiang, Inner Mongolia, and Jilin in the north and Sichuan and Yunnan in the south. A new genotype named CN-11 (Gpi 100/111, Pep 100/100, IIb mtDNA haplotype) was less frequent in south China. Three isolates were the US-1 genotype (Gpi 86/100, Pep 92 or 100/100, Ib mt DNA haplotype). Remaining genotypes (Gpi 100/100, Pep 100/100, Ia mtDNA haplotype) had unique RFLP fingerprints and were named CN-9 and CN-10. Genotype diversity was highest in Yunnan. The predominant genotype in China was SIB-1 (IIa mtDNA haplotype) and identical to those from Siberia suggesting later migration of the pathogen from Russia into China. Mitochondrial haplotype evidence suggests multiple migrations of the pathogen into China after the introduction of the Ia haplotype in the 1930's.

The *galU* gene is required for survival of *Xanthomonas axonopodis* pv. *citri in planta* and its pathogenicity

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Xanthomonas axonopodis pv. *citri* (Xac) is the causal agent of citrus canker and has a wide host range. In this study, one mutant of Xac (264D12), which had lost pathogenicity on citrus, was identified in Xac EZ-Tn5 <R6Kγori/KAN-2>Tnp transposon library. Sequence analysis and southern blot indicate that one single copy of EZ-Tn5 transposon inserted into the gene *galU* on Xac chromosome. Gene *galU* encodes UTP-glucose-1-phosphate uridylyltransferase which plays an important role in biosynthesis of polysaccharides such as lipopolysaccharides (LPS), capsular polysaccharides and exopolysaccharides (EPS). The mutant showed the similar growth rate as the wild type strain 306 (wt 306) in liquid medium but died quickly *in planta*. The plasmid pUFR053 containing the intact *galU* gene complemented the phenotype of the mutant and recovered the pathogenicity *in planta*. EPS production test showed that the *galU* mutant remarkably decreased the level of EPS production (only 6% of the amount in wild type strain). Capsule stain demonstrated the capsule of the *galU* mutant was disrupted. The *galU* mutant also shows the LPS pattern different from that of wt 306 in M9 glucose medium and less biofilm formation. Co-inoculation of *galU* mutant with wt 306 (1:1 ratio) did not help the growth of the *galU* mutant *in planta*. These data indicate that *galU* contributes to Xac survival *in planta* by producing capsule. Our results suggest that *galU* is required for the survival *in planta* and pathogenicity of Xac.

Effect of fungi-toxicants on spore germination of *Puccinia graminis* f. sp. *tritici*

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Phytopathology 99:S48

Puccinia graminis f. sp. *tritici* (*Pgt*), causal organism of wheat stem rust is one of the most important diseases of wheat in North America and other parts of the world. This has become more significant because of the evolution of more virulent race TTKS (Ug99) in Uganda, which is found to infect almost all available resistant cultivars. Management of disease by chemicals is one of the main approaches. Laboratory experiment was conducted using 4 different races (QFCS, QTHJ, RKQQ & TPMK) of *Pgt* and one field collection (collected during 2007 from rust nursery at SDSU fields-race unknown) to see the effect of seven different fungicides *viz.* Caramba (Metconazole), Folicur (Tebuconazole), Proline (Prothioconazole), Tilt (Propiconazole), all belonging to group 3 and Gem (Trifloxystrobin), Headline (Pyraclsotrobin) and Quadris (Azoxystrobin) belonging to group 11, along with control. Gem, Headline & Quadris were mixed with 10 per cent water agar @ 10, 1.0, 0.1, 0.01, 0.001 ppm while Proline and Caramba were used @ 100, 50, 25, 10, 7.5, 5.0, 2.5, 1.0 ppm and Folicur & Tilt @ 100, 50, 25, 20, 15, 10, 1.0 ppm along with one control for each fungicide. Per cent germination inhibition of spores was observed after 12 hours of plating and it was observed that EC-50 for Headline ranged between 0.001–0.0001 ppm, Gem & Quadris 0.01–0.001 ppm, Caramba 2.5–3.0 ppm, Proline 2.5–4.5 ppm, while for Folicur 15.0–20.0 ppm and Tilt 16.0–18.0 ppm depending upon the race of *Pgt*.

Infection and development of spot blotch and tan spot on timely and late seeded wheat

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Phytopathology 99:S48

Foliar blight (FB) of wheat, a complex disease caused by *Cochliobolus sativus* (Cs), and *Pyrenophora tritici-repentis* (Ptr), is an economically important disease of wheat in South Asia. There is limited information available on the effects of wheat genotypes on FB epidemic under rice-wheat cropping system. Field experiments were conducted in 2 yrs to determine infection potential and epidemic development of Cs and Ptr on timely (Nov-26) and late (Dec-26) seeded susceptible and resistant wheat genotypes. The dynamics of airborne conidia were studied by collecting plant tissues as well as using air samplers. The highest conidial concentration and maximum number of leaf infection by both pathogens were detected during the first three weeks of March in both years. Cs became predominant in early maturing susceptible wheat genotypes (Sonalika, and BL1473) during Zadok's DC 51 to 58 growth stage, while Ptr occurred after Zadoks' DC 71 growth stage. Both Cs and Ptr became predominant in resistant late maturing wheat genotypes (Milan/Shanghai#7 and NL750) before Zadoks' DC 51 and after Zadoks' DC 71 for all seeding dates. Resistant genotypes had higher disease severity when planted after Dec-26. The disease complex reduced an average of 25% grain yield in both years. The findings suggest that wheat seeding date in South Asia should be adjusted between Nov-26 and Dec-11 in order to reduce the effect of FB epidemics on grain yield.

Single sequence repeat diversity of *Mycosphaerella graminicola* populations from California and Kansas

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Phytopathology 99:S48

A total of 186 isolates of *Mycosphaerella graminicola* collected from spring and winter wheat growing in California and Kansas was assayed by polymerase chain reaction (PCR) amplification of the two mating type idiomorphs. Frequencies of the two mating type genes did not differ significantly from a 1:1 ratio for the Kansas population, whereas the ratio differed significantly from a 1:1 ratio for the California population. The Kansas population was subdivided into six geographic subpopulations and none of which differed significantly from a 1:1 ratio except one subpopulation from the Central region. These populations or subpopulations were further analyzed using 17 simple-sequence repeat (SSR) markers, and analysis of genetic diversity and gene flow between the populations or subpopulations will be discussed.

Broad-spectrum disease resistance in winter and spring wheat

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Phytopathology 99:S49

A total of 825 spring and winter wheat accessions from the USDA-ARS National Small Grains Collection were evaluated for resistance to tan spot (caused by *Pyrenophora tritici-repentis*) and Stagonospora nodorum blotch (SNB) (caused by *Phaeosphaeria nodorum*) at the seedling stage in a growth chamber during 2006 to 2008. Eighty-eight accessions exhibited resistant reaction to both tan spot and SNB. Data from the Germplasm Resources Information Network (GRIN) further suggested that 28 of the accessions also had resistance to multiple diseases of wheat including leaf and stripe rusts. Resistance gene analog polymorphism primers were used to assess the genetic relationship among the 88 resistant accessions, and accessions with similar growth habit grouped together based on the marker analysis despite differences in country of origin. Resistance to SNB and tan spot was more common in winter wheat accessions than in spring wheat accessions. These newly identified genetically diverse wheat accessions have high levels of resistance to multiple diseases and can be used in wheat breeding programs to develop resistant cultivars.

Molecular variability of *Grapevine fanleaf virus* in Washington State vineyards

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Phytopathology 99:S49

Grapevine fanleaf virus (GFLV, genus: *Nepovirus*, family: *Comoviridae*), responsible for fanleaf degeneration disease, has been documented in four different wine grape varieties in Washington State vineyards. In this study, we determined the nucleotide sequence of the genomic RNA1 and RNA2 of two GFLV isolates, designated as WAPN61 and WAPN17, from overlapping cDNA clones and compared these isolates with each other and with corresponding sequences available in the GenBank. In pairwise comparisons, the RNA1-encoded polyprotein (6855 nucleotides and 2284 amino acids) of WAPN61 and WAPN17 showed 89% and 94% identity at the nucleotide and amino acid level, respectively. The RNA2-encoded polyprotein of WAPN61 (3757 nucleotides and 1109 amino acids) and WAPN17 (3776 nucleotides and 1110 amino acids) showed 87% and 91% identity at the nucleotide and amino acid level, respectively. The RNA1-encoded polyprotein of WAPN61 and WAPN17 showed 87–89% and 93–94% identity at the nucleotide and amino acid level, respectively, with corresponding sequence of GFLV F13 isolate. The RNA2-encoded polyprotein of WAPN61 and WAPN17 showed 81–88% and 84–90% identity at the nucleotide and 86–92% and 91–96% at the amino acid level, respectively, with corresponding sequences of other GFLV isolates. Among the three proteins encoded by RNA2, the homing protein ($2A^{HP}$) of WAPN 61 was found to be more divergent than WAPN17.

Virulence spectrum to barley cultivars in *Cochliobolus sativus* from North Dakota

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Phytopathology 99:S49

Cochliobolus sativus (Ito & Kurib.) is an important and frequently isolated root and leaf pathogen in barley in North America. The virulence spectrum of *C. sativus* isolated from roots in North Dakota is unknown. Several sub crown internodes (SCI) were collected from farmers' fields and two research stations to isolate *C. sativus* from barley in 2006. Six to twelve virulent isolates of *C. sativus* from root and leaf were selected to study the virulence spectrum on roots and leaves of 7 to 12 barley cultivars. The experiments were conducted in a randomized complete block design with split plot arrangement of treatments and 4 replicates. In differential experiments on CRR, none of the cultivars showed a high level of resistance to root isolates 111, 417, 802, 1408, 1457, and 4008. The cultivars Conrad, Eslick, and breeding line ND20448 showed less disease severity than the cultivars Arizona, Argyle, Robust, and Golf. The comparison of virulence spectrum of same *C. sativus* isolates causing CRR and spot blotch revealed that there could be different origins of CRR and spot blotch resistance in barley. Isolates 4008 and 1457 were highly virulent on both root and leaf of currently deployed resistant cultivars and had overcome resistance to spot blotch in NDB112. Current finding on virulence spectrum of *C. sativus* and resistance to CRR in barley will be important in breeding resistance cultivars to CRR and spot blotch.

Genome wide association mapping of resistance to common root in barley breeding germplasm from the Upper Midwest of USA

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Phytopathology 99:S49

Common root rot caused by *C. sativus* and *Fusarium* spp. in barley is a serious disease in the USA. Identification of resistance to root rot disease using bi-parental mapping populations is limited due to complex and partial nature of the resistance. Association mapping of root rot resistance was performed using 384 advanced breeding lines from 4 breeding programs in the Upper Midwest of the USA. These lines were phenotyped for resistance to CRR in an RCBD experiment with 8 replicates in 2007. All lines were genotyped with 1536 SNP markers at the USDA-ARS Fargo. Quantitative Trait Loci (QTL) were mapped using the mixed linear model in the software Tassel. Population structure and kinship relatedness were accounted for to reduce the false positives. Ten to twelve QTL were mapped in different barley lines. The effects of QTL were small and explained 3–8% of the phenotypic variation. These studies showed that association mapping can complement bi-parental mapping studies and are potentially useful in barley for scanning the whole genome for QTL for resistance to plant disease.

Survey of Barley Yellow Dwarf vectors in Alabama and the Panhandle of Florida

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Phytopathology 99:S49

Barley Yellow Dwarf is a major disease problem of wheat in Alabama and is estimated to cause yield loss of 21–42 bushels per acre. Barley Yellow Dwarf is caused by a complex of luteoviruses comprising several strains that will be referred collectively as Barley Yellow Dwarf Virus (BYDV). BYDV is exclusively transmitted by aphids. Aphids were surveyed in the beginning of the planting season in several wheat plots throughout Alabama and the panhandle of Florida for three consecutive years. Collected aphids were identified and bioassayed to detect whether they transmitted BYDV to new plants. This survey was designed to identify the aphid species that serve as primary vector of BYDV. From 2005 to 2007, bird cherry - oat aphid, *Rhopalosiphum padi* (Linn.), and rice root aphid, *Rhopalosiphum rufiabdominalis* (Sasaki), were consistently abundant on the sampled fields, especially between October and December. The species of aphids and their timing of appearance in wheat plots reported here are consistent with flight data collected in north Alabama between 1996 and 1999. Of the three sampling years, viruliferous aphids were detected in 2005 and 2006. BYDV strain PAV and RPV were detected in these years. Low overall numbers of aphid collected throughout the sampling period and even lower proportion of viruliferous aphid made it difficult to conclusively identify the primary vector of Barley Yellow Dwarf in Alabama.

Non-host disease resistance: Signals and nuclear protein changes associated with the activation of pea PR genes

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Phytopathology 99:S49

The pea endocarp/Fusarium solani (Fs) interaction has been a model system for detecting the very early changes in pea chromatin/nucleoproteins following inoculation. The fungal challenge alters nuclear density at 30 min and induces the ubiquitination and depletion of the nuclear proteins: HMG A and histones H2A and H2B within 4 h. These changes were monitored with: HMG A-, histone H2A/H2B- or ubiquitin-specific antisera via imunoprecipitation or western analyses. The dis-association of these proteins from gene start sites is currently viewed essential to enable the polymerase complex (PC) to progress through the ORFs of some genes. The associations of nuclear proteins with PR gene DNA were detected with chromatin imunoprecipitation (ChIP). FsphDNase can induce complete resistance in peas to Fs-f. sp. pisi. Direct detection and computer predicted potential (SignalP- from N-terminal sequence info) indicate a potential for its action directly on chromosomal DNA to provide PR gene access to the PC. The potentials for transport and localization for all functional PR and pathogen virulence gene products were summarized.

Summarization and assembly of functional attributes of known genes in the Non-host resistance and susceptible reaction of pea to *Fusarium solani* sp.

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Phytopathology 99:S49

The contributions of several laboratories have identified and cloned genes that have central functions in the pea/F. solani interactions. These contributions

afford a nearly complete picture of functions developing both the resistance and susceptible result (Phytopath. 98:373). Products of pathogen genes PDA1, PEP1, PEP2, PEP5, FsphDNase, and cutinase, assist virulence by detoxifying pisatin, cleaving fungal DNA, or penetrating the cuticle. The latter 3 products can exit the fungal cell. Plant products of PR genes DRR39 and DRR 230 are defensins that exit the cell and have direct antifungal activity. Chitinase and β-glucanase gene products exit the cell and attack the fungal wall, releasing chitosan oligomers that signal PR gene activation. DRR49 and its homologs are RNase-related and are not secreted. Products of secondary enzyme genes such as DRR206, PAL and chalcone synthase help generate pisatin or lignin. Nuclear protein genes, HMG A, histones H2A/H2B and ubiquitin appear functional in PR gene transcription. The N-terminal sequences of these genes predict their localization and potential for excretion. A scenario developed from this information suggests that FsphDNase, when released, activates the PR gene response rapidly to give non-host resistance. Non-released FsphDNase accumulates and can cause nuclear degeneration and fungal death. A slower response allows hyphal tips retaining intact nuclei to escape and grow as the defense response weakens.

Performance of peanut disease risk index programs at two locations in Alabama

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Phytopathology 99:S50

In 2008, a study was conducted to evaluate the performance of Artisan and Convoy against peanut diseases when used according to the Peanut Disease Risk Index. Studies were conducted at the Wiregrass Research and Extension Center in Headland, AL and at the Gulf Coast Research and Extension Center in Fairhope, AL. Peanuts were rated for early and late leaf spot, rust, and stem rot. Based on Peanut Disease Risk Index, the WREC study site would be rated as a high risk for leaf spot and stem rot and the GCREC study site would be rated as a medium risk for leaf spot, rust and stem rot. At WREC, leaf spot AUDPC values for the index treatment schedules with Artisan were not significantly different from each other but the high risk Convoy program gave the best results. The low risk Convoy program had higher a higher leaf spot AUDPC rating than all other treatments. At GCREC, leaf spot AUDPC results showed no differences among any of the indices. Stem rot incidence was similar for all risk levels for all programs at both locations. At WREC, highest yields were reported for the low index with Artisan, the high index with Convoy and were similar to the Echo/Provost, Echo/Moncut, Headline/Folicur/Headline/Echo, and Echo + Eminent/Echo + Muscle programs. At GCREC, the high risk index with Convoy had higher yields than all other programs.

Impact of tillage, row spacing, and variety on diseases and yield of dryland continuous corn in Alabama

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Phytopathology 99:S50

In 2006, 2007, and 2008, corn varieties were planted on the same site cropped to corn in the previous two years. Experimental design was a split-split plot with conservation or conventional tillage as the whole plot, corn variety (3) as the split plot, and row spacing (single or twin on 30 in. centers) as the split-split plot. Each splt-split plot had four 50-ft rows. The study was not irrigated. Occurrence of diseases was rated on a 0 to 10 scale on the ear leaf at black layer. Due to dry weather in 2006, disease activity was minimal. While noticeable Physoderma brown spot and southern rust was observed in 2007, minimal southern rust and Northern corn leaf blight were seen in 2008. On all varieties in 2007, Physoderma brown spot ratings were higher on the conservation than conventional till corn. Tillage had no impact on southern rust or Northern corn leaf blight. Row spacing had no impact on any disease. While southern rust occurrence was similar on all varieites in 2007 and 2008, Physoderma brown spot severity was higher on Pioneer 31N26 and DeKalb 69-72 than Pioneer 33M53 in 2007 but was similarly low on all varieties in 2008. In two of three years, yields were higher for conservation than conventional-till corn. Over the study period, row spacing did not influence yield. In two of three years, significant differences in yield were noted between corn varieties. In 2007, highest yield was recorded for Pioneer 33M53, while the latter varieity and Pioneer 31N26 yielded best in 2008.

Experimental evolution of an avirulent *Soybean mosaic virus* toward virulence on *Rsv1*-soybeans imitates mutations selected through natural evolution

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Phytopathology 99:S50

In search of virulence determinants, virologists traditionally utilize comparative analyses of the genomes of a virulent and an avirulent virus strain and construct chimeras to map their locations. Subsequent comparison reveals sequence differences and through analyses of site-directed mutants, critical residues are identified. Through a similar approach, we recently constructed a series of chimeras and mutants between virulent *Soybean mosaic virus* (SMV)-G7 and avirulent SMV-N and mapped the virulence determinants on *Rsv1*-soybeans resistant to SMV-N to one amino acid in HC-Pro and two in P3. Furthermore, through adaptation of avirulent SMV-N-derived chimeras containing P3 sequences from two virulent strains to *Rsv1*-soybeans, we also identified HC-Pro residues essential for virulence. To reveal if adaptation of molecularly cloned SMV-N to *Rsv1*-resistant soybeans leads to selection of the same determinants, we have now adapted the virus through an experimental evolutionary approach initially to a unique soybean recombinant line containing a specific crossover within the *Rsv1*-locus (L800) and subsequently to PI 96983 (*Rsv1*), L78-379 (*Rsv1*) and L81-4420 (*Rsv1*). Interestingly, some of the identified virulence determinants were identical to those discovered through comparative analyses with the naturally evolved virulent SMV-G7. Thus, our observation vindicates an experimental evolutionary approach to identify virulence determinants of an RNA virus.

From herbicide to antibiotic: A novel role for Germination-Arrest Factor (GAF) in the *in vitro* inhibition of *Erwinia amylovora*

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Phytopathology 99:S50

The naturally-occurring herbicide GAF (Germination-Arrest Factor) is a compound secreted by certain strains of *Pseudomonas* bacteria. GAF specifically inhibits the germination of a wide range of grassy weeds. GAF activity in culture filtrates is associated with a low molecular weight, hydrophilic compound that stains with ninhydrin on thin-layer chromatograms, suggesting that GAF may be a small peptide or amino acid analog. In a search for possible additional biological properties of GAF, GAF-containing bacterial culture filtrate was screened *in vitro* for potential antimicrobial activity against 28 strains or races of 14 different species of bacteria. Although most of the bacteria tested exhibited little if any response to GAF-containing culture filtrates, the growth of *Erwinia amylovora*, the causal agent of fire blight disease, was strongly inhibited. In this report, we demonstrate that GAF is the agent directly responsible for the antibiotic effect of these culture filtrates on the growth of *E. amylovora*. Mutagenesis that results in loss of GAF activity in culture filtrates also results in a loss of antibiosis activity against *E. amylovora*, demonstrating that GAF is the agent responsible for the antimicrobial effects of these culture filtrates. Reversal of the GAF-induced antibiosis reaction was possible using particular amino acids, suggesting that GAF may exert its cytotoxic effect against *E. amylovora* via an effect on nitrogen metabolism.

Understanding interactions between phytopathogenic *Phytophthora* effector IpiO and the host resistance protein RB

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Phytopathology 99:S50

Species of phytopathogenic *Phytophthora* are well known for their ability to cause disease on economically important crops, with almost 100 recognized species targeting close to 300 different hosts. The host resistance protein RB, isolated from wild potato, specifically recognizes the *P. infestans* IpiO effector to elicit resistance. Using *P. infestans* isolates collected worldwide, we have found that IpiO is universally present, explaining the broad-spectrum phenotype of RB. However, we have found that multiple *IpiO* variants exist within a given *P. infestans* isolate. Importantly, some *IpiO* variants are recognized by *RB* (*IpiO1* and *IpiO2*) and some are not (*IpiO4*). We have determined that *IpiO* alleles not recognized by *RB* can be recognized by *RB*-like genes from other potato species. Most importantly, we have found that IpiO alleles are present in other phylogenetically distinct *Phytophthora* species. Therefore, *RB* may not only confer resistance to late blight of potato and tomato, but is potentially a source of resistance to other *Phytophthora* species on related or unrelated hosts. Further elucidation of the molecular events involved in *IpiO* recognition will be presented.

DNA barcoding of Septoria species from leaf spots and stem cankers of Poplar in British Columbia, Canada to assess risk of spread

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Phytopathology 99:S51

Widespread use of hybrid poplar in north-central and northeastern North America is limited by occurrence of a leaf spot and canker disease caused by the haploid Coelomycete fungus Septoria musiva (teleomorph Mycosphaerella populorum). Molecular marker analyses previously allowed us to document the occurrence of genetically differentiated *S. musiva* subpopulations in north-central and northeastern North America, with both asexual and sexual recombination contributing to the genetic structure. With the extension of poplar cultivation and the use of hybrid poplars, stem cankers have recently been reported in new bioclimatic domains in the province of Québec and in the Fraser valley in British Columbia (BC) previously considered to be *S. musiva*-free. By harvesting and mapping infected poplars and conducting DNA barcoding to assess Septoria species distribution we can evaluate the risk of damage of this plant pathogen in these newly infected area and assess the potential for eradicating the pathogen. DNA barcoding of poplar leaves harvested in the Fall of 2008 using ITS rRNA indicated that the native *Populus trichocarpa* is almost exclusively infected by the native *S. populicola* while hybrid poplars are infected by the non-native *S. musiva*. Although further sampling is necessary to confirm these trends, it appears that *S. musiva* is mostly restricted to planted hybrid poplars and do not display a propensity to spread to native *P. trichocarpa*.

Self-assembly of *Maize rayado fino virus* particles in bacteria and in plants: Towards an understanding of marafivirus gene expression and biology

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Phytopathology 99:S51

The leafhopper-borne *Maize rayado fino virus* (MRFV; genus *Marafivirus;* family *Tymoviridae*) is restricted to the Americas and replicates in both the plant and insect host. Among viruses that replicate in the insect, MRFV's simple genome presents a unique opportunity to investigate the molecular interactions between the virus and its hosts. In this initial study of MRFV gene expression, we addressed the requirements for capsid protein assembly and genome packaging. MRFV contains two components: empty shells and complete virus particles (encapsidating the 6.3 kb genomic RNA). Isometric virions of 30 nm in diameter contain two serologically related, carboxy co-terminal coat proteins (CP) of 21 kDa (CP2) and 25 kDa (CP1) found in a molar ratio of 3:1, respectively. CP1 is a 37 amino acid N-terminal extension of CP2. Recombinant CP1 and CP2 each self-assembled into virus-like particles (VLPs) in *Escherichia coli* and *Nicotiana benthamiana*. Expression of each protein alone in *E. coli* resulted in VLPs that appeared empty (CP2) (stain penetrated particles) or complete (CP1) in electron microscopy. In *N. benthamiana*, expression of CP1 resulted in translation of both CP1 and CP2, in contrast to what was found in *E. coli*, and production of VLPs that appear to be complete. Co-expression of CP1 and CP2 in *E. coli* resulted in VLPs which encapsidated the CP mRNA, suggesting that the N-terminal 37 amino acid residues of CP1 are involved in the assembly of complete virion particles.

An improved method for DNA sequence based identification of nematodes

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Phytopathology 99:S51

Accurate identification of soil nematodes can be a challenging problem, especially for people who are not trained in nematology. Yet, a need exists to rapidly and accurately identify nematodes isolated from soil and plant samples in order to identify nematodes associated with potential crop loss and quarantine issues. Several systems have been described for identification of nematodes based on DNA sequences including those from the nuclear small subunit ribosome gene. Using an extensive alignment from the Nematode Tree of Life project we designed and evaluated several primer sets that were theoretically capable of amplifying the small subunit sequence from virtually all nematode species. Analysis of this alignment also suggested that this sequence would be useful for genus and possibly species level identification of any known nematode. The work presented here describes the evaluation of these primer sets and the optimization of lysis, PCR, and sequencing procedures to develop an effective single tube assay capable of identifying single nematodes at the genus and species level based on small subunit sequences. Data presented includes characterization of nematode populations present in random soil samples to evaluate the effectiveness and utility of this procedure.

Multi-gene phylogeny and genetic diversity within *Phytophthora capsici* in New Mexico

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Phytopathology 99:S51

The oomycete pathogen *Phytophthora capsici* is the most destructive pathogen on chile peppers grown in the desert Southwest. P. capscici can cause up to 100% losses in affected fields. Classical breeding programs have not succeeded in identifying resistance to *P. capsici* in chile pepper cultivars. A high level of diversity among *P. capsici* strains in the region has been postulated to be one of the main challenges in resistance breeding programs. While preliminary studies have indicated a high level of diversity among *P. capsici* strains isolated from chiles in the desert Southwest a rigorous molecular level analysis of diversity has not been performed. In this study four nuclear and one mitochondrial loci, comprising ~4.5 Kb in total sequence, were analyzed for to analyze diversity in a collection of chile infecting *P. capsici* strains. While all loci showed variation B-tubulin generally displayed a higher level of diversity than the nuclear ITS region, the RAS intron, or the LSU locus. Phylogenies constructed from individual and combined sequences were similar and showed that there is a great deal of diversity among chile infecting strains of *P. capsici* in the desert Southwest. Ongoing work will include studies on strain occurrence and distribution to determine which strains of *P. capsici* are the most damaging to chile crops. The results of these studies will guide future work on developing strategies to mitigate P. capsici caused damage on chile peppers.

Synergistic biofilm formation between *S. enterica* and *X. vesicatoria*

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Phytopathology 99:S51

Incidents of Salmonellosis associated with raw produce consumption have increased dramatically in the last few years. Growth of *Salmonella enterica* on healthy plant leaves has only been reported in combination with the bacterial plant pathogen, *Xanthomonas vesicatoria*. *X. vesicatoria* is a successful tomato leaf spotter that utilizes a biofilm matrix to sustain steady and effective epiphytic populations before entering plant cells. Biofilms contribute to the attachment and persistence of pathogens on plant leaves in order to withstand harsh environments like the leaf phyllosphere or antibacterial treatments. *S. enterica* is not a plant pathogen, therefore an efficient mechanism that may allow and eventually promote population growth and survival on plants is important. Biofilm formation may be part of the synergistic interaction observed between *X. vesicatoria* and *S. enterica*. Experiments have been conducted to test the interaction of biofilm formation between the two bacteria on glass test tube walls within different media. Both pathogens formed weak biofilms separately, while stronger biofilm formation was observed when both bacteria were co-inoculated. A key component of *S. enterica* survival as an epiphyte may be biofilm formation in combination with bacterial plant pathogens.

Building diagnostic capacity in Central America: A cooperative effort with the Southern Plant Diagnostic Network, USDA-FAS, and Ministries of Agriculture

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Phytopathology 99:S51

The Southern Plant Diagnostic Network (SPDN) and the USDA-Foreign Agriculture Service have established a cooperative project to provide plant pest diagnostic training for phytosanitary laboratories in Central America. The trainings help laboratories develop best practices for plant diagnostics. Participating countries are signatories to the Dominican Republic-Central American Free Trade Agreement (CAFTA-DR). Participation in the program and compliance with the agreement require laboratory assessments and cooperation between Regional Ministries of Agriculture. The SPDN provided advanced diagnostic training to personnel from expert and/or phytosanitary laboratories in these countries in the form of regional workshops held in the Dominican Republic, Honduras, and Guatemala in 2008 and 2009. Participants were selected by their countries to attend intensive week-long hands-on laboratory training. Participants developed standard operating protocols (SOP) for the diseases and insects on the import/export priority pest lists. Fourteen SOPs are complete or will be completed by the end of 2009. These protocols will be shared and implemented among participating countries. The establishment of standard protocols and reporting requirements for baseline monitoring and detection programs will foster trust between cooperating organizations and will facilitate safer and more accessible agricultural trade.

TonB dependent receptors of *Pseudomonas fluorescens* Pf-5: Roles in siderophore and iron uptake
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Phytopathology 99:S52

TonB-dependent receptors (TBDRs) are outer membrane proteins with essential roles in iron uptake by Gram-negative bacteria. The biological control strain *Pseudomonas fluorescens* Pf-5 has 45 predicted TBDRs in its genome, which far exceeds the number of TBDRs in most published bacterial proteomes. 18 TBDRs have the N-terminal extension domain characteristic of transducers, a subclass of TBDRs that typically initiate a signaling pathway and function in siderophore uptake. Phylogenetic analysis indicated that five of the 18 putative transducers are related to TBDRs that function in the uptake of ferric-pyoverdines; a structurally-diverse group of fluorescent siderophores produced by *Pseudomonas* spp. Mutants deficient in each of the five transducers were derived and their capacities to obtain iron from a diverse set of pyoverdines were assessed. Each of the five transducers recognized a specific subset of pyoverdine siderophores, highlighting their specificities for certain pyoverdine structures. Other transducers identified in the Pf-5 genome have predicted specificities for siderophores (enterobactin, aerobactin, ferrichrome, and ferrioxamine) produced by diverse groups of fungi and bacteria, and Pf-5 was shown to utilize each of these siderophores as sources of iron. These transducers may contribute to the environmental fitness of *P. fluorescens* Pf-5 by facilitating iron acquisition from siderophores produced by its coinhabitants in soil and on plant surfaces.

Transmission of the huanglongbing pathogen *Ca.* Liberibacter spp. from Citrus by dodder, *Cuscuta indecora* Choisy, to periwinkle, *Catharanthus roseus* G. Don.
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Phytopathology 99:S52

The transmission of bacterial plant pathogens by dodders has been demonstrated, but the phenomenon has not been adequately characterized. Dodder plants were grown on *Ca.* Liberibacter-infected hosts and cut into 2 cm segments. We show by Q-PCR and electron microscopy that the pathogen replicates in dodder and may reach very high concentrations in phloem, with Ct values occasionally less than 20. However, as in infected citrus, the distribution in dodder is erratic, and consecutive 2 cm segments may have widely different titers of the pathogen. Individual dodder tendrils from infected plants may contain bacteria or not. Dodder does not show obvious disease symptoms when infected by *Ca*. Liberibacter, but the life cycle of dodder on infected citrus is accelerated compared to growth on healthy citrus. Both *Ca.* Liberibacter asiaticus and *Ca*. Liberibacter americanus can be transmitted by *C. indecora*, although one strain from Florida was not transmitted. The *Ca.* Las pathogen is very pleomorphic in dodder sieve tubes with both round and long thin morphologies present. In some sections, individual bacteria can be seen transitioning between the two morphologies which shows that both forms are the same organism. The pathogen also was found passing through phloem sieve plates. Starch granules were seen in the healthy and infected dodder without evidence of plastid membranes. The origin of the starch is probably from citrus.

Evaluating known and unknown mycoparasites as biological control agents for sugar beet root pathogens
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Phytopathology 99:S52

Root diseases routinely damage sugar beets in Nebraska and other areas of the Central High Plains, and it is becoming more common to find fields infested simultaneously with multiple pathogens. Due to the lack of available chemicals for economic control of soilborne diseases, other alternative techniques, such as biological control are being evaluated for disease management. Over the last several years, two distinct groups of naturally-occurring fungi have been collected and tested for their ability to inhibit several common root pathogens in-vitro. All fungi have been found in association with root disease infections of various crops. One group consists of three different pyrenomycetous ascomycetes, and the other group includes several unknown, sterile fungi. The most promising candidates have then been challenged against members of a root disease complex in field studies as biological control seed treatments. Results obtained from 2006 and 2008 are encouraging and suggest that some of these fungi are providing some level of protection for the entire season against this group of pathogens. For example, plots planted with a root rot susceptible cultivar treated with the unknown sterile fungi resulted in sucrose yields of 2000 and 2500 kg/ha higher than untreated plots of the same cultivar.

New hosts for the dry bean bacterial wilt pathogen in western Nebraska?
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Phytopathology 99:S52

Bacterial wilt of dry beans, caused by *Curtobacterium flaccumfaciens pv flaccumfaciens* (*Cff*), was an economically important problem in western Nebraska over forty years ago, but then became rarely found. The disease has now re-emerged and has been documented from almost 400 separate dry bean fields since 2004. Additionally, over the last 3–4 years, bacterial isolates from necrotic lesions of soybeans, wheat, and corn have been shown to cause wilt-like symptoms and disease on dry beans after artificial inoculation. Biolog® identifications matched these isolates and *Cff* with high probabilities. Based on these findings, a survey of production fields in western Nebraska was begun in 2008 to document the incidence of bacterial wilt isolates found in association with other crops grown in rotation with dry beans. More than 200 production fields in 11 counties were scouted for symptoms consistent with bacterial infections. From these fields, 270 symptomatic samples were collected and processed for identification. Approximately 10% of isolates obtained from the survey were judged to be potential *Cff* candidates based on several criteria and are being tested for pathogenicity on dry beans. Also, a newly evaluated crop from this survey that has yielded isolates pathogenic to dry beans is alfalfa.

Role of "*Candidatus* Liberibacter"-infected seed tubers in epidemiology of potato zebra chip
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Phytopathology 99:S52

An emerging disease of potato in the US called "Zebra Chip" or "Zebra Complex" (ZC), vectored by the potato psyllid, results in tuber defects that render them unmarketable. The putative causal pathogen is a fastidious bacterium "*Candidatus* Liberibacter solanacearum (CLs)", similar to that which causes citrus greening. A low percentage of CLs-infected seed tubers may germinate, but typically produce small, deformed plants. The impact of CLs-infected seedlings on the epidemiology of ZC is currently unknown. CLs-infected seedlings may contribute to within-field spread of ZC should CLs-free psyllids feed, acquire, and spread CLs to other plants. Therefore, studies were performed in two potato fields to assess the incidence of ZC near seedlings arising from CLs-infected tubers, seedlings infected by CLs-infective psyllids, and disease-free controls. Isolated seedlings, psyllid-infected plants and healthy plants were marked and revisited after three weeks, when new infections near marked plants were counted. Results showed that ZC incidence near infected seedlings was no different than healthy controls ($P > 0.05$), indicating that ZC was not being spread from CLs-infected tuber seedlings. In contrast, psyllid-infected plants had significantly more ZC-infected plants nearby than either infected seedlings or healthy controls ($P < 0.05$). Overall, the density of CLs-infected seedlings was deemed too low to be an epidemiologically important source of CLs in potato fields.

Yellow-cedar decline: Key landscape features and snow modeling of a climate-induced forest decline on a dormant volcano
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Phytopathology 99:S52

Yellow-cedar decline is the most widespread and ecologically important forest health issue in southeast Alaska. Recent research indicates that the extensive tree death is related to a warming climate that results in root freezing injury during sporadic spring thaw-freeze events when there is no protective snow covering roots. We tested the association of healthy and dead yellow-cedar with several terrain factors on Mt. Edgecumbe, an inactive volcano near Sitka, AK. Elevation was a primary factor associated with the forest decline as delineated from aerial photographs. Intensive tree death peaked at 100 to 200 m; yet, cedar forests > 300 m were healthy. Aspect and slope were secondary factors; the former modified the elevation effect. Observations from helicopter of 96 virtual plots on contour grids confirmed the presence and health of yellow-cedar at higher elevations, which grew close to timberline at about 600 m. Snow modeling with PRISM and a downscaling elevational adjustment used weather station data as input for models from 1900 to the present, and a conservative global circulation model (CGCM2 B2) for future projections. Adequate snow to protect yellow-cedar was determined to be about 2500 mm/yr (precipitation as snow). This annual accumulation was extensive at all but the lowest elevation in the early 1900s, but diminished progressively through time, leaving only a small suitable habitat for cedar near the cone of the volcano by 2080.

Status of fungicide resistance in orchard populations of *Venturia inaequalis* in Chile

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Phytopathology 99:S53

Apple scab is the most serious fungal disease in Chile and fungicides are widely used depending on climatic conditions and location. The disease has been present in some orchards despite fungicide use in years of few predisposing conditions and, therefore resistance is believed to occur. Practical resistance to QoI fungicides was reported recently. Sensitivity to the fungicides difenoconazole, dodine, fenarimol, kresoxym-methyl, mancozeb, myclobutanil, triflumizol, and pyrimetanil was assessed on orchard and wild isolates obtained from the south central region where the disease is prevalent. Sensitivity of monosporic isolates was measured on synthetic media upon conidia germination or mycelial growth depending on the fungicide. Reduced sensitivity in orchard isolates was observed for all the fungicides tested, but the impact on practical resistance needs to be assessed. Baseline population sensitivity to fenarimol was lower than previously reported and the corresponding monitoring dose higher.

Elm yellows detection in trees and insects

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Phytopathology 99:S53

Elm yellows (EY) is caused by a phytoplasma. The common strain was found in the vicinity of State College, PA in 2007. A probe and primer pair for TaqMan real time PCR chemistry (EY1332 minor grove binding probe: 6FAM - CTT TTG GCG GGA CTA GT; EY1332 forward primer: TTTAGCAGGAAGTGCAATATCTAAACA; EY1332 reverse primer: GATTCTAAAGCAACACCGACAATAATT) were designed for detecting the common strain of EY in elms and insects. Five to 10 mm diameter twigs were collected, from American (*Ulmus americana*) and red (*U. rubra*) elms, phloem harvested, cells macerated using a TissueLyser, and EY DNA was successfully extracted following the standard Qiagen Dneasy Plant Mini Kit protocol. Following the same protocol, EY DNA was extracted from insects trapped among elms on yellow sticky cards. CT values for the target EY DNA ranged from CT15 to CT37 in tree samples and CT25 to CT37 in insects. Of the13 different morphological groups of insects including leafhoppers and sharpshooters collected during 2008, 11 harbored EY. In June 2008, EY was found in every bark phloem sample removed from 2 trees sampled at 1–3 meter intervals from the soil line to the top-most twigs. No gradient in CT values was detected from the bottom to the top of the trees. EY was found in some but not all leaf vein samples. Experiments are planned to determine which of the EY positive insects are capable of vectoring EY.

Effect of cultivar on the relationship between Fusarium head blight severity and deoxynivalenol concentration in winter wheat

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Phytopathology 99:S53

Fusarium head blight (FHB) caused by *Fusarium graminearum* is a destructive disease of wheat. *F. graminearum* produces the mycotoxin deoxynivalenol (DON) which accumulates in grain. The relationship between FHB severity (FHBsev) and DON can be used to estimate the level of DON to expect in grain, enabling producers to make informed decisions early regarding the marketing of grain from fields affected by FHB. This study was undertaken to determine if this relationship is cultivar-dependent. The cultivars Jagalene, Harry, and 2137 were planted following corn in October 2007. In addition to natural inoculum, plots were inoculated with 1×10^5 spores/ml of *F. graminearum* at early anthesis and were not irrigated. Cultivars were arranged in a randomized complete block design with three replications. FHB severity was determined 21 days after inoculation on 20 heads tagged in each of 13 disease severity categories in each plot: 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 70, and 90%. Linear regression analysis with DON as the dependent variable showed that the relationship between FHBsev and DON was strongest for Jagalene ($R^2 = 0.84$, $P < 0.0001$) followed by 2137 ($R^2 = 0.75$, $P = 0.0001$) and Harry ($R^2 = 0.41$, $P = 0.0191$). Regression coefficients were 0.35, 0.19, and 0.09 for Jagalene, 2137, and Harry, respectively. Knowledge of this relationship for specific cultivars can enable producers to more accurately estimate DON.

Characterization and mapping of a gene component for durable leaf rust resistance in chromosome arm 7BL

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Durable resistance to leaf rust (*Puccinia triticina*) is governed by genes that confer slow rusting and have small to intermediate effects but act additively. Identification of individual slow rusting resistance genes is limited to two catalogued genes *Lr34* and *Lr46*. 'Parula' is known to confer high levels of durable leaf rust resistance. Previous QTL analysis using a F6 population from the cross with susceptible 'Avocet' showed that the resistance in Parula involved three independent loci on chromosome arms 1BL (*Lr46*), 7DS (*Lr34*) and 7BL. Two F6 lines from the above population, identified to carry the single resistance allele on 7BL, were crossed with a highly susceptible line and single gene based mapping populations containing 396 F5 RILs developed for further mapping studies. The populations were characterized for resistance for two years in Cd. Obregon, Mexico, under high leaf rust pressure and polymorphic SSR and other 7BL markers used for genotyping. Leaf rust resistance in RILs could be classified as a simply inherited Mendelian trait and confirmed the location of resistance gene based on close linkage with several markers on 7BL. Deletion mutants are being developed for fine mapping to develop tightly linked molecular markers.

Molecular analysis of cultivated soybean germplasm resistant to frogeye leaf spot

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Frogeye leaf spot (FLS), caused by *Cercospora sojina* Hara, is a persistent threat to soybean *Glycine max* (L.) Merr. across the US. Resistant varieties are the most cost effective means of managing yield loss from FLS which is mainly the result of reduced photosynthetic area and premature defoliation. The development of simple sequence repeat (SSR) markers presents new opportunities for molecular diversity analysis of soybean. The current study was conducted to identify diverse resistant germplasm for the development of mapping populations and for their introduction into breeding programs. One-hundred and twenty SSRs were screened across 41 soybean genotypes with differing levels of resistance to FLS. Several methods were applied to identify candidate SSR loci that may be valuable for mapping FLS resistance: Multi-dimensional scaling and cluster analyses to reveal different groups of genotypes, Shannon entropy for defining single- and multi-locus diversity as well as SSR informativeness, along with AMOVA and Kruskal-Wallis one-way ANOVA. The molecular diversity analysis provides valuable information for soybean breeders designing strategies for incorporating FLS resistance and for molecular biologists wishing to create recombinant inbred line populations to map these traits.

Detection of complex soil-borne disease interactions by hyperspectral foliar surface monitoring in sugar beet

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In sugar beet fields, damage due to soil-borne nematode and fungal root pathogens often appears in clusters. In addition, these soil-borne disease agents can occur either alone or simultaneously on one plant making detection and optimum treatment difficult. In greenhouse experiments susceptible, tolerant or resistant sugar beet varieties were inoculated with the fungus *Rhizoctonia solani* and/or the nematode *Heterodera schachtii* alone or concomitantly. Attempts were made to discriminate between the occurrence of each disease alone or in combination. Concomitant infections of these two pathogens in sugar beet plants can lead to synergistic based symptoms that cause accelerated symptom development which over time can lead to increased yield loss and even plant death. To determine variation in symptoms in different sugar beet varieties caused by the two pathogens hyperspectral measurements were made at different times after infection. Hyperspectral vegetation indices were calculated from the leaf reflectance data to determine vitality changes and accordingly the injury level of the plants.

NPDN First Detector education: Traditional and multimedia training

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Invasive arthropods, plant diseases, and weeds cost U.S. agriculture billions of dollars annually through direct pest damage and indirectly via eradication and management programs. Each year, integrated pest management (IPM) programs are disrupted by the introduction of new, unwanted invaders. In

order to raise awareness concerning the threat of invasive species and appropriate sampling as well as communication protocols, the National Plant Diagnostic Network (NPDN) launched an extensive First Detector training program in 2003. First Detector training occurs through traditional, face-to-face training (2003–09), interactive, content-management-based e-Learning modules (2008–09), and a wiki platform based series of pest information pages (2008–09). First Detectors completing training receive certificates of completion, and the national First Detector newsletter. Outcomes from the three NPDN training delivery platforms will be presented.

A disease forecasting and management strategy against *Monilinia fructigena* in organic apple orchards

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Temporal development of brown rot *Monilinia fructigena* on apple fruits was analysed in Eastern Hungary (2002–2006) and a brown rot forecasting and management strategy (BRFMS) was developed for organic apple orchards. The three-parameter logistic function gave the best fit to brown rot over four non-linear growth functions. The disease variables of final disease incidence (Y_s), the relative rate of disease progress (beta) and the standardized area under the disease progress curve ($AUDPC_S$) were derived from the logistic function and were used to develop a fundamental model for predicting temporal brown rot development and then to construct a BRFMS. The fundamental model contained four parts i) data insertion and analyses by computer simulation of pathogen submodels, ii) calculation of yield loss threshold levels based on disease incidence, iii) determination of epidemic intensity levels and iv) decision modul with suggestions of disease management practices for each epidemic intensity level. The fundamental model was supplemented with prediction of occurrence of first fruit rot symptoms and with insect injury prediction related to brown rot development in order to complete a BRFMS for organic apple orchards. Season-long application of BRFMS treatments from 2006 to 2008 reduced the number of sprays against brown rot by 22.2–33.3% compared with the treatments of general spray schedules in organic apple orchards.

Can tree pruning reduce the incidence of Cercospora leaf spot in integrated and organic elderberry orchards?

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In a two-year study, the temporal development of Cercospora leaf spot (*Cercospora depazeoides*) was evaluated in two winter pruning treatments (trees pruned to four and eight scaffolds) in two integrated and organic elderberry orchards in Hungary. Under integrated production, leaf spot onset occurred two to four weeks later (early- and mid-August) in both years and both orchards compared with the organic program. Disease then continuously progressed until late-September in both years, reaching a maximum final disease incidence of 15.9% in the integrated system and of 38.2% in the organic system. In general, disease progress after late-August was greater on trees pruned to eight scaffolds than trees pruned to four scaffolds in both production systems. Final disease incidence was significantly lower ($P \leq 0.001$) in the integrated treatments compared with organic ones. Across all treatments, final disease incidence values were significantly ($P \leq 0.05$) lower on trees pruned to four scaffolds compared with trees pruned to eight scaffolds. However, when the effect of pruning on final disease incidence was analyzed separately for integrated and organic systems, pruning caused uniformly significant differences in disease development only for the organic system. In conclusion, winter pruning may be useful as a Cercospora leaf spot management practice in organic elderberry orchards.

hypE: A gene predicted to be involved in the late steps of aflatoxin biosynthesis

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Aflatoxins are carcinogenic, polyketide-derived mycotoxins produced by members of the genus *Aspergillus*. Their biosynthesis is performed by the coordinated action of more than 25 genes contained within a ~70 kb aflatoxin gene cluster. We identified a small open reading frame, *hypE*, situated between *aflM(ver-1)* and *aflN(verA)* that is predicted to encode a 127 amino acid protein. Gene expression studies of *A. flavus* grown under aflatoxin conducive and nonconducive conditions indicated that the transcriptional regulation of *hypE* is similar to that of other aflatoxin biosynthetic genes. A *hypE* deletion mutant of *A. flavus* produced less aflatoxin B1 and B2 than control strains and accumulated an unknown metabolite which we assigned as a tentative HypE substrate (HESUB). Aflatoxin biosynthesis could be restored in this *hypE* mutant by a *hypE* overexpression construct. Restored aflatoxin biosynthesis was associated with loss of HESUB accumulation. However, addition of a *hypE* overexpression construct to the parental strain did not increase aflatoxin production. These data suggest that the *hypE* gene product is involved in formation of aflatoxins, although its precise biochemical function remains unclear.

Four records of *Phytophthora* species for nursery irrigation water in Virginia

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Phytophthora species are commonly regarded as 'water moulds' and pose a constant threat to the health of ornamental crops as well as surrounding forests. We previously reported isolation of twelve species of *Phytophthora* from nursery recycled irrigation water in Virginia. These include two new species: *P. irrigata* and *P. hydropathica*. In an investigation into the aquatic ecology of these pathogens, we recently recovered four genetically distinct groups of *Phytophthora* as indicated in single-strand conformation polymorphism (SSCP) analysis of PCR amplified ribosomal DNA internal transcribed spacer 1 (ITS). These groups produced DNA fingerprints typical of *P. insolita*, *P. polonica*, *P. pseudosyringae* and *P. sansomeana*, respectively. These identities were confirmed by morphological examination and ITS sequence data. Cultures of these four species all were homothallic. In addition, *P. pseudosyringae* produced semipapillate and caducous sporangia while the other three species produced nonpapillate and noncaducous sporangia. These species except for *P. insolita* were described recently and data on their plant health risk are lacking. Tests on the pathogenicity of selected isolates to a universal suscept plant are underway.

Biological control of Pierce's disease in grapevines propagated from mother vines infected with *Xylella fastidiosa* strain EB92-1

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Pierce's disease (PD) of grapevine, caused by *Xylella fastidiosa*, limits the grape industry in much of the southern U.S. In Florida, injection of a benign strain (EB92-1) of *X. fastidiosa* into transplants has controlled PD in a *Vitis vinifera* cv. Cabernet Sauvignon planting for 12 years. However, injection of every vine is costly and labor intensive. A more efficient treatment is desirable. Rooted cuttings were propagated from *V. vinifera cv.* Chardonnay and cv. Chambourcin (French/American hybrid) mother vines infected with strain EB92-1 and from mother vines not infected with EB92-1. Treatments included 12 rooted cuttings from an infected vine, 12 from an uninfected vine, and 12 from an uninfected vine that were injected with EB92-1 in the greenhouse. Two weeks later, all plants were inoculated with a pathogenic PD strain and rated weekly for symptoms. After 8 weeks, there was significantly less disease in the 'Chambourcin' cuttings from mother vines infected with EB92-1 than in cuttings from uninfected control vines. In 'Chardonnay', there was no reduction in PD in cuttings from mother vines infected with EB92-1. Injection of EB92-1 reduced PD development in both cultivars. These greenhouse tests indicate that the biocontrol strain can be transmitted in cuttings from an infected mother vine, but further research is needed to attain consistency. This could be a more efficient method of utilizing strain EB92-1 for the biological control of PD.

Characterization of potential biological control bacterial strains

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The use of natural antagonists lessens the dependence on synthetic agrochemicals. These materials continue to face increased regulation and phase-out. The development of alternative, biologically-based materials is increasing in importance. Two bacterial strains were isolated during routine soil dilution plating in 2004. The observed anti-fungal activity lead to further characterization and the isolates, *Burkholderia pyrrocinia* (FL728) and *Paenibacillus lentimorbis* (FL95), were identified using 16S rDNA sequencing. Petri plate bioassays were used to identify inhibition of several fungal plant pathogens, including *Fusarium oxysporum*, *Colletotrichum*

acutatum, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, and *Verticillium albo-atrum*, by one or both of the bacteria. Several approaches have been used to attempt to characterize the observed antifungal activity. Using *Bacillus subtilus* (GB03) as the positive control, attempts to amplify selected fengycin, surfactin, iturin, and bacillomycin genes identified in that biological control strain were unsuccessful. Recently, the use of Tn5 mutagenesis has resulted in positive transformants, exhibiting kanamycin resistance with a loss of the previously observed antifungal activity, that are currently being characterized.

Isolation and sequencing of conditionally dispensable chromosomes from *Alternaia* spp.

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The fungal genus *Alternaria* is well known as containing many notoriously destructive plant pathogens. A closely-related group, *Alternaria* spp. produce host-specific toxins (HSTs), genes conding for which are found to be carried on extra small, conditionally dispensable chromosomes (CDCs). Subsequent studies show that CDCs are not required for normal saprophytic growth but essential for virulence. Another character of CDCs is that DNA on them does not hybridize to any other host genomic sequence, indicating that these sequences are possibly acquired through horizontal gene transfer (HGT), a lateral movement of stable genetic material between two individuals. It is demonstrated that asexual fungi are capable of recombination or HGT. These processes offer an alternative solution of introgression of novel genetic information to *Alternaria* since majority of them are asexual. In this project, we are evaluating the entire CDC to address the origin, maintenance and spread of these genetic elements in the *Alternaria alternate* group. The first step is to sequence and generate a draft sequence assembly of the CDCs. After that, we will apply functional analysis of genes predicted to condition host specificity, virulence, and chromosome maintenance or transfer. Here we present our progress in CDC sequencing, including optimization of CHEF-gel conditions, isolation, and preliminary sequencing results.

Field evaluation of virginia-type peanuts transformed with a barley oxalate oxidase gene for resistance to Sclerotinia blight

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Three virginia-type cultivars (Perry, Wilson, NC 7) and two modified lines of each cultivar with a barley oxalate oxidase gene were evaluated in a field with a history of Sclerotinia blight in 2007 and 2008. Expression of the oxalate oxidase gene was confirmed in all six transformed lines by enzyme assays of leaf discs from 80 randomly selected plants of each line. The transformed lines possessed the same agronomic characteristics of the corresponding non-transformed parent. Disease incidence was recorded at 2-wk intervals until harvest by counting disease foci in each 60-ft plot. There were no significant differences in susceptibility of transformed lines and their corresponding non-transformed parent to tomato spotted wilt, early leaf spot, web blotch, southern stem rot, or Cylindrocladium black rot. Incidence of Sclerotinia blight in transformed lines was significantly lower than parent lines. Four transformed lines yielded significantly more than the non-transformed parents. Additional comparisons indicated that transformation resulted in little or no change in desired market traits or quality of kernels. These data agreed with results of previous trials in that the oxalate oxidase gene provides resistance to Sclerotinia blight. Due to the high economic value of peanut and the importance of Sclerotinia blight, the engineered Sclerotinia-blight resistance is of useful tool for improving the efficiency of disease control in commercial cultivars.

Specific detection and identification of *Xylella fastidiosa* strains causing oleander leaf scorch by polymerase chain reaction

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A pair of PCR primers, QH-OLS05/QH-OLS08, was developed that is specific for strains of Xylella fastidiosa causing oleander leaf scorch. The primers were designed based on DNA sequence of a randomly amplified polymorphic DNA (RAPD)-PCR product unique to oleander strains. The PCR assay using primer pair QH-OLS05/QH-OLS08 allowed quick and simple detection and identification of oleander strains in cultured bacterium and infected plant samples. The assay can also be applied to insect samples. Specific detection and identification of oleander strains of X. fastidiosa by PCR is useful for epidemiological and etiological studies of oleander leaf scorch by identifying what plants and insect vectors harbor or carry this particular strain of X. fastidiosa, especially in locations where mixed natural infections by oleander and other strains of X. fastidiosa occur.

Antifungal proteins and potential mode of action of endophytic bacteria from wheat as biocontrol agent of take-all disease

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Phytopathology 99:S55

Endophytic *Bacillus subtilis* strains E1R-j and EDR4 showed significantly biological control efficacy on take-all disease, caused by *Gaeumannanyces graminis* var. *tritici* (Ggt), in the greenhouse and filed trails. In the presence of E1R-j hyphae of Ggt showed leakage, appeared ruptured, swollen and shriveled. TEM studies showed that E1R-j cells were present in roots of wheat seedlings and effectively retarded infection and colonization of Ggt. Suppression of Ggt was accompanied by disintegration of hyphal cytoplasm. In the presence of E1R-j cells in Ggt-infected root tissue defense reactions were triggered such as formation of wall appositions and papillae. Antifungal proteins were purified from the culture filtrates. The antifungal protein MW from E1R-j was 51.9 kDa and the pI value was 8.7. It showed protease activities but not β-1, 3-glucanase activities. The purified proteins exhibited inhibitory activity on mycelium growth of Ggt. SEM observation showed that hyphae of Ggt treated with the antifungal proteins were deformation. The antifungal protein isolated from EDR4 demonstrated neither β-1, 3-glucanase or β-1, 4-glucanase or chitinase activities nor protease inhibitory activity. However, it exhibited ribonuclease and hemagglutinating activities as well as a trifle protease activity. The MW was about 377.0 kDa and the pI value 6.59. The mode of action of antifungal proteins needs to be clarified.

Isolation and evaluation of endophytic bacteria from wheat as biocontrol agent of take-all disease

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Sixty endophytic bacterial strains isolated from wheat roots were screened *in planta* and 44 strains showed significant biocontrol activity against wheat take-all caused by *Gaeumannanyces graminis* var. *tritici* (Ggt). Two of these strains, E1R-j and EDR4, were identified as *Bacillus subtilis* based on morphology and 16S rDNA sequence analysis as well as physiological, biochemical characteristics. Two strains inhibited mycelium growth *in vitro* of Ggt. In the greenhouse, soil drenches of E1R-j cell suspension reduced significantly take-all disease incidence in wheat seedling by 60–70% compared to the inoculated control, 4 weeks after sowing. Growth parameters such as lengths and fresh weight of roots and shoots of treated plants were significantly higher compared to the inoculated plants. Field experiments in years 2006–2008, treatments with strain E1R-j and the fungicide Triadimefon reduced take-all disease in wheat roots by 50–55% and 60–62%, compared to the inoculated control plants. Plant height in inoculated control was significantly lower and also the yield parameters seeds per head and especially TKW were drastically reduced compared to the other treatments. Strain EDR4 treatments showed the similar results in greenhouse and field trial. The treatments alleviated the detrimental effects of take-all on grain yield parameters to a similar extent as Triadimefon application.

Population structure of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in Florida inferred from vegetative compatibility groups and microsatellites

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Phytopathology 99:S55

One hundred and twenty isolates of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) were collected from three main tomato-growing counties in Florida from 2006 to 2008. Vegetative compatibility groups (VCGs) and ten microsatellite loci were used to infer the population genetics of FORL. Sixty-eight percent of the isolates could be assigned to one of three VCGs, 0094, 0098 and 0099. The frequencies of VCGs, 0094, 0098 and 0099 were 38%, 23%, and 7%, respectively, indicating that VCG 0094 was predominant among the isolates assessed. No significant difference in virulence was found between VCGs 0094 and 0099. However, VCG 0098 was more virulent but less frequent than VCG 0094, suggesting a trade-off between virulence and

saprophytic competitive advantage. Interestingly, VCG 0098 was previously reported in Collier County, but has now been found in Manatee and Hendry Counties, whereas VCG 0099 seems restricted to Collier County in this study. Occasionally, all three VCGs (0094, 0098, and 0099) were recovered from a single sampling site in Collier County, probably resulting from multiple introductions. The population structure and migration of these VCGs among the three counties revealed by microsatellite data will be discussed.

Phylogenetic relationships between *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici* inferred from IGS, EF1-α, and a SSR locus

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Fusarium oxysporum f. sp. *lycopersici* (FOL) and *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) are two important soilborne pathogens of tomato. Sequence analysis of the nuclear ribosomal DNA intergenic spacer (IGS) region, translation elongation factor 1-α (EF-1α), and a microsatellite locus with high gene diversity was conducted to resolve phylogenetic relationships between FOL and FORL. Neither EF-1α nor the microsatellite locus provided useful phylogenetic resolution. However, IGS was capable of grouping most known vegetative compatibility groups (VCGs) of FORL and FOL into five clades. Within the five clades, two clades contained the dominant VCGs of FOL and FORL in Florida. A clade formed by VCG 0033 of FOL was separate from the clade consisting of the FORL VCGs 0094, 0098, and 0099, suggesting that the IGS region may be used to differentiate the predominant VCGs of FOL from FORL in Florida. The mating-type (MAT) idiomorph of VCG 0033 was typed as *MAT-1*. VCG 0099 was assigned to *MAT-2*, whereas VCGs 0094 and 0098 were typed as *MAT-1*. Of the three loci used for phylogenetic analysis, IGS revealed considerable sequence polymorphisms and thus contributed to resolving the phylogenetic relationships of the two closely related *formae speciales*.

Expressed sequence tags from a SSH cDNA library identified genes involved in adult-plant resistance to stripe rust in 'Xingzi 9104' wheat

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Phytopathology 99:S56

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is a destructive disease of wheat (*Triticum aestivum*) worldwide. 'Xingzi 9104' (XZ) is a wheat cultivar with resistance to race CYR32 of *P. striiformis* f. sp. *tritici* in the adult-plant stage while susceptible at the seedling stage. To gain a better understanding of the mechanism of adult-plant resistance, the suppressive subtractive hybridization (SSH) approach was used to identify wheat genes induced by *P. striiformis* f. sp. *tritici* inoculation at adult-plant stage. A total of 1,250 positive cDNA clones were obtained and sequenced. After the contig analysis with the Cap3 assembler, 427 unique sequences were obtained and compared to the NCBI no-redundant protein database using the BlastX program. The sequences were putatively categorized as genes belonging to signal transduction, transcription regulation, protein synthesis and storage, membrane transport, and cell growth and division. Based on the putative functions of the induced genes, we propose a special defense-related pathway that is triggered during the expression of adult-plant resistance in XZ after we compared our results with the research on high-temperature adult-plant (HTAP) resistance and race specific resistance in the literature. The time-course expressions using quantitative RT-PCR confirmed the induction of seven selected genes by *P. striiformis* f. sp. *tritici* infection and determined their expression patterns.

Biofilm formation and motility by strains of *Xanthomonas axonopodis* pv. *citri* causing differential symptoms on citrus leaves

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Citrus bacterial canker causes severe diseases on leaves, stems, and fruits of various citrus cultivars and is a limiting factor in citrus-production worldwide. This disease is caused by *Xanthomonas axonopodis* pv. *citri*. Biofilm formation and motility have been indicated to be important for various bacteria to successfully develop pathogenic relationships with their host. To investigate the mechanisms of X. *Xanthomonas axonopodis* pv. *citri* causing differential symptoms on citrus leaves, the biofilm formation and motility by four strains were assessed. In this study, strains belonging to three types of symptoms on Mexican lime, grapefruits, Liucheng, and lemon were examined. Strain XW19 belonged to type A which induced typical erumpent canker lesions with watersoaked margin on leaves of all four citrus species; strain XW47 belonged to type A^r which induced typical erumpent canker lesions with watersoaked margin on Mexican lime, but induced flat necrotic lesions with watersoaked margin on grapefruit, Liucheng, and lemon; strains XW16 and XW121 in type A^r induced restricted and raised corky lesions with no watersoaked margin on leaves of all the four citrus species. Our results showed that biofilm formation by strain XW47 was significantly reduced, while swimming motility was enhanced compared to that by strains XW19, XW16 and XW121. The molecular mechanisms were under investigation.

Soil and stem populations of *Phialophora gregata* f. sp. *sojae* following the monoculture of brown stem rot-resistant and susceptible soybean cultivars

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Phytopathology 99:S56

Quantitative PCR was used to study the influence of monocropping brown stem rot (BSR)-resistant and susceptible soybean cultivars on the quantity and genotypic composition of *Phialophora gregata* f. sp. *sojae* (*Pgs*) populations in soil and stem samples. The overall quantity of *Pgs* was similar in soil from plots monocultured to a BSR-resistant or BSR-susceptible soybean cultivar. However, the quantity of *Pgs* genotype B was greater ($P < 0.0001$) in soil from a resistant monoculture than from a susceptible monoculture and the quantity of *Pgs* genotype A was greater ($P < 0.0001$) in soil from a susceptible monoculture than from a resistant monoculture. The overall quantity of *Pgs* was less ($P < 0.0001$) in stems of a BSR-resistant cultivar than in stems of a BSR-susceptible cultivar. Similar to soil populations, the quantity of *Pgs* genotype B was greater ($P < 0.0001$) than *Pgs* genotype A in stems of a BSR-resistant cultivar and the quantity of *Pgs* genotype A was greater ($P < 0.0001$) than *Pgs* genotype B in stems of BSR-susceptible cultivar. BSR-resistant cultivars derived from plant introduction (PI) 88788 had higher ($P < 0.0001$) quantities of *Pgs* genotype B and lower quantities of *Pgs* genotype A than BSR-resistant cultivars derived from PI 84946-2 or PI 437833. The influence of host genetics on the genotypic composition of *Pgs* populations was less pronounced among BSR-susceptible cultivars. These results demonstrate the influence of host genetics on both stem and soil populations of *Pgs*.

Effects of seed size, seeding date and seeding depth on seedling blight of canola in Alberta, Canada

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Successful canola establishment continues to be one of the greatest challenges for canola production in Alberta, Canada. Field plots were established near Edmonton, Alberta in 2007 and 2008 to determine the effects of seeding date, seed size and seeding depth on the severity of seedling blight caused by *Fusarium*, *Rhizoctonia*, and *Pythium* species. Inoculation with *Rhizoctonia* resulted in the greatest reduction in seedling establishment and yield, followed by *Fusarium* and then *Pythium*. In 2007, the earliest-seeded treatment showed lower establishment but similar yield relative to subsequent dates. In 2008, the early date showed similar establishment and higher yield. The lowest establishment and yield were obtained with small seeds (0.7 mm in diameter or less) in inoculated soils relative to medium or large seeds. Seedling establishment decreased with each 1.2 cm increase in seeding depth, but yield was not affected. The results demonstrate that conditions at the time of seeding and the size of the seed both affect seedling establishment and yield.

***Botryosphaeria* species from California tree nut crops: Exploration of species limits using multiple genes and isolates from 29 hosts on five continents**

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Phytopathology 99:S56

Species of the *Botryosphaeria* group of fungi cause serious diseases of California tree nut crops, such as band canker of almond, and panicle and shoot blight of pistachio. We used phylogenetic analyses based on six loci to investigate the genetic diversity of strains isolated from diseased tissues of California tree nuts. We evaluated species boundaries, whether species as currently defined were genetically homogenous, or they contained genetically distinct groups constituting possible cryptic species. To this purpose, we used phylogenetic analyses based on six genetic loci that we sequenced from a diverse set of 131 isolates from five continents and 29 different hosts, including 64 isolates from California almond, pistachio, and walnuts, and 13

representatives of type species. Isolates from California tree nuts fell into eight different species, *B. dothidea*, *Neofusicoccum parvum* (formerly *B. parva*), *N. mediterraneum*, one group close to *N. mediterraneum*, *Macrophomina phaseolina*, *Dothiorella sarmentorum* (‘*B.’ sarmentorum*), *Diplodia seriata* (‘*B.’ obtusa*), and *Lasiodiplodia theobromae* (‘*B.’ rhodina*). We found evidence for cryptic species, as in several instances the genetic diversity within species was greater than the genetic difference between established, morphological species. We are currently evaluating intraspecific groups that may warrant designation as separate species.

Silver scurf caused by *Helminthosporium solani* can be a polycyclic disease on potato tubers, below ground

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Phytopathology 99:S57

A lot of Yukon Gold seed potatoes having a high incidence of silver scurf, was planted in May 08 in crop environments at Mount Vernon, WA (rainfed) and Hermiston, OR (dryland). Plots were replicated 4 times, and maintained by typical cultural practices. Three plants per plot were destructively sampled 7 times in WA and once in OR during the growing season. Tissue samples were immediately observed (without washing) for signs of *Hs* using a dissecting microscope, and photographed. Select progeny tubers from one date were photographed using SEM. In the WA environment *Hs* sporulated on seed tubers prior to and following planting. Cracks and depressions near the stolon end, at eyes, and on surfaces of adjoined seed and malformed progeny tubers had highest sporulation, possibly because these tissues remain moist for extended periods. Conidia were viable as evidenced by pure cultures obtained from them. Progressive movement of conidia onto emerging roots and stolons and developing progeny tubers also was observed. New infections and sporulation by *Hs* occurred more frequently on progeny tubers than on stolon or root tissues. Of 67 and 138 progeny tubers sampled in WA and OR before harvest, 60 and < 0.01% were positive for *Hs*, respectively. The soil temperature and moisture conditions which favor events in the disease cycle of *Hs* below ground are not known. However, multiple sporulation and infection cycles of *Hs* can occur on tubers in the soil, and lead to direct spread of *Hs* from the field to storage.

Data trends and results from an HLB testing laboratory that has processed over 64,000 commercial and research samples over a two year period in Florida

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Phytopathology 99:S57

Since the discovery of Huanglongbing (HLB) in Florida in 2005, the disease management procedures recommended by the University of Florida have included scouting and removal of infected trees, control of the psyllid vector, and use of healthy material for replanting. In October 2006, Southern Gardens Citrus opened its diagnostic laboratory to the Florida citrus industry for submission of samples for analysis by real-time polymerase chain reaction testing (RT-PCR). The samples are run free-of-charge and results are typically available to the grower in 2–4 weeks depending on the sample load at the laboratory. During the first two years of operation, 64,905 samples have been submitted and tested. These represented samples from over 1265 groves, over 200 different submitters, and 27 counties. As part of the sample submission process, sample information was requested from the submitter which included among other things, location, variety, age, and temporal information. Some of key findings from the database are: 1) a gradient of the incidence of positive samples in the state from south to north, 2) visual symptoms are most evident from July to March, 3) using Ct values as a proxy, the highest titer of HLB bacteria is from July to Feb, 4) the highest incidence of infection occurs in trees 6–9 years old or 6–9 ft tall and most often in round oranges and grapefruit, least in tangerines and tangelos 5) infected trees are most likely to be found at the perimeter of blocks.

Quantitative analysis of susceptibility to *Wheat streak mosaic virus* among alternate hosts and winter wheat varieties in the Great Plains

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Phytopathology 99:S57

Grassy weeds are known to be hosts of *Wheat streak mosaic virus* (WSMV) and its vector, the wheat curl mite (WCM, *Aceria toshichella*). Although weeds have been tested by mechanical inoculation and found to be naturally infected in the field, their relative quality as a source for WCM and WSMV transmission to the wheat has not been reported. Common grassy weeds and widely planted winter wheat varieties were obtained from six Great Plains states (Montana, Colorado, Idaho, Nebraska, Oklahoma, and Texas) which vary in the frequency of WSMV epidemics. Each weed species was mechanically inoculated to determine relative susceptibility to a strain of WSMV from Conrad, Montana. *Bromus tectorum* (downy brome) from Montana and Nebraska and *Aegilops cylindrica* (jointed goatgrass) from Colorado and Nebraska had higher ELISA absorbance values than same species from the other states. This study also showed *Avena fatua* (wild oat) from Montana was as susceptible to WSMV as wheat, and *Agropyron repens* (quackgrass) from Montana, which in the literature was reported as immune, was susceptible to WSMV. These findings suggest regional differences in susceptibility of alternate hosts to WSMV. Preliminary data also suggest variation in winter wheat varieties. Each host species is currently being tested for efficiency of mite transmission. Results of this study will contribute to our understanding of regional differences in virus epidemiology.

First report of tomato foliar blight caused by *Rhizoctonia solani* AG-3 basidiospore infection in North America

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Phytopathology 99:S57

A foliar blight of tomato was observed in several organic production fields in North Carolina in 2005 and 2006. Distribution of symptomatic leaves within the plant canopy and microscopic observation of basidia on diseased tissue indicated that the disease resulted from basidiospore infection. Isolates from symptomatic tissue were identified as members of *Rhizoctonia solani* anastomosis group 3 (AG-3) based on hyphal anastomosis reaction with tester isolates and sequence analysis of the internal transcribed spacer region of ribosomal DNA. Isolates were characterized morphologically and by somatic compatibility reactions with each other. Koch’s postulates were fulfilled using a novel method for generating basidiospore inoculum of *R. solani* AG-3. Oat grains colonized with the fungus were placed in the potting mix near the base of 4–5 week old tomato plants (cv. Mountain Fresh) in 22°C growth chamber. Tape mounts of the stem at the base of the each plant were taken within a week after inoculation to confirm the presence of basidia. Infected stem tissue was then suspended over healthy tomato plants to eject basidiospores onto the leaves. Symptoms resulting from basidiospore infections were consistent with those initially observed in the field. The isolates obtained from symptomatic leaf tissue were similar in growth and morphology to the original field isolates. This is the first report of foliar blight of tomato by basidiospore infection of *Rhizoctonia solani* AG-3 in North America.

Sensitivity of *Phytophthora capsici* isolates from bell pepper and cucurbits in Georgia to mefenoxam, fluopicolide and mandipropamid

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Phytopathology 99:S57

Phytophthora blight induced by *Phytophthora capsici* is a major concern in vegetable production in Georgia and many other states in the U.S. *P. capsici* were isolated from bell pepper and cucurbits at various locations in GA. Mating types of ninety isolates were determined, which indicated that 53% of the isolates were A1 and 47% were A2 with both mating types present in most of the fields. Greenhouse studies showed that the isolates were pathogenic on squash plants and there were significant differences among the isolates in aggressiveness. Isolates resistant, intermediately sensitive, and sensitive to 100 ppm of mefenoxam were 6%, 23%, and 71%, respectively, based on *in vitro* mycelial growth. None of the isolates was resistant to 10 ppm of fluopicolide or mandipropamid. Field experiments were conducted to evaluate the efficacy of fluopicolide (Presidio) and mandipropamid (Revus) for control of *P. capsici* in 2008. Presidio applied alone or in conjunction with mefenoxam (Ridomil Gold), copper hydroxide (Kocide), and famoxadone and cymoxanil (Tanos) significantly reduced disease incidence on squash and bell pepper. Application of Ridomil Gold for soil treatment in conjunction with foliar applications of Revus, Kocide, and Ridomil Gold Copper enhanced disease suppression compared with Ridomil Gold applied alone. The results indicated that some new active ingredients could be viable alternatives to mefenoxam for managing Phytophthora blight on vegetables.

Comparative gene expression profile analysis of temperate and tropical strains of *Ralstonia solanacearum*

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Bacterial wilt is a major source of crop losses on diverse hosts, primarily in warm-temperate and tropical zones. However the Race 3 biovar 2(R3bv2) subgroup of the pathogen, *Ralstonia solanacearum*, attacks plants in

temperate zones and the highland tropics. We found that a phylotype II R3bv2 strain, UW551, and a phylotype I tropical strain, GMI1000, grew equally well in culture at tropical and temperate temperatures and caused comparable wilting on tomato at tropical temperatures. In contrast, at a temperate 20C, the R3bv2 strain was a much more aggressive pathogen. Although a large core of ORFs are conserved across *R. solanacearum* strains, about 10% of the R3bv2 ORFs are not present in the GMI1000 genome; the functions of these genes may explain the biological differences between the strains. We designed whole-genome microarray chips for UW551 and GMI1000 to test the hypothesis that the strains express different transcriptomes at different temperatures in culture and during pathogenesis. Multiple familiar factors contribute to *R. solanacearum* virulence, but little is known about the specific functions this pathogen needs to succeed in its understudied habitat, the plant xylem tissue. Characterization of plant-induced and low-temperature-induced genes should identify mechanisms underlying both wilt pathogenesis in general and the specific temperate ecological trait of R3bv2.

A new *Fusarium* species in the *Gibberella fujikuroi* species complex from pineapples with fruit rot in South Africa

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Pineapple (*Ananas comosus*) is native to South America and widely planted as a fruit crop in the tropics and sub-tropics. This plant is susceptible to a number of fungal diseases of which the most severe is fusariosis. The disease is caused by *Fusarium guttiforme* and occurs only in South and Central America and Hawaii. The occurrence of a similar disease on pineapples in South Africa has prompted a re-evaluation of the *Fusarium* spp. associated with pineapple fruit rot. Phylogenetic relationships of isolates from pineapples collected in Brazil and South Africa were assessed based on sequence data for the translation elongation factor-1α, histone H3 and β-tubulin gene regions. Analyses showed that the South African isolates represent a species distinct from Brazilian isolates. The South African isolates are characterised by a concentration of aerial mycelium at the centres of the colonies, different to the Brazilian isolates that have an even distribution of aerial mycelium. Both phylogenetic and morphological data indicate that the disease on pineapple in South Africa is caused by a new *Fusarium* species described here as *F. ananatum*, sp. nov.

Effect of application time on displacement of aflatoxin producers by the atoxigenic strain *Aspergillus flavus* AF36

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Phytopathology 99:S58

Aflatoxins are toxic and carcinogenic secondary metabolites produced by members of *Aspergillus* section *Flavi*. Aflatoxin contamination of cottonseed is a perennial problem in Arizona. Use of the atoxigenic strain *A. flavus* AF36 to competitively displace aflatoxin producers as biocontrol has been used to limit aflatoxin contamination in commercial cotton fields for over a decade. The biocontrol product has better persistence and sporulation when applied at or after canopy closure, but displacement of aflatoxin producers may not be optimal. The objective was to determine the application timing for optimal displacement of aflatoxin producers. The biocontrol was applied at different crop stages in three areas of southern Arizona in 2007. Displacement was quantified by pyrosequencing. Results indicate that crop stage at application influences efficacy of AF36. Application at initial flowering has low persistence and sporulation of the product in the field. However, these early applications have higher displacement (85%) of aflatoxin producers than applications made at later stages. Applications made when first bolls have developed but prior to opening have good residence and sporulation, and good displacement (~ 75%). Applications made after 25% of bolls open have poor displacement (< 33%) even though the product persists in the field well and sporulates extensively. Improving persistence of early applications should improve aflatoxin management with atoxigenic strains.

Characterizing culturable microflora of nectarines: Bacteria and their potential for biological control of postharvest fruit decays

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Microorganisms isolated from fruit surfaces have been used to control postharvest decays of fruit, however, there is little information on microflora colonizing surfaces of fruits other than grapes, apples and citrus. We characterized culturable bacteria on nectarine fruit surfaces during fruit development. The most frequently occurring genera were *Curtobacterium* (21.31%), *Pseudomonas* (19.99%), *Microbacterium* (13.57%), *Clavibacter* (9.69%), *Pantoea* (6.59%), and *Enterobacter* (4.26%). The frequency of isolation of some bacteria such as the major Pseudomonads (*P. syringae*, *P. putida* and *P. savastonoi*) or *Pantoea agglomerance* tended to decline as fruit developed. As *Pseudomonas* declined, *Curtobacterium* become more dominant. Time of isolation was a significant factor in the frequency of occurrence of different bacteria, indicating a succession of the genera. Some of the bacteria showed strong antagonistic activity against brown rot caused by *M. fructicola* after harvest, suggesting that these natural microflora from fruit may be useful in developing biocontrol of postharvest diseases.

Influence of temporal separation on the interaction of *Meloidogyne incognita* and *Thielaviopsis basicola* on cotton

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Phytopathology 99:S58

A synergistic interaction between *Thielaviopsis basicola* and *Meloidogyne incognita* on cotton often increases disease losses. This study characterized the nature of this interaction by examining the effect of the temporal separation of the two pathogens in controlled environmental studies conducted over six weeks. Treatments consist of: 1) plants grown in non-infested soil; 2) soil infested with one or both pathogens at planting, 10 days after planting (DAP) or 20 DAP; 3) soil infested with one or both pathogens for the first 10 or 20 DAP only; 4) and soil infested with *T. basicola* or *M. incognita* at planting, followed by inoculation with the other pathogen 10 or 20 DAP. Results showed that plant height and top dry weight were reduced when both pathogens were present earlier and for a longer duration. The interaction also occurred when the two pathogens were temporally separated. An earlier presence of *M. incognita* in the soil increased galling, while galling was reduced by the earlier occurrence of *T. basicola*. Root colonization by *T. basicola* often increased in the presence of the nematode. These results suggest that the interaction on cotton does not require an intimate contact between the two pathogens.

Impact of rotation and fungicide application on blackleg and Sclerotinia stem rot of canola

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Phytopathology 99:S58

North Dakota accounts for 90% of the nation's annual canola (*Brassica napus* L.). Canola production in North Dakota is affected by: blackleg (caused by *Leptosphaeria maculans*) and Sclerotinia Stem Rot (SSR, caused by *Sclerotinia sclerotiorum*). Growers frequently follow a two-year rotation using wheat/barley with a blackleg-resistant cultivar and occasional fungicide application at flowering for SSR to maximize profits. In 2000, a twelve-year rotation study began at the North Dakota State University-North Central Research Extension Center in Minot, ND with the objectives of studying the impact of crop rotations and fungicide use on these diseases. Six rotations were arranged in a replicated randomized complete block design. Each rotation includes canola every one, two, three, or four years alternated with canola, flax, wheat, or barley. Incidence and severity of blackleg and SSR were assessed visually. Test weight and yield were determined at harvest for all crops. Blackleg incidence and severity increased with shorter rotations, and in 2007, both were significantly higher in continuous canola than in all other rotations. In 2007, canola plots planted every four years and treated with fungicide had significantly less SSR than not treated plots. No statistical impact was detected in all other years.

Biocontrol activity by *Myrothecium verrucaria* improved by surfactant activity

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Phytopathology 99:S58

Myrothecium verrucaria is a plant pathogen of many important weed species, including kudzu. For the fungus to infect weeds and provide bioherbicidal activity, it must be co-applied with a surfactant. We evaluated several commercially available spray adjuvants and polyoxyethylene tridecyl ether (TDA) in a plant bioassay for bioherbicidal activity. In the bioassay on the weed Senna obtusifolia, all of the surfactants improved the activity of M. verrucaria over the water-only treatments and TDA formulations with a hydrophilic – lipophilic balance (HLB) number of 8 or 10 had the highest

activity. The mechanism for improved bioherbicidal activity with these adjuvants was investigated in vitro, and TDA HLB 8 and 10 did not significantly improve conidia dispersal or accelerate spore germination relative to other surfactants. It is possible that the role of the surfactant is in the alteration of the plant cuticle or otherwise preparing the infection court. Better adjuvant selection and integration with affordable synthetic herbicides should aid in the development of more cost-effective biological control of weeds.

Correlation between anthracnose leaf blight and anthracnose stalk rot as affected by corn residue level

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Corn residue is an inoculum source for important diseases, including anthracnose. Anthracnose, caused by *Colletotrichum graminicola*, occurs as either a leaf blight or stalk rot. Field experiments were established in WI to study associations among cultural practices, residue cover, and the incidence and severity of corn anthracnose leaf blight and stalk rot. The experimental design was a split-split plot with factors being tillage, corn hybrid, and the rotation of corn and soybeans. Anthracnose leaf blight incidence and severity were assessed over the season six and three times, respectively. Stalk rot was evaluated at black layer by sampling 10 stalks from each plot. Spearman's correlation analysis was used to study the associations between residue cover and anthracnose. Positive correlations were observed between residue cover and anthracnose leaf blight incidence for five of six assessments (r = 0.48, 0.34, 0.30, 0.19, 0.31; $P < 0.05$). A negative correlation was found between anthracnose leaf blight and stalk rot incidence for three of five assessments (r = –0.21, –0.24, –0.25, $P < 0.02$), and severity for all three assessments (r = –0.06, –0.07, –0.11, $P < 0.03$). There was an appearance of increased stalk rot incidence and severity in chisel-plowed plots. Results from 2008 suggest that cultural practices that result in more surface residue may have a higher incidence of leaf blight, while burying the residue may result in more stalk rot.

Effect of rotation and tillage on the development of foliar fungal diseases of corn in WI

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Phytopathology 99:S59

Field experiments were established in WI to monitor the development of foliar fungal diseases of corn as affected by cultural practices. The experimental design was a split-split plot with factors being tillage, corn hybrid, and the rotation of corn and soybeans. Incidence and severity of foliar fungal diseases were assessed over the growing season six and three times, respectively. Of seven diseases documented, three, common rust, anthracnose, and eyespot, had levels of disease at the plot scale greater than 5% incidence and 0.5% severity. Peak incidences were: anthracnose, 18% at 71 days after planting (DAP); common rust, 100% at 77 DAP; and eyespot, 18% at 87 DAP. Regardless of peak incidence values, severity remained low at < 3% plot average throughout the season for all diseases. A nested ANOVA was used to determine the effects of rotation and tillage on disease development (AUDPC). Common rust development was not affected by cultural practices. Anthracnose leaf blight and eyespot development were affected by rotation (p = 0.057, 0.041), but not tillage. Corn disease development in WI during 2008 was reduced by cool and dry weather during the corn reproductive growth period. Understanding that residue borne disease development is augmented by continuous corn cropping is important for crop management decisions during the season, especially when environmental conditions are more favorable for leaf blight.

Effect of type III and type II secretion on *Acidovorax avenae* subsp. *citrulli* colonization of watermelon seed and seedling tissue

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Acidovorax avenae subsp. *citrulli* (*Aac*), causes bacterial fruit blotch (BFB) of cucurbits. Infested seed is an important source of *Aac* inoculum, however little is known about the factors that affect seed to seedling transmission of *Aac*. The aim of this study was to determine the roles of type III and II secretion systems in the colonization of watermelon seed and seedlings by *Aac*. The type III secretion system (T3SS) facilitates translocation of virulence proteins directly into plant cells. Deletion of the AAC00-1 T3SS structural gene, *hrcC*, resulted in loss of pathogenicity and failure to develop a HR. Interestingly, AAC00-1Δ*hrcC* still colonized watermelon seed at wildtype levels during germination. Seedlings produced from seed inoculated with AAC00-1Δ*hrcC* remained asymptomatic, while seedlings from seed inoculated with AAC00-1 developed symptoms. The type II secretion system (T2SS) facilitates secretion of degradative proteins into the extracellular matrix. The role of T2S in seed colonization was investigated using a prepilin peptidase (*pulO*) mutant. AAC00-1*pulO*::TnGFP attained populations 10^6-fold less than AAC00-1 on germinating seed and caused reduced disease incidence (11% compared to 91.5% for AAC00-1) on resulting seedlings. These results suggest that the T3SS is important for *Aac* pathogenicity, but is not required for colonization of germinating seed. Additionally, the T2SS is required for seed colonization and efficient seed to seedling transmission of *Aac*.

Alternative methods to control *Pythium* in tobacco transplant production in 2008–9

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Phytopathology 99:S59

Pythium species limit tobacco transplant production in hydroponic greenhouses. Terramaster (etridiazole) provides excellent control, but is expensive, can result in plant growth regulator effects, and fungicide-treated nutrient solution must be appropriately disposed of after transplanting. Therefore, greenhouse tests were conducted to evaluate alternative methods of *Pythium* control. A spring 2008 trial compared damping off and root rot among seedlings of burley tobacco cultivar 'TN90' after incorporating PGPR (BioYield) into the plant growth medium or after applying H_2O_2 or Terramaster (etridiazole) to the nutrient solution upon which seedlings were grown. BioYield did not reduce damping-off incidence compared to the untreated control. Nutrient solutions containing 100 ppm H_2O_2 lowered (k-ratio = 100) disease incidence 39 days after seeding versus the untreated control, but not on 2 earlier dates and at the final observation. Curative application of Terramaster minimized *Pythium* incidence at all dates. In two subsequent trials, 1000 ppm Naiad (a commercially-available surfactant) suppressed (k-ratio = 100) early disease levels versus the untreated control and comparably to curative application of Terramaster. Inoculations 23 & 41 days after seeding in a 3rd and final test resulted in disease incidences ≥ 85% in all treatments. Further research is needed to confirm the possibility of commercially acceptable control of *Pythium* on tobacco seedlings through use of surfactants.

Development of a Real-Time RT-PCR assay for the detection of *Cucumber mosaic virus*

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Phytopathology 99:S59

Cucumber mosaic virus (CMV) infects over 800 species in 85 families, has a worldwide distribution, and is considered as one of the most important viruses infecting horticultural and ornamental crops. A number of diverse strains of CMV have been reported and characterized and can be divided into two main subgroups by biological and serological properties, and by nucleotide sequence homologies. Although serological and biological methods for the detection of CMV are available, they may not offer the sensitivity for reliable detection of low levels of CMV in early infections and/or in asymptomatic plants serving as reservoirs for transmission to symptomatic crops. In addition, a sensitive, quantitative nucleic acid-based method is needed to determine the expression levels of virus-related transgenes in genetically-modified plants. We have developed a highly-sensitive, quantitative Real-Time Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) assay that can detect genetically diverse CMV strains (from both serogroups I and II) and virus transgenes in genetically-engineered gladiolus. Primers include sets for viral replicase and for coat protein genes. Variations of the method use either a single conserved TaqMan probe or the green fluorescent dye SYBR Green I to determine the absolute (fg level) and relative copies of CMV genomic RNAs or transgenes contained in purified virions, or in total RNA extracts from infected or transgenic plant tissues.

Characterization of kudzu (*Pueraria* spp.) resistance to *Phakopsora pachyrhizi*, the causal agent of soybean rust

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Phytopathology 99:S59

Soybean rust (SBR) has the potential to be one of the most devastating diseases for soybean in North America. Kudzu, (*Pueraria* spp.), is an accessory host for SBR that is widespread throughout the Southeast U.S. The SBR pathogen overwinters on kudzu and is a likely source of initial inoculum each year. In Florida, some kudzu sites have appeared symptomless for SBR

since 2004. To further assess the variable susceptibility of kudzu to SBR, we evaluated and characterized SBR resistance in wild kudzu populations. Ten accessions of kudzu from North Central Florida were characterized for their response to foliar inoculation by *Phakopsora pachyrhizi*. Three responses were observed: 6 accessions had tan lesions with profuse sporulation (susceptible), 1 accession had slightly larger, reddish-brown lesions with delayed, reduced sporulation (resistant), and 3 accessions did not develop lesions (immune). QRT-PCR was performed on *P. pachyrhizi*-inoculated leaf tissue to quantify colonization. After 15 dpi, the amount of SBR-pathogen DNA (26.7 ng/µl) was nearly 7X greater in the susceptible than in the resistant kudzu (3.8 ng/µl); in immune kudzu, the pathogen DNA quantity was below the detection level. Susceptible kudzu had half the amount of pathogen DNA present (26.7 ng/µl) when compared to a susceptible soybean variety (52.6 ng/µl). The cytology of the SBR-kudzu interaction and the production of H_2O_2 also were examined. This is the first report of SBR resistance in kudzu.

Construction of a DNA-based virus induced gene silencing (VIGS) system for functional genomics of soybean seed development using *Tobacco streak virus*

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Phytopathology 99:S60

Virus induced gene silencing (VIGS) is an effective tool for reverse genetics in plants. Suppression of gene expression is achieved by infection of plants with a virus vector carrying a fragment of an exon of a plant gene. Plant viruses that are suppressed by post-transcriptional gene silencing (PTGS) and show strong recovery from infection (associated with degradation of viral RNAs) are the best candidates for VIGS vectors. *Tobacco streak virus* (TSV), a seed transmitted member of the *Bromoviridae*, has a tripartite single-stranded, positive-sense, RNA genome and requires coat protein along with RNAs 1, 2 and 3 for infectivity. Full-length cDNAs of RNAs 1, 2 and 3 and subgenomic RNA 4 of an Illinois isolate of TSV were cloned into pHST40 and sequenced. When biolistically inoculated into soybean plants, the clones were highly infectious and showed strong recovery and cultivar-specific rates (0–98%) of seed transmission. To construct VIGS vectors, a multicloning site (MCS) was introduced into RNA 2 at the end of the intact and two truncated versions of the open reading frame that encodes the 2b protein. The 2b protein has been implicated in cell-to-cell movement and suppression of PTGS. The modified TSV clones were highly infectious. The complete coding region of the green fluorescent protein gene and portions of the phytoene desaturase gene were inserted into the MCS to produce fusion proteins with 2b. These clones are being evaluated for infectivity and silencing.

Evaluation of wild apple (*Malus sieversii*) germplasm from Kazakhstan for resistance to *Penicillium expansum* and *Colletotrichum acutatum*

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Penicillum expansum and *Colletotrichum acutatum* cause postharvest decay of apple fruit resulting in significant economic losses during storage. However, little resistance to both fungi exists in the domesticated apple gene pool. Therefore, a collection of wild apple (*Malus sieversii*) germplasm from Kazakhstan (apple center of origin), located and maintained in Geneva, N.Y., was evaluated for resistance. Fruits from over 175 Kazak accessions were harvested at the preclimacteric to climacteric stage and were wound-inoculated with conidial suspensions of *P. expansum* and *C. acutatum* at 10^3 and 10^4 mL^{-1}. Twenty Inoculated fruit per conidial concentration from each accession were incubated at 24°C for 5 (for *P. expansum*) or 6 days (for *C. acutatum*) and then evaluated for decay incidence and severity. For *P. expansum,* 7 accessions were classified as immune (no decay), 38 as resistant (no decay at 10^3 mL^{-1}), 142 as moderately resistant (lesions <10 mm at 10^3 mL^{-1}), and 3 as susceptible. For *C. acutatum,* 1 accession was categorized as immune, 12 were resistant, 97 were moderately resistant, and 65 were susceptible. Differences in individual host resistance against both pathogens were expected due to differences in fungal lifestyles exhibited by *P. expansum* and *C. acutatum*. Both resistant and immune Kazak accessions can serve as a source of genetic material in breeding programs and may be used in molecular studies to identify the genetic component(s) of host resistance to these important postharvest pathogens.

Characterization of three isolates of *Pyrenophora-tritici repentis* collected from winter wheat in Oklahoma in 1983, 1996, and 2006

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Using plant pathogen isolates representative of field populations is important in developing disease resistant germplasm. Three isolates of *Pyrenophora tritici-repentis*, cause of tan spot of wheat, were compared for hyphal growth, sporulation, reproduction, and virulence on wheat. These isolates, OKD-1, RBB6 and OK06-1, were collected in Oklahoma in 1983, 1996 and 2006, respectively. Hyphal growth and sporulation were tested on clarified V-8 juice agar (CV8) and on wheat leaves. Formation of pseudothecia was tested on wheat straw, and virulence was determined on the hard red winter wheat cultivar Deliver in the greenhouse and in a field trial. Greatest radial growth was observed for OK06-1, which also produced significantly ($P < 0.05$) more conidia on CV8 and on wheat leaves. Isolates were similar in number of pseudothecia formed; OK06-1 produced the highest percent of mature pseudothecia (22.0%), followed by OKD-1 and RBB6. RBB6 produced significantly less conidia on CV8 and on leaves than OKD-1, but was more virulent in the field. Maximum disease severity was recorded for OK06-1 in both greenhouse and field studies. In the field, OK06-1 reduced yield by 20.7% compared to the control, whereas RBB6 and OKD-1 reduced yield by 13.8 and 4.9%, respectively. These results indicate that selection of *P. tritici-repentis* isolates used in germplasm testing programs or in disease management and epidemiological studies is important as isolates vary with time.

Loss of efficacy of fungicides in the management of coffee berry disease in Kenya

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There are five commercial varieties of coffee (*Coffea arabica* L.) grown in Kenya. All except one are susceptible to *Colletotrichum kahawae* the cause of Coffee Berry Disease (CBD). The disease attacks green expanding berries resulting in 80% crop loss or more. The disease is managed with various recommended fungicides applied in an established protective spray programme. In two fungicide evaluation trials conducted in 2007 and 2008, the efficacy of chlorothalonil and copper formulations declined in the second year. After the first year sprays, *Colletotrichum kahawae* inoculum on mature bark of cropping branches was tested for sensitivity to chlorothalonil (Daconil 720 SC, 0.3%). The resultant data indicated that bark samples from sprayed plots maintained >22.84% sporulation of *C. kahawae* in the presence of Daconil 720 SC, 0.3% sensitivity treatment. This was an indication that a low population of *C. kahawae* tolerant to chlorothalonil was building up as a result of continuous application in coffee plantations for a period not less than 38 years. Although mature bark of cropping branches is not the most important source of *C. kahawae* inoculum as is the lesions on green berries, it is responsible for initial berry infections where out-of-season crop is absent. Thus, there is a possibility for fungicide tolerant *C. kahawae* population to become dominant resulting in disease stimulation as happened in 2008. The implications of these findings will be discussed.

Pathogenic and genetic diversity of *Alternaria alternata* isolates from Tangerine hybrids of Iran, based on RAPD-PCR technique

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Phytopathology 99:S60

Alternaria brown spot is one of the most worldwide important diseases of citrus (Tangerine Hybrids). The disease is caused by *Alternaria alternata* (Fr.) Keissl, and make serious economical losses. In order to study of pathogenic variability and genetic diversity of the pathogen 42 isolates of *Alternaria alternata* from Tangerine hybrids collected from different regions of Iran. Pathogenic variability was evaluated through in vitro conditions. The results revealed considerable variation in aggressiveness of the isolates. Cluster analysis of the isolates classified them into three categories: highly, moderate and weekly virulent groups. Genetic diversity was analyzed based on RAPD-PCR using 15 random 10mer primers and Cluster analysis of DNA fragments was performed using NTSYSpc V2.2 based on UPGMA method and Jacard coefficient. The isolates divided into distinct groups at 80% similarity level. However The RAPD- PCR technique could not separate isolates based on pathogenicity but showed high correlation between geographical origins of isolates.

Genomic structure and organization of a plant pararetrovirus (family *Caulimoviridae*) infecting *Rubus* species

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Badnaviruses often cause devastating economic losses of infected crops. They have a bacilliform particle with a circular dsDNA genome encompassing a

long intergenic region followed by three open reading frames (ORF). Most of the biological information known about badnaviruses originates from the characterization of ORF 3, a large polyprotein consisting of the movement protein, coat protein, protease and the polymerase. Characterization and the functions of ORFs 1, 2 and other putative genes remain largely unexplored. Here, we present the complete genomic sequence of *rubus yellow net virus* (RYNV) and confirm the sequence through the development of a full length infectious clone. The 7936 bp genomic sequence is organized in a similar manner to other badnaviruses but with the presence of additional putative ORFs 4 and 5 downstream of ORF 2. We examined the various RYNV ORFs through genetic and secondary structural analyses combined with sequence comparisons to other related viruses. Availability of a full length infectious clone will enable us to study the function of these genes *in planta*.

A new *Rhizoctonia* sp. pathogenic to seashore paspalum turfgrass

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Phytopathology 99:S61

A wide diversity of *Rhizoctonia* species, anastomosis groups (AG), and varieties cause diseases of both warm and cool season turfgrasses. *Waitea circinata* is a teleomorph of some *Rhizoctonia* spp. Over sixty *Rhizoctonia*-like isolates were collected from thinning patches of seashore paspalum from a few inches to a few feet in diameter and with orange to yellow borders, on eight different golf course fairways in Florida over an 18-month sampling program. A group of four isolates from three golf courses resembled *Rhizoctonia* morphologically and had profuse pink to yellow mycelia in culture and produced small dark brown sclerotia. Three of four of these isolates were obtained from the 3 January, 2008 sampling. Isolates were insensitive to thiophanate-methyl fungicide and were difficult to get into pure culture. Amplified fragments of rDNA including internal transcribed spacers from the isolates were sequenced bi-directionally using primers ITS1/ITS4. Consensus sequences for these 4 isolates matched with 98% homology isolates NUK-3BG and DAI-5BG of *Waitea circinata* var. *circinata* (GenBank accession numbers AB213567 and AB213569), the Waitea reddish-brown patch pathogen of bentgrass first identified in Japan. Pathogenicity was confirmed on two cultivars of seashore paspalum and 'Penncross' creeping bentgrass in incubator inoculations. This is the first report of Waitea reddish-brown patch on a warm season grass and the first reported isolation of this strain of *Rhizoctonia* in the United States.

Monitoring the effectiveness of *Phytophthora ramorum* eradication treatments in southwest Oregon tanoak forests

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Phytopathology 99:S61

Phytophthora ramorum, the cause of sudden oak death was first discovered in Oregon forests in July 2001, and has since been the focus of an aggressive eradication effort. Eradication treatments consists of cutting and burning infected and exposed host plants, and where possible, injecting herbicide into tanoaks to prevent sprouting. The effort has slowed, but not stopped, long-distance dispersal of the pathogen. To monitor the effectiveness of eradication treatments we are revisiting treated sites and sampling soil and vegetation in fixed plots centered on stumps of known infected trees. All samples are assayed for *P. ramorum* at the Oregon State University lab and confirmed by the Oregon Department of Agriculture. To date we have visited 25 SOD-infested sites that had received treatments between 2001 and 2008. Time since treatment for the sampled sites ranged from 1 to 5 years. *Phytophthora ramorum* was not recovered from 17 (68%) of the 25 sites sampled. Eight (32%) of the sites yielded cultures of *P. ramorum* from soils, and 4 (16%) yielded P. ramorum from vegetation. Positive vegetation samples were from tanoak and rhododendron and were always associated with sites that also had positive soil samples. Positive samples were recovered only from sites that were treated within the past two years, and most were from sites that were treated one year prior to sampling. Additional sampling will provide a better basis to infer persistence of *P. ramorum* on treated sites.

Early detection and eradication of *Phytophthora ramorum* (sudden oak death) in Oregon forests

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Phytopathology 99:S61

Sudden Oak Death (SOD), caused by *Phytophthora ramorum*, was first discovered in Oregon forests in July 2001. An interagency team has been working with landowners to eradicate the pathogen by cutting and burning all infected and nearby host plants. During the first four years eradication (2001–2004), the number of new infested sites remained steady or decreased each year, indicating modest success at containment and eradication. In 2005 the area infested began increasing. Delays in completing treatments and consecutive years of unusually wet spring and early summer weather contributed to spread of the disease, forcing the expansion of the quarantine zone from 26 mi^2 to 162 mi^2 in 2008. Between 2001 and 2008 we have treated more than 2,400 acres at a cost of over $4 million. Eradication costs have been funded by the USDA Forest Service, the Oregon Department of Forestry, USDA-APHIS, and the USDI-Bureau of Land Management. There is no compensation to landowners for the value of timber or other resources lost as a result of the eradication treatments. We have eliminated the pathogen from many sites and we have restricted spread to a relatively small area. A stream-bait early detection program and repeated aerial and ground surveys throughout southwest Oregon have failed to detect the pathogen in forests beyond the quarantine area.

Construction and ESTs analysis of a cDNA library of wheat leaves challenged by *Puccinia striiformis* f. sp. *tritici*

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Phytopathology 99:S61

Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), has been one of the most important wheat diseases worldwide. In order to elucidate the wheat resistant mechanism at the molecular level, wheat cultivar shuiyuan11 and *Pst* isolate CY23 were used as the starting materials to construct an cDNA library using mixed RNA samples isolated from wheat leaves 16 h, 24 h, 36 h and 48 h after inoculation, respectively. A total of 5163 positive clones were sequenced and assembled into 1515 unigenes including 494 contigs and 1021 singlets. The results of function annotation showed that approximately 46% unigenes could not be identified, the remainder 54% was mainly assigned to 10 categories. Remarkably, about 18% unigenes were putatively encoded for the resistance- and defense-related genes, including phenylalanine ammonia-lyases, ascorbate peroxidases, catalases, Zn finger protein, hypersensitive-induced reaction protein, wound induced genes, and so on. Real-time PCR analysis of 32 genes showed differential expression patterns, suggesting that these genes play different roles in incompatible interaction between wheat and *Pst*.

***Nicotiana benthamiana* as a model plant to study aphid transmission of plant viruses**

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Phytopathology 99:S61

To facilitate and standardize molecular genetic studies of plant virus transmission by aphids, we adapted two colonies of green peach aphid, *Myzus persicae* Sulz., to a model plant, *Nicotiana benthamiana* Domin. The first colony, A1, was a sub-population of aphids reared on potato, and the second colony, A2, was an accidental find. Both colonies, A1 and A2, were shown to successfully feed and breed on greenhouse-grown *N. benthamiana* plants, and able to transmit viruses in an experimental setting. Three types of transmission were tested: non-persistent with *Turnip mosaic virus* (TuMV); semi-persistent with *Beet yellows virus* (BYV); and persistent with *Potato leafroll virus* (PLRV). The efficiency of green peach aphid transmission for both colonies, A1 and A2, was similar for TuMV and BYV, and varied from 4% for TuMV to 10% for BYV, while transmission efficiency significantly differed for the persistently transmitted PLRV, from ca. 20% for A1 to ca. 80% for A2 colony. Colonies A1 and A2 differed in their fecundity and mortality when kept on *N. benthamiana*, with colony A2 demonstrating greater affinity to the new host. Our prolonged experience with the A2 colony of *M. persicae* indicated that it can be stably maintained on *N. benthamiana* and used for studies of different unrelated viruses infecting *N. benthamiana*, including virus mutants in genes affecting aphid transmission. We believe an *N. benthamiana*-adapted colony of *M. persicae* aphids would be a useful tool for studies of plant virus vector transmission.

Characterization of naturally avirulent strains of *Burkholderia glumae*, the causative agent of bacterial panicle blight of rice
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Phytopathology 99:S62

Burkholderia glumae is the bacterial pathogen of rice causing bacterial panicle bight (BPB). *B. glumae*, whose growth and pathogenicity is favored by high temperatures, has become a serious threat to rice production around the world due to the current global climate changes. Previously, we isolated more than 240 *B. glumae* strains from the rice fields in Louisiana, Texas, and Arkansas. These strains were tested for virulence on both rice seedlings and panicles. Remarkably, at least 22 field strains (ca. 9%) showed avirulent or drastically reduced virulent phenotypes in the virulence tests. In this study, the naturally occurring avirulent and near avirulent strains were characterized in respect to the production of known and potential virulence factors including toxoflavin (the major toxin produced by *B. glumae*), lipase, and polygalacturonase. The ability to elicit a hypersensitive response (HR) on tobacco leaves and the motilities mediated by flagella and type IV pilli, which are also involved in bacterial pathogenesis, were also tested. Interestingly, considerable phenotypic variation was observed among the naturally avirulent and near avirulent strains. This observation suggests that the occurrence of avirulent strains in nature is not a rare event, probably because many genes are involved in the pathogenicity of *B. glumae*. In this presentation, diverse phenotypic characteristics associated with avirulent and near avirulent *B. glumae* strains will be presented.

Management of diseases in vegetable crops by using *Trichoderma* and *Pseudomonas*
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Phytopathology 99:S62

Vegetable farmers, particularly smallholders, confront a number of constraints in the vegetable production. The production risks are high primarily because of considerable production losses caused by pests. These are estimated to be about 30% of the total vegetable output. The farmers end up giving 15–30 sprays with pest control below their satisfaction level. Demonstration of IPM package involving seed and seedling treatment with *Trichoderma* and *Pseudomonas* for disease management in vegetable crops viz. eggplant (brinjal), okra (lady finger), and tomato, were carried out in 15 villages of Uttar Pradesh, Karnataka, and Andhra Pradesh in India under USAID IPMCRSP program. The IPM practices not only helped in reducing the reliance on pesticide by 50–70% reduction in pesticide spray but also enhanced the quality of the produce, production and income of the farmers. Farmers got 2–3 times higher price for their produce.

A survey for citrus blight diseases in the Eastern Mediterranean region of Turkey
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Phytopathology 99:S62

The surveys for Citrus Blight (CB) disease have been done in various citrus orchards which are more than twenty of years old in different locations in the Eastern Mediterranean region. Trunks of trees showing CB-like symptoms have been water injected by syringe within 60 seconds and samples from their fibrious roots have been collected for molecular analysis of p12 gene. The surveys have been completed in 23 different orchards and locations in Adana, Karatas, Misis, Kozan, Tarsus and Mersin during 2006–2008. One hundred and ninty-eight trees have been water injected on their trunks within 60 seconds and water uptake by the trunk has been calculated as ml per second. Based on water injection test, 0,0-0,3 ml of water has been uptaken by 81 trees, 0,5-1,5 ml of water has been uptaken by 76 trees, over 0,3 ml of water has been uptaken by 12 trees, and over 1,5 ml of water has been uptaken by 29 trees within one second. It has been concluded that 81 trees out of 198 have been found suspicious for CB disease based on water syringe test. Among these trees, 45 of them have uptaken 0,0-0,3 ml of water in one second and these trees have been studied for p12 gene which is specific for CB associated protein p12. Thirty eight trees out of 45 gave positive cDNA band for the 396 bp of p12 ORF gene amplification by RT-PCR.

An establishment of *in vitro* shoot-tip culture system in some stone fruit rootstocks for future development of disease-free rootstocks
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Phytopathology 99:S62

In this research, *in vitro* propagation of apricot and plum rootstock, Myrobolan 29 C (*Prunus cerasifera*), peach and nectarin rootstocks, Garnem (GN) and GF 677, and sweet cherry rootstocks, Maxma and PHLC was studied. Shoot-tips between 3 and 5 mm length were exiced from all tested rootstocks and cultured on modified MS, MST and MNJ mediums. The number of adventive shoot formation from all rootstoks was evaluated 6–8 weeks after culturing. The best results were obtained in adventitous shoot formation for Myrabolan 29 C when 0.5 and 1.0 mg/l BAP concentrations were added to the modified MS medium. The best adventitious shoot development for Garnem, GF-677, Maxma, and PHLC was obtained with addition of 2.0 mg/l of BAP into the MST and MNJ mediums respectively and the longest shoot development was acquired with the combination of 2.0 mg/l of BAP, 0.05 mg/l of GA_3 in the mediums. The average number of shoot siblings in Myrabolan 29 C, GF-677, Garnem, Maxma, and PHLC were 6.07, 1.8, 2.2, 3.1, and 6.1 respectively. The developed shoots were rooted within two weeks with the addition of either 1.0 mg/l or 1.5 mg/l of IBA to the their own mediums separately. Then rooted plantlets were transferred into pots including commercial torf for acclimatization.

Identification of some fungal diseases of canola (*Brassica napus* L.) in the Eastern Mediterranean region of Turkey
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Phytopathology 99:S62

Canola has been grown as a seed crop in Adana, Osmaniye and Hatay provinces in the Eastern Mediterranean region for five years or so. Since the Eastern Mediterranean region has warm climate condition several plant diseases take place in any field crops. In this scheme, at first, fungal diseases have been identified in canola crops surveyed in Adana, Osmaniye and Hatay during 2007–2008. Survey period has lasted from flowering to harvesting period. Four different fungal pathogens have been identified in canola plants from the flowering to harvesting stage. Whereas lower leaves have shown circular black spot syptoms with a varying in size infected by *Alternaria alternata* during flowering stage, pods have shown similar black spots symptoms, but small in size by the infection of *Alternaria brassicae*. During the drought years, 2007–2008, powdery mildew has covered almost all plants and idendified as *Erysiphe cruceferarum.* Additionally, more than 50% of the canola crops has been infected by *Pseudocercosporella capsellae* (*Mycosphaerella brassicicola*) showing starry speckled grey to black in colour appearance on the stems.

The effect of sulfur in fungicide trials for the control of *Erysiphe polygoni* causal agent of powdery mildew on sugar beets
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Phytopathology 99:S62

Erysiphe polygoni, the causal agent of powdery mildew (PM) on sugar beets, has caused significant economical losses in commercial fields. In the past, sulfur was applied as foliar treatment for the control of PM. Recently the University of Idaho introduced and evaluated several new fungicides for their effectiveness for the control of PM. In 2008, prothioconazole (Proline 480 SC), trifoxystrobin (Gem 500 SC), a combination of the individual products, and sulfur (S) were tested for the control of PM. Treatments were replicated 6 times and applied before disease onset (July 10), followed by a second application on July 31. Disease ratings were taken July 9, 30, and August 20. When compared to the non-treated control, (UTC) GEM+S decreased the percentage of diseased mature leaf area by 53%, a 14.5% increase of control when compared to GEM alone. Proline and Proline+S achieved 100% control of PM. The effects of additional S were even more obvious for the area under the disease progress curve (AUDPC), root yield, and estimated recoverable sugar (ERS) when treatments were compared to the UTC. Additional S decreased the AUDPC in GEM treated plants by 15.6% from 38.7 to 30.3, increased the root yield by 6.1% for both fungicides (GEM: 37.3 to 38.6 tons/A, Proline: 40.2 to 42.3 tons/A), and increased ERS by 2.8 and 7.1% for GEM (4.66 to 4.78 tons/A) and Proline (5.11 to 5.40 tons/A) respectively.

First report from South Carolina of boscalid-insensitive isolates of *Didymella bryoniae* on field-grown watermelon treated with boscalid-pyraclostrobin
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Several isolates of *Didymella bryoniae*, causal agent of gummy stem blight on cucurbits, were found to be insensitive to boscalid in Georgia in 2007. In 2008, four isolates of *D. bryoniae* from South Carolina were used to inoculate watermelons in replicated field plots in Charleston, SC. Two isolates were sensitive to boscalid and pyraclostrobin in vitro; one isolate sensitive to boscalid had reduced sensitivity to pyraclostrobin; and the fourth isolate had reduced sensitivity to boscalid and was insensitive to pyraclostrobin. At the

last harvest, mean diseased leaf area did not differ (P = 0.01) between nonsprayed plots (91.5%) and plots sprayed with 3 applications of boscalid-pyraclostrobin (Pristine®) rotated with 4 applications of chlorothalonil (83.5%). Of 12 isolates recovered from diseased leaves, 8 were insensitive to boscalid and 1 was insensitive to pyraclostrobin, based on germination of >50% of conidia and ascospores on water agar plus 10 mg/l boscalid or 10 mg/l pyraclostrobin plus 100 mg/l SHAM. All 8 isolates insensitive to boscalid in vitro caused disease on muskmelon seedlings sprayed with 855 mg/l boscalid-pyraclostrobin and on seedlings sprayed with 567 mg/l boscalid, the rate of boscalid in Pristine. For 2 isolates, disease severity on boscalid-treated seedlings did not differ significantly from severity on seedlings sprayed with water. Two isolates sensitive to boscalid in vitro did not cause disease on seedlings sprayed with boscalid.

Location affects suppression of Fusarium wilt in seedless watermelon grown after winter cover crops of hairy vetch and hybrid common vetch

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Fusarium wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum* is a widespread problem on seedless watermelon. In Maryland, incorporating winter cover crops of hairy vetch (*Vicia villosa*) reduced Fusarium wilt and increased yield. The objectives of this study were to i) test vetch incorporation in South Carolina and Maryland and ii) compare 'Cahaba White' hybrid common vetch (*V. sativus* × *V. cordata*) to hairy vetch. Field plots of the vetches and rye were seeded in fall 2006 and 2007 in naturally infested fields in South Carolina and Maryland. Cover crops were incorporated into soil in spring 2007 and 2008 2 to 4 weeks before seedless watermelons cv. Sugar Heart (susceptible) and Revolution (resistant to race 1) were transplanted into each cover crop whole plot. In South Carolina, wilt incidence was lower after 'Cahaba White' than after rye in 2007; wilt incidence did not differ among cover crops in 2008. In Maryland, both vetches reduced wilt incidence by 28% compared to rye in both years. Marketable weight of fruit was 59% greater after vetch than after rye in Maryland in 2007; yields did not differ among cover crops in the other experiments. In all experiments, wilt incidence of 'Revolution' was 50% lower than wilt incidence of 'Sugar Heart,' and yield of 'Revolution' was double the yield of 'Sugar Heart.' Further research is needed to elucidate how location and environment influence the reduction in Fusarium wilt in watermelons grown after vetch.

First report of *Fusarium oxysporum* f. sp. *niveum* race 2 in South Carolina watermelon fields

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Fusarium wilt of watermelon is caused by *Fusarium oxysporum* f. sp. *niveum* races 0, 1 and 2. Most triploid seedless watermelon cultivars are susceptible to all races. As the acreage of seedless watermelon has increased in South Carolina, outbreaks of Fusarium wilt have become more frequent. *F. oxysporum* was isolated from discolored vascular tissue in wilted watermelon vines collected from commercial fields in 1999, 2005 and 2008; from a research field in 2005; and from diseased seedlings in a commercial transplant greenhouse in 2006. Isolates were recovered from triploid (40 isolates), diploid (5), and unknown (2) cultivars and a pollenizer watermelon (11). Twenty seedlings each of 3 differential cultivars Black Diamond or Sugar Baby (susceptible to all races), Charleston Gray or Crimson Sweet (susceptible to races 1 and 2), and Allsweet (susceptible to race 2) were inoculated by dipping roots in a suspension of 10^6 microconidia/ml and evaluated 2 and 3 weeks later. Of 58 isolates tested, 2 were nonpathogenic, 0 were identified as race 0, 17 were identified as race 1, and 39 were identified as race 2. Race 2 isolates were found in 4 counties in all 6 fields sampled. The oldest race 2 isolates were collected in 1999. The 11 isolates from the greenhouse were all identified as race 1. This is the first report of *F. oxysporum* f. sp. *niveum* from greenhouse-grown seedlings in South Carolina and the first report of race 2 of *F. oxysporum* f. sp. *niveum* in South Carolina.

Large scale field screening of transgenic anthuriums for bacterial blight resistance

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Anthuriums are the most important cut flower for the Hawaiian floriculture industry today, with sales valued at 4.8 million dollars. Anthuriums in Hawaii were virtually disease-free until the early 1980s, when bacterial blight caused by the pathogen *Xanthomonas axonopodis* pv. *dieffenbachiae* struck the industry, causing production figures to decrease by over 75%. Blight control and management adds approximately 20–30% to the cost of production. Transgenic anthuriums were developed to determine if any of the available transgenes would provide some degree of protection against the bacterial blight pathogen. A large scale screening method to test for bacterial blight resistance in transgenic 'Marion Seefurth' and 'Midori' anthuriums was developed. Humidity chambers were designed and constructed, and inoculation methods were compared, and the most reliable method was selected for all future experiments. To date, more than 2,500 plants, from more than 150 lines, have been inoculated and evaluated for disease resistance. Data and photographs have been compiled and compared. Certain lines show some promise and have been selected for further testing. Developing bacterial blight resistant lines of the highest value cultivars has the potential to save the anthurium industry millions of dollars in manpower and chemical control.

Integrated community outreach programming to prevent spread of pine wilt into western Kansas

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Phytopathology 99:S63

Pine wilt, caused by the pinewood nematode (*Bursaphelenchus xylophilus*) is a fatal disease that is a threat to *Pinus nigra* and *P. sylvestris* windbreaks, conservation plantings, and landscapes in western Kansas. Pine wilt was discovered in 1979 in extreme southeast Kansas. The disease has moved west at approximately 10 miles per year, killing thousands of pines, and is now present approximately halfway across the state. We have developed an integrated Pine Wilt Initiative to disseminate information about pine wilt in communities directly along the leading edge of disease and beyond. Timely sanitation (removal and destruction of infected pines) will reduce the likelihood of establishment in western KS plantings, where tree resources are scarce and thus highly valued. We have developed printed materials in English and Spanish (to reach a growing Spanish-speaking population in southwest Kansas). We have held several community-based workshops with homeowners, arborists, and city foresters to raise awareness of pine wilt and train them as early detectors. The program closely integrates university, forest service, regulatory, and county personnel in addition to private tree care professionals and private landowners.

The role of necrosis and infection inducing compounds by germinating spores of *Botrytis cinerea* in pathogenesis

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The infection strategy of *Botrytis cinerea* is thought to include killing of host cells and feeding on dead tissue. It secretes cell wall degrading enzymes and toxic metabolites during infection. Herein, pathogenicity of several field isolates of *Botrytis* was examined with water-suspended conidia. An isolate BC02.RO from rose petal exhibited strong pathogenicity in several hosts. We have devised a method to prepare the secretomes by BC02.RO during spore germination. The spore germination fluid (SGF) accelerated lesion development in *Nicotiana benthamiana*, 3 days post treatment and induced accessibility of a non pathogen *Alternaria alternata* (15B). Spores of 15B with SGF were able to infect and develop lesion on leaves of several crops. That is, BC02.RO-SGF contained non-specific toxic and infection-inducing factor(s). Both activities are exhibited in 10–30 kDa fraction of SGF. H_2O_2 generation and superoxide accumulation was occurred in histochemical staining of SGF (>40 µg/ml) treated leaves (>12 h). Quantitative measurements also confirmed the microscopy data. Moreover, SGF is able to cause host cell death and concomitant lesion formation in *N. benthamiana*, suggesting a primary pathogenicity determinant(s) in SGF. Pharmacological approach could be modulated the SGF-induced H_2O_2 generation. Characterization of SGF in the establishment of successful infection is in progress. Altogether, this piece of work describes a new type of virulence factor of *Botrytis*.

Resistance to pyraclostrobin and boscalid in *Botrytis cinerea* populations from apple in Washington State

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Gray mold caused by *B. cinerea* is a major postharvest disease of apples. Pristine, a mixture of pyraclostrobin and boscalid was recently registered for

use on apple. Pristine applied within two weeks before harvest has been shown effective in controlling gray mold in stored apples. To determine the distribution of sensitivity of *B. cinerea* populations to the fungicides, 40 isolates from organic and 80 from conventional apple orchards in which Pristine has not been used were tested for mycelial growth or conidial germination on fungicide-amended media. The EC_{50} values of sensitive isolates ranged from 0.008 to 0.132 (mean = 0.043, n = 116) µg/ml for pyraclostrobin and from 0.003 to 0.183 (mean = 0.075, n = 117) µg/ml for Pristine based on a mycelial growth assay and from 0.065 to 1.538 (mean = 0.631, n = 29) µg/ml for boscalid based on a conidial germination assay. The minimum inhibitory concentration of these fungicides was 5 µg/ml. Four isolates were resistant to pyraclostrobin, with resistant factor (Rf) ranging from 12 to 4193. Of the four pyraclostrobin-resistant isolates, one also was resistant to boscalid (Rf = 14) and Pristine (Rf = 373), and two exhibited reduced sensitivity to Pristine (Rf = 16 and 17). Pristine applied at the label rate in the orchard failed to control gray mold on apples inoculated with the Pristine-resistant isolates. This is the first report of multiple resistance to pyraclostrobin and boscalid in field populations of *B. cinerea*.

Comparison of real-time PCR vs. microscopy with image analysis to quantify colonization of sclerotia by a fungal biocontrol agent
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Phytopathology 99:S64

The potential biocontrol agent *Trichoderma harzianum* colonizes sclerotia of the plant pathogenic fungus *Sclerotinia sclerotiorum* in soil. Plating of sclerotia previously has been used to determine the incidence of mycoparasitism, but does not quantify the extent to which individual sclerotia are colonized. We developed real-time quantitative PCR primer/probe sets for the GFP-transformant *T. harzianum* ThzID1-M3 as well as for *S. sclerotiorum*. The primer/probe sets exhibited high precision and reproducibility over a linear range of six orders of magnitude. Real-time PCR using these primers and probes was compared with epifluorescence microscopy plus image analysis, to quantify dynamics of colonization of sclerotia that were incubated in nonsterile soil. Amounts of ThzID1-M3 DNA and *S. sclerotiorum* DNA inside sclerotia were quantified using real-time PCR. Also, sclerotial thin-sections were obtained with a microtome, epifluorescence micrographs were captured, and GFP fluorescence was quantified using computer image analysis. As determined by either method, ThzID1-M3 effectively colonized sclerotia in soil, and both methods quantified colonization dynamics over time. Although epifluorescence microscopy combined with image analysis provided useful information on the spatial and temporal dynamics of colonization, the real-time PCR method provided a more precise assessment of the extent and progression of sclerotial colonization.

FDL1*, a putative cytochrome P450, is involved in macroconidiation, conidia germination, and fumonisin B1 production in *Fusarium verticillioides
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Phytopathology 99:S64

Fusarium verticillioides is an important pathogenic fungus that causes ear rot and produces mycotoxin fumonisin B1 on maize. To better understand fungal development and toxin production, we generated random insertional mutants by REMI strategy. In this work, we describe the isolation and characterization of REMI strain RG147, which displayed concentric bands when grown under a light/dark cycle in addition to other aberrant phenotypes. Molecular analysis revealed that the REMI vector was inserted within FVEG03297.3 locus, designated *FDL1*. *FDL1*, which encodes a putative protein of 576 amino acids with two introns, shows homology with cytochrome P450 monooxygenases. Several cytochrome P450 genes are known to be associated with virulence and toxin production in phytopathogenic fungi, e. g., *F. sporotrichioides* and *Aspergillus flavus*. RG147 grew slower than wild type in both complete dark and constant light conditions. The mutant produced macroconidia on V8 agar and cracked-corn media while wild-type strain produced few. RG147 also showed deficiency in conidia germination in YEPD broth. Significantly, RG147 produced 80% less FB1 than the wild type. Stalk rot and ear rot virulence assays are in progress. In addition, we are analyzing the expression profile of *FDL1* under a variety of host/environmental conditions. We are also investigating functional conservation of RG147 in *Fusarium* species.

Intercontinental phylogeographic structure of the white-pine-blister-rust fungus, *Cronartium ribicola*
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Phytopathology 99:S64

The white-pine-blister-rust fungus, *Cronartium ribicola*, was introduced into North America in the late 1800s and has caused significant mortality of five-needled, white pines. Presently, little is known about the worldwide genetic structure, diversity, or evolutionary relationships of this fungus. A collaborative international effort is underway to determine the phylogeographic structure among Asian, European, and North American sources of *C. ribicola* and closely related taxa. We have preliminary information on phylogenetic relationships among selected Eurasian and North American populations of *C. ribicola* using DNA sequences from four nuclear loci. Geographic sources included eastern and western North America, northern Germany, Korea, Japan, and northeastern China. Phylogenetic analyses suggest *C. ribicola* comprises at least three distinct clades. Isolates from Korea and China formed one clade, and isolates from Japan formed a second clade that was intermediate the third clade, which comprised isolates from USA and Germany. Identifying the evolutionary relationships and potential origin(s) of *C. ribicola* that spread through Eurasia and North America, and determining the phylogenetic relationships of its hosts are critical toward evaluating risks of cryptic introductions, contributing to the development of biological controls, identifying sources of host resistance, and developing appropriate regulatory practices.

GRIN-Global: An international project to develop a global plant genebank and information management system
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Phytopathology 99:S64

The mission of the GRIN-Global Project is to create a new, scalable version of the Germplasm Resources Information Network (GRIN) to provide crop genebanks with a powerful, flexible, easy-to-use plant genetic resource (PGR) information management system. The system will help safeguard PGR and information vital to global food security, and encourage PGR use. Developed jointly by USDA-ARS, Bioversity International and the Global Crop Diversity Trust, GRIN-Global will be deployed in selected plant genebanks worldwide by 2011. The .NET Framework/Visual Studio development environments were chosen for the project. Core web services, enterprise services or other technologies will update data stored locally or on networks, distribute centralized data to off-site systems, and enable third party data sharing. The database and interface(s) will accommodate commercial and open-source programming tools, be database-flexible (MySQL, MS SQL Server, Oracle), and require no licensing fees. The database will be deployable on stand-alone computers or networked systems. Iterative programming strategies will support continuous product evaluation and refinement. Bioversity International will deploy GRIN-Global internationally, working cooperatively to document the new system in Arabic, English, French, Russian and Spanish, translate its interface, and implement it in developing countries. The impact of system use will be evaluated by users during and following database implementation.

Impact of different US genotypes of *Phytophthora infestans* on potato seed tuber rot and plant emergence in different cultivars and breeding lines
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Seed pieces of different potato cultivars and advanced breeding lines (ABLs) from north central US breeding programmes were inoculated with different genotypes of *Phytophthora infestans* (US-1, US-1.7, US-8, US-11 and US-14). The effect of these genotypes of *P. infestans* on seed piece rot severity after re-storage was assessed using an image analysis technique. *P. infestans* genotypes demonstrated variable ability to cause seed piece rot, to reduce plant emergence and the relative area under the plant emergence curve (RAUEPC). The *P. infestans* US-8 genotype was the most aggressive genotype, followed by US-14 in both years. The other genotypes were the least aggressive, causing only moderate seed piece rotting across cultivars/ABLs tested. Similar trends were observed in two field experiments. Values of final plant stand (%) and RAUEPC demonstrated that the cultivars/ABLs Atlantic, MSJ453-4Y and Torridon were the least susceptible across all *P. infestans* genotypes. In both experiments cv. Pike was the most

susceptible. Other cultivars/ABLs demonstrated variable responses to different genotypes of *P. infestans*. The variability of susceptibility of tubers to different genotypes of *P. infestans* has implications for plant breeding efforts, focused in the past to breed for foliar resistance, with limited emphasis on the reaction of the tuber. Results from this study suggest that highly aggressive genotypes of *P. infestans* such as US-8 may lead to severe tuber rotting and deterioration of tubers before emergence.

Effect of different genotypes of *Phytophthora infestans* and temperature on tuber disease development of advanced breeding lines

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Phytopathology 99:S65

Potato late blight, caused by *Phytophthora infestans* is limiting disease for potato production, and often affecting in early stages due to inoculum survival in the seed tubers. The objective of this study was to get seed tubers from advanced breeding lines (ABL) and challenge them with different genotypes of *P. infestans* at three different temperatures used in storage (3, 7 and 10°C) in four production seasons. The results were analyzed as mean tuber disease response affected by components of isolates genotype, variety and storage temperature. Results showed that most of the varieties were resistant to US-1 genotype, regardless of the storage temperature and US-8 genotype was the most aggressive followed by US-14. It was also noticed that tuber resistant breeding lines were evaluated having little to no late blight infection. The ABL's differed in their resistance to the different genotypes of *P. infestans*, although the resistance has been increased, the disease response is still high. These results showed the importance of different components involved in the disease development and inoculum source, including the pathogens variability as an important component to take in advance. This study is an important approach to overcome new *P. infestans* genotypes equally or more aggressive than US-8 genotype.

The relationship between colonization by *Verticillium dahliae* and symptom expression in strawberry genotypes resistant to Verticillium wilt

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Phytopathology 99:S65

Eleven strawberry genotypes from the UC breeding program known to be resistant to Verticillium wilt were inoculated with *Verticillium dahliae*. Individual plants were given a resistance score based on visual symptoms, and the extent of colonization was quantified as the percent of petioles not colonized by the pathogen. Resistance scores and the percent of non-infected petioles decreased significantly over the growing season. Resistance scores decreased less over time than the percent of non-infected petioles, but genotype by date interactions were not significant, suggesting consistency of disease development over the growing season. Significant genotypic variance was detected for the percent of non-infected petioles, but not for resistance score. The percentage of non-infected petioles had a strong genotypic correlation with resistance score, indicating that about 60% of genotypic variation for visual symptoms in this set of resistant genotypes is explained by the extent of colonization in individual plants. Conversely, the genotypic correlation between the percent of non-infected petioles and resistance score for plants sampled in May was smaller than that for plants harvested in July. These results suggest that both resistance and tolerance mechanisms influence the impact of *V. dahliae*, but tolerance may be less stable over the course of a season. Differentiating between these mechanisms may require evaluations that supplement visual assessments of resistance.

Predicting potential impacts of climate change on Armillaria root disease in the inland northwestern USA

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Phytopathology 99:S65

Climatic changes in weather patterns, especially temperature and precipitation, will undoubtedly impact large-scale features of forest health. Forest pathogens are especially influenced by changing climate; their distribution and pathogenicity will almost certainly change as climatic factors shift. Methods are needed to predict these changes in association with their impacts and responses. With such predictions, forest managers can make prudent decisions about management practices aimed at mitigating adverse impacts or augmenting potentially positive impacts. If precise locations of accurately identified pathogens, their hosts, and climatic factors are spatially referenced, then climate surfaces can be used to determine which factors influence and determine pathogen distributions. Bioclimatic models can predict the potential occurrence and distribution of suitable climate space for host and pathogen species under present and projected future climate scenarios. For most forest pathogens, predictive capacity is extremely limited because precise distribution data are lacking. In an ongoing study, we used existing data from survey plots to develop spatial modeling approaches for predicting Armillaria root disease in the inland northwestern USA. Continued surveys and research are needed to further refine bioclimatic models to predict influences of climate and climate change on forest disease.

Verticillium comparative genomics -- Understanding pathogenicity and diversity

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Phytopathology 99:S65

Verticillium dahliae is the primary causal agent of Verticillium wilt that causes billions of dollars in annual losses worldwide. This soil-borne fungal pathogen exhibits extraordinary genetic plasticity, capable of colonizing a broad range of plant hosts in diverse ecological niches. We have employed a genomic approach to compare *V. dahliae* to a related species, *V. albo-atrum*, that has distinct phenotypes with respect to pathogenicity, and exhibits host range differences. A 7.5X assembly of the 33.8 Mb genome of a lettuce isolate of *V. dahliae*, in addition to a 4X assembly of an alfalfa isolate of *V. albo-atrum* are now publicly available via the Broad Institute. About 38,000 EST reads from three cDNA libraries of *V. dahliae* were generated, and the genomes of both species have been annotated. Through comparative analyses, we have identified four major regions on two chromosomes that are specific to *V. dahliae*. Each of these span approximately 300 kb and are enriched in repetitive DNA. The expression of genes encoded in these regions was confirmed by the presence of corresponding ESTs. We are currently examining these regions to gain insight into diversity, pathogenicity, and other aspects of *Verticillium* spp. biology.

Identification of lettuce genes differentially expressed in a *Verticillium dahliae*-lettuce interaction by suppression subtractive hybridization

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Verticillium wilt, caused by the fungus *Verticillium dahliae*, is an emerging threat to the U.S. lettuce industry. Lettuce germplasm with resistance to race 1 of *V. dahliae* is available for breeding programs, although germplasm with resistance to race 2 of the pathogen has not been identified. The objective of this work was to identify lettuce genes that are differentially expressed in leaves displaying Verticillium wilt symptoms. To accomplish this objective, the technique of suppression subtractive hybridization (SSH) was applied. The cDNA populations used for the SSH were prepared from symptomatic leaf tissue (tester), and asymptomatic leaf tissue (driver) of lettuce line PI 251246. cDNAs from the forward and reverse subtraction were cloned, sequenced, and analyzed in database searches. The results of these searches indicated an abundance of sequences encoding plant pathogenesis-related proteins, including endochitinase and thaumatin-like proteins, in the forward subtracted library. Further evaluation of these genes, and others identified in this library, may provide insight into mechanisms of resistance or susceptibility in lettuce-*V. dahliae* interactions.

Release of genetically engineered organisms: A role for plant pathology in the evolution of new ecological, regulatory, and legal paradigms

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Phytopathology 99:S65

Genetically engineered (GE) organisms have potential to transform agriculture. Concerns persist in the scientific and legal communities about whether released GE organisms can effectively be evaluated in a long-term context for subtle ecological effects including: gene flow into native populations, nontarget

activity, or disruption of indigenous communities. Effects occurring in non-agricultural habitats are even less likely to be considered or readily detected. Last year, the Ninth Circuit court affirmed an injunction on all planting of GE 'Roundup Ready' alfalfa pending a full Environmental Impact Statement, with the decision resting largely on balancing agricultural and economic interests of the opposing parties. Missing from the case was significant consideration of potential long-term environmental effects. In November, the Supreme Court reiterated a standard requiring that injunctive relief be based on demonstration that irreparable environmental injury is likely rather than just possible. Analysis of recent case history suggests a need for more stringency in predicting ecological effects of released GE organisms. Therefore, challenges for plant pathology and related disciplines include development and refinement of technology to track specific microbial genotypes in the environment, and further elucidation of microbial roles in natural ecosystem processes. Programs such as NSF's LTER and the United Kingdom's Farm Scale Evaluation program may help provide research approaches.

Fungicide concentration analysis on creeping bentgrass leaf blades using commercially available ELISA kits for the control of *Microdochium nivale*
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Phytopathology 99:S66

Microdochium nivale is an important winter pathogen of turfgrass in temperate climates. Traditionally, a single fungicide application made in the fall is expected to control winter turfgrass pathogens until the spring. In the absence of snow cover, fungicides applied in the fall can break down and fail to provide protection to the plant. Measuring the fungicide concentration on the leaf blade allows turfgrass managers to determine if adequate disease protection remains and if another fungicide application is required. In the past, accurate fungicide concentration analysis has been obtained through methods such as high performance light chromatography. These methods are effective but not conducive to analyzing large numbers of samples due to the time and cost required. Commercially available enzyme-linked immunosorbent assay (ELISA) kits produced by Horiba Ltd can determine fungicide concentrations on fruits and grains quickly and accurately for a large number of samples. Significant changes to the method had to be made for use on turfgrass. Preliminary testing on turfgrass has shown that it can effectively determine differences in the degradation rate of the fungicides iprodione and chlorothalonil under snow cover and in the absence of snow. Continued study of ELISA kits on turfgrass can help determine the rate of fungicide breakdown under winter conditions with the goal of making more accurate and effective fungicide applications in the future.

Flagellar stators MotAB function in biofilm formation of *Erwinia amylovora*
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Phytopathology 99:S66

Biofilm development is an essential component of infection by *Erwinia amylovora*, the causal agent of fire blight. Our scanning electron microscope (SEM) analysis of *E. amylovora* during infection suggests that surface structures are present during attachment to the interior walls of apple xylem. Attachment of bacterial cells to a surface requires the formation and proper function of appendages such as fimbriae, pili, or flagella. Aiding in this process are flagellar stators which comprise the stationary component within the flagellar motor that allows the motor to turn. The MotAB flagellar stators present in *E. amylovora*, function in initial polar attachment in biofilm formation by *Pseudomonas aeruginosa*. Present upstream of motAB is *flhC*, a DNA-binding transcriptional activator. We generated single gene deletion mutants in *flhC*, *motA*, and *motB*, as well as a deletion mutant of *motAB* in *E. amylovora* Ea1189. Quantitative reductions in biofilm formation on glass surfaces in vitro of 33.6%, 58.1%, 36.1% and 88.4% were observed for the *flhC*, *motA*, *motB* and *motAB* mutants, respectively. A similar reduction in aggregation on glass slides was also observed. The *flhC*, *motA*, and *motB* mutants all exhibited decreased virulence in an immature pear fruit assay and corresponding decreases in population of 1000-fold for *flhC*, and of 100-fold for *motA* and *motB* compared to the wild type strain after 3 days incubation. The *motAB* mutant was almost completely inhibited in pathogenesis.

Influence of climatic conditions on the efficacy of early season fungicide applications to manage dollar spot
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Phytopathology 99:S66

Dollar spot caused by *Sclerotinia homoeocarpa* is one of the most important diseases of turfgrass. Although variable, previous research has shown that fungicide application to asymptomatic turfgrass in the fall and/or spring may reduce dollar spot severity the following season. Replicated field studies were established at two locations in 2006 and 2007 to determine the relationship between climatic conditions at or surrounding the time of fungicide application with the ability of imposed treatments to suppress the development of dollar spot later in the season. Weather monitoring stations were used to record on-site climate conditions. Sequential applications of propiconazole and chlorothalonil as a combination treatment were applied in the fall and spring of each year to asymptomatic turf. Disease severity was assessed by counting dollar spot infection centers and using digital imagery analysis. Areas under disease progress curves were calculated and the differences among treatments determined. Fall fungicide applications had no impact on disease severity the following season. The lack of consistent disease suppression observed with the fall fungicide applications in this study is consistent with previous reports. Applications made in late March to early April in both years of the study resulted in significantly less dollar spot. The relationship between climatic conditions at or surrounding these early spring fungicide applications will be discussed.

Detection of TCDVd and PSTVd in seeds of tomato
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Phytopathology 99:S66

Tomato chlorotic dwarf viroid (TCDVd) and *Potato spindle tuber viroid* (PSTVd) are important viroids of tomato. Several outbreaks by both viroids have been recorded in Europe; however, the primary source of infection generally was not identified. The TCDVd and PSTVd infections in tomato crops might have originated from both contaminated seeds and infected (non symptomatic) ornamentals such as *Brugmansia* spp., *Petunia hybrida* and *Solanum jasminoides*. Reliable tests are available for the detection of both viroids in leaves of tomato and various ornamentals. However, internationally accepted protocols for the detection of PSTVd and TCDVd in seeds of tomato are still not available. Therefore, the development of a real-time RT-PCR for this purpose was initiated. Preliminary results show that both viroids can be detected in contaminated tomato seeds. The sensitivity of the assay is under investigation by testing mixtures of one contaminated seed and variable amounts of healthy seeds. In addition, for TCDVd a grow-out experiment was performed. In total 4000 seedlings were grown from the TCDVd-contaminated seeds. Leaves of 25 seedlings were pooled, total RNA was extracted and real-time RT-PCR was performed to determine transmission of TCDVd. However, no viroid transmission was found, suggesting that the viroid detected in the seed tests probably only was present at the surface of the seeds.

Improvement of semi-selective media for the detection of *Clavibacter michiganensis* subsp. *michiganensis* in seeds of tomato
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Phytopathology 99:S66

Clavibacter michiganensis subsp. *michiganensis* (*Cmm*) is the causal organism of bacterial canker of tomato. Dilution plating of concentrated seed extract on the semi-selective media D2ANX or CMM1 and SCM or mSCM is routinely used for the detection of seed-borne *Cmm*. Spiking of seed extracts with *Cmm* revealed that recovery of the target pathogen can be seriously hampered by the presence of antagonistic bacteria. We investigated whether semi-selective media for *Cmm* could be improved. Sixty antagonistic bacterial isolates from tomato seed were characterized by 16S rDNA sequencing and a collection of 20 of these isolates was used to screen alternative antibiotics for their efficacy. A set of 20 *Cmm* strains, selected for genetic diversity on the basis of AFLP, was used to determine whether *Cmm* was sufficiently tolerant to these antibiotics. Two antibiotics, trimethoprim and cephradine were identified to best fulfill the requirements. We noted that the growth inhibiting effect of antagonists, at least partly, was due to acid production. Therefore we also investigated the possible benefit of stronger buffering of media. Interestingly, a high Tris concentration was not only found to take away their inhibitory effect but also prevented many antagonists to grow at all. On the basis of our findings new media for detection of Cmm are proposed.

Expanded host range of *Fusarium virguliforme*
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Phytopathology 99:S66

Fusarium virguliforme (*Fv*) is the causal agent of sudden death syndrome (SDS), a soybean disease capable of causing severe yield loss. The known host range of *Fv* is limited to soybean (*Glycine max*), mung bean (*Vigna radiata*), and green bean (*Phaseolus vulgaris*) when inoculated without wounding and includes lima bean (*P. lunatus*) and cowpea (*V. unguiculata*)

when plants are wounded. Crop rotation does not appear to reduce *Fv* inoculum or SDS, suggesting *Fv* may have a larger host range. Greenhouse inoculations of 15 species representing crops, weeds, and prairie plants were conducted without wounding using a layer of *Fv*-infested sorghum placed below seeds prior to planting. Root and foliar symptoms and plant biomass were measured 5–6 weeks later. The presence and quantity of *Fv* in root tissue was determined with isolations on media and quantitative PCR using *Fv*-specific primers, respectively. Our data suggests an expanded host range for *Fv* consisting of symptomatic and asymptomatic plant species. Ten species, including alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), and pinto bean (*P. vulgaris*) developed symptoms, or had reduced plant biomass, or both. Five species including corn (*Zea mays*) and lambsquarter (*Chenopodium album*) appear to serve as asymptomatic hosts capable of increasing or sustaining *Fv*. The range of hosts could limit the effectiveness of crop rotation, and many hosts could also suffer deleterious effects from *Fv* infection.

Selection and evaluation of microbial strains with potential for biologically controlling pink rot of potatoes in storage

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Phytopathology 99:S67

Pink rot, incited by *Phytophthora erythroseptica*, is a field and post harvest disease of potatoes that, in recent years, has had a negative impact on potato growers in many regions of the world including North America. The ineffectiveness of many fungicides against pink rot due to the development of pathogen resistance justifies evaluating the development of biological control measures. In this study, the microbiota of 84 different agricultural soils was individually transferred to separate samples of gamma irradiation-sterilized field soil enriched with potato periderm. After microbial community proliferation in similar, enriched field soil environments, samples of each were assayed for biological suppressiveness to pink rot and zoospore production using tuber and soil extract assays. Zoospore production was reduced by 14% to 93% and disease severity on tubers was reduced by 6% to 21% by the most suppressive soils tested. Over 270 isolates of bacteria and yeast were recovered from the 13 most suppressive soil samples. The most promising candidate strains against pink rot in storage will be selected based on strain possession of both favorable liquid culture growth kinetics and disease suppressiveness when produced in commercially feasible liquid culture media. Results from these studies will clarify the importance of incorporating the liquid cultivation aspect of industrial practice into the strategy used to choose strains for commercial development.

Evaluation of systemic acquired resistance inducers for control of *Phytophthora capsici* on squash

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Phytopathology 99:S67

Phytophthora blight induced by *Phytophthora capsici* is a major constraint in vegetable production. Limited information is available regarding systemic required resistance (SAR) inducers that may provide protection of squash plants against the disease. In this study, the effect of DL-3-aminobutyric acid (BABA), 2,6-dichloroisonicotinic acid (INA), Saver (a.i. salicylic acid), and acibenzolar-*S*-methyl (ASM) on mycelial growth, zoospore germination and sporangium production of *P. capsici* was evaluated. The products were tested in *in vitro* studies at concentrations ranging from 25 to 2000 ppm. Mycelial growth and zoospore germination were not significantly affected by BABA and ASM and sporangium production was not affected by BABA. INA and Saver reduced mycelial growth and sporangium production at 100 ppm or higher concentrations and zoospore germination at 500 and 1000 ppm. In greenhouse studies, all the products applied as a soil drench or foliar spray at 25 or 50 ppm reduced disease severity on squash, compared with the pathogen-only control, when zoospores at a concentration of 10^3 spores/ml were used to inoculate the leaves. INA, BABA, and ASM also reduced disease significantly when zoospores at 10^3 spores/ml were used to inoculate the root. The results indicated that some SAR inducers did not inhibit the growth of the pathogen at concentrations generally recommended for use but had the potential to suppress the disease on squash significantly.

Baseline sensitivity of *Phytophthora capsici* isolates from the southeast US to mandipropamid

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Phytopathology 99:S67

The plant pathogen *Phytophthora capsici* is rapidly becoming an important limiting factor in vegetable production in the southeastern United States. In 2008, a new fungicide, mandipropamid (trade name: Revus) was labeled for managing *P. capsici* on vegetable crops. In this study we used a collection of 28 *P. capsici* isolates from the southeastern United States to determine baseline sensitivity values to this new fungicide. Of these 28 isolates, 5 were from NC, 8 from SC, 9 from GA, and 6 from FL. All isolates were confirmed as *P. capsici* based on morphology and by using *P. capsici*-specific PCR primers. An *in vitro* biological growth assay using V8-juice agar amended with four concentrations of mandipropamid (0, 0.0015, 0.015, 0.15 mg/L) was used to determine EC_{50} values. The EC_{50} values for mycelial growth on amended media ranged from 0.0125 mg/L to 0.0292 mg/L (mean = 0.0219 mg/L). Mycelial growth of all isolates was completely inhibited at 0.15 ppm. EC_{50} values for production of sporangia ranged from <0.0015 mg/L to 0.032 mg/L. EC_{50} values for zoospore germination ranged from <0.0015 mg/L to 0.0105 mg/L. These baseline values will be useful in monitoring changes in sensitivity of *P. capsici* populations as mandipropamid is used across the southeastern United States in vegetable production.

Antimicrobial activity of Snakin-1 and Defensin-1 as a hybrid protein

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Phytopathology 99:S67

To enhance plant protection properties, we constructed a hybrid antimicrobial protein gene (*sap*) for simultaneous expression of Snakin-1 and Defensin-1 in plant cells. Prior to testing *in vivo*, SAP was tested on a wide variety of phytopathogenic microorganisms *in vitro*. SAP exhibited significant antibacterial activity against *Clavibacter michiganensis* subsp. *sepedonicus* (IC_{50} approx. 3.5 µM) and antifungal activity against *Colletotrichum coccoides* (IC_{50} approx. 2.5 µM). To evaluate antimicrobial properties of SAP *in vivo*, we exploited transient-expression from a *Potato virus X* (PVX)-based vector. Stable expression of SAP was confirmed by RT-PCR and Western blot analysis. Antimicrobial activity against *C. coccoides,* causing anthracnose, was assessed on *Nicotiana benthamiana*. Anthracnose symptoms, visible on the leaf surface and stems at 4 days post inoculation (dpi) in all experimental variants, appeared as dried papery spots (about 1 mm). Spot size increased to 4–6 mm at 8 dpi in the control. On plants carrying the empty PVX-vector, lesions quickly coalesced resulting in death of leaves and, eventually, in death of whole plants. In contrast, lesion size on plants expressing SAP was about 1 mm and remained unchanged until 18 dpi. Light microscopy showed germination of conidia at 2 dpi, and development of appressoria and formation of acervuli at 5 dpi in all experimental plants. Plants expressing SAP did not exhibit any severe signs of anthracnose infection.

Multi-state assessment using window pane analysis confirming weather variables related to Fusarium head blight epidemics

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Phytopathology 99:S67

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, is a sporadic disease that is dependent, at least in part, on weather and climatic conditions. The goal of this research was to determine the consistency of the relationship between FHB and environment across multiple locations. Data on FHB were collected, using an ordinal rating scale, for each of 44, 36, 28, and 23 years in Ohio, Indiana, Kansas, and North Dakota, respectively. Weather data were gathered from local weather stations, and summary variables (such as average RH, precipitation, temperature) were calculated for a wide range of time windows and starting times of the windows during the growing season. The windows ranged from 10 to 280 days in duration, beginning around physiological crop maturity and proceeding backwards to the fall of the previous year. Based on Spearman rank correlations, FHB was significantly ($P < 0.05$) associated with total daily precipitation for all locations, and average daily relative humidity for three of the four locations (with a similar, but non-significant, trend in the fourth location), around the time of heading and flowering for various time windows. However, high correlations found for Ohio at other time periods (e.g., winter dormancy and first node observed), were not found at other locations. There was no significant effect of temperature on FHB for any time window.

More than 40 years of observations from Ohio confirm the importance of relative humidity and precipitation for Fusarium head blight epidemics

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Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum*, is a sporadic disease that is dependent, at least in part, on weather and climatic

conditions. The goal of this research was to identify environmental variables that are related to FHB over time. For each of 44 years (1965 - 2008), an ordinal assessment of FHB in Ohio was performed, based on the magnitude of disease symptoms, DON in grain, and yield-loss estimates. Weather data were gathered from local weather stations, and summary variables were calculated for a wide range of time windows and starting times of the windows during the wheat growing season. The windows ranged from 10 to 280 days in duration, beginning at June 30 (physiological maturity) and proceeding backwards to September 24 of the previous year (planting time). Based on Spearman rank correlations, FHB was significantly ($P < 0.05$) associated with average daily relative humidity and total daily precipitation for short- and long- time windows. FHB rating and RH were significantly correlated throughout the growing season, including both early and late spring, but FHB and precipitation were significantly correlated in late spring only. Weather variables that have been identified in this analysis may be used to potentially improve the national FHB forecasting system.

Use of molecular beacons for direct detection of Loop-mediated isothermal AMPlification (LAMP) amplicons of the plant pathogen *Ralstonia solanacearum*

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Phytopathology 99:S68

Isothermal DNA amplification techniques, such as Loop-mediated isothermal AMPlification (LAMP), are suitable for rapid detection of bacteria with simple field devices due to the ability to amplify DNA with high specificity, efficiency, and speed without thermal cycling. However, an additional step to visualize the product helps confirm the presence of the amplicons and ensure specificity of the reaction. Molecular beacons were designed to detect specific LAMP amplicons that distinguished several unique populations of the bacterial wilt pathogen *Ralstonia solanacearum* (Rs). Sequence-specific molecular beacons were designed to target loop regions, which are unique single-stranded DNA sequences generated by the LAMP primer sets. The reaction of the molecular beacon with the loop region distinguished group-specific amplicons generated by LAMP primer sets from a variety of closely related Rs strains. Group-specific LAMP was evaluated against 274 strains of Rs representing three phylotypes, four races and four biovars. Rs-specific LAMP products were detected in samples containing target strains but not in samples containing other Rs strains or non-target species. Early prototypes suggest that the LAMP reaction coupled with DNA hybridization probes can form the basis of a simple rapid diagnostic system for subgroups of *R. solanacearum*.

Coat protein-based genealogy of *Banana bunchy top virus* in the sub-Saharan Africa

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Banana bunchy top virus (BBTV; genus *Babuvirus,* family *Nanoviridae*) is emerging as a serious pathogen of banana (AAA genome) and plantain (AAB genome) (*Musa* sp.) in the West-Central and Southern regions of sub-Saharan Africa. A study was conducted to document the occurrence of BBTV in Angola, Cameroon, Gabon, Democratic Republic of Congo and Malawi and determine its relationship with BBTV isolates from other banana-growing countries around the world. A 538 nucleotide fragment of the coat protein (CP) encoded by BBTV DNA-S genomic segment was cloned and sequenced from virus-infected samples collected from the five African countries. Pair-wise comparison of CP sequences from these countries showed nucleotide sequence identity between 98 and 99 percent. Pair-wise comparison with corresponding sequences in the GenBank indicated high degree of homology (99%) with BBTV isolates from India. Phylogenetic analysis of currently documented BBTV isolates showed clustering into "South Pacific" and "Asian" groups with BBTV isolates from Africa aligning with "South Pacific" group. These results suggest the likely introduction of BBTV into Africa through infected suckers from South Asia, in particular India. Subsequent spread between African countries could have occurred through the distribution of infected suckers and spread by the banana aphid (*Pentalonia nigronervosa*).

Identification of pathogenesis-related genes in *Phytophthora phaseoli* during infection of lima bean

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Phytopathology 99:S68

Lima bean (*Phaseolus lunatus*) is an important crop in the State of Delaware and is grown primarily for the processing industry. Lima bean is susceptible to the Oomycete pathogen *Phytophthora phaseoli* that causes downy mildew disease on flower racemes and pods. Phytophthoras are known to secrete several effector or elicitor proteins that have some effect on the host. Next generation sequencing (Illumina) was used to study the expression of these proteins in *P. phaseoli* during pathogenesis. Messenger RNA from both the plant-grown and plate-grown mycelium was used for this study. Overall, we found a two-fold increase in the number of short nucleotide sequences, or signatures, in plant-grown mycelium when compared with plate-grown mycelium, and identified distinct signatures from *P. phaseoli* that matched transcripts from *P. infestans, P. ramorum,* and *P. sojae*. Further, we identified several effector or elicitor proteins that were over-expressed by *in-planta* mycelium. Full length cDNAs of several *P. phaseoli* transcripts were isolated using either 3' or 5' RACE-PCR, and subsequent RT-PCR reactions were carried out to confirm gene expression patterns. The results reported herein will help us to understand this pathosystem and will serve as a basis for further research.

A new plant picorna-like virus related to torrado viruses causes chocolate spot disease of tomato in Guatemala

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A new virus-like disease of tomato, characterized by necrotic spots and lesions in leaves and petioles, has recently emerged in Guatemala. The disease was named chocolate spot (Cs), based on the diagnostic symptoms. Antibody-based tests ruled out a role for known tomato-infecting viruses, including those that induce necrosis (tospoviruses and ilarviruses). A sap-transmissible virus was associated with Cs, and it primarily infected solanaceous indicator plants, e.g., *Nicotiana benthamiana* and *Nicotiana glutinosa*. The virus was graft-transmitted to tomato, where it induced Cs symptoms. Icosahedral virus-like particles, 28–30 nm in diameter, were purified from infected *N. benthamiana*. The viral genome consists of two (+) ssRNA molecules, RNA1 (7473 nts) and RNA2 (5095 nts). RNA1 was 74% identical with RNA1 sequences of *Tomato marchitez virus* (ToMarV) and *Tomato apex necrosis virus* (ToANV), and 69% identical with that of *Tomato torrado virus* (ToTV). The RNA2 sequence was ~70% identical to those of ToMarV and ToTV. In phylogenetic analyses, the Cs-associated virus clustered with ToANV, ToMarV and ToTV, which are newly described picorna-like viruses that induce necrosis symptoms in tomato. Together, these results indicate that the Cs-associated virus is a new member of the recently proposed genus *Torrado virus*, which includes ToANV, ToMarV and ToTV, and the name *Tomato chocolate spot virus* (ToCsV) is proposed.

Global identification of cellular and excreted proteins of *Rhizoctonia solani*

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Phytopathology 99:S68

Rhizoctonia solani, anastomosis group (AG) 4 (Tele: *Thanatephorus cucumeris, T. praticola*) is a soilborne and broad host range basidiomycetous fungus pathogenic to ornamentals, herbs, legumes, vegetables, turfgrasses and forest trees. Limited information on characterized genes and proteins of *R. solani* makes it difficult to carry out global genomic investigations to understand its biology and host-pathogen interactions. We earlier reported two cellular protein extraction methods optimized for *R. solani*. In this investigation, we have developed an extraction method and two-dimensional (2-D) gel resolution of excreted proteins of *R. solani* AG 4, isolate Rs23. We have detected over 50 secreted proteins from the (2-D) gel covering pH 4-7 and 6.5 – 205 kDa. In addition, 150 of the resolved, major and minor cellular protein spots were analyzed using MALDI-TOF-MS and LC-MS/MS, by comparing with cross-species fungal protein and Gene Ontology (GO) databases and with MASCOT (Matrix Science), BLAST (NCBI) and AmiGO (GO) search utilities. At least 140 protein spots were positively identified which could be associated with nucleus, mitochondria, cytosol, vacuole, plasma membrane, cell wall, and surface proteins. Identification of proteins even in absence of a homologous annotated genome database demonstrates the feasibility of carrying out *R. solani* protein profiling and pathogenic investigation.

Modeling sporulation of *Fusicladosporium carpophilum* on nectarine twig lesions

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Phytopathology 99:S68

Sporulation of *F. carpophilum*, causal agent of scab on peach and nectarine, was examined under various abiotic environmental conditions. During the

peak sporulation period in spring of 2007 and 2008, one-year-old twigs harboring overwintering scab lesions were harvested from a heavily infected 'Redgold' nectarine orchard. Twigs were washed, dried, placed in sealed plastic trays at >95% RH, and incubated at constant temperatures of 1, 5, 10, 15, 20, 25, 30, and 35°C. Twigs were removed for assessment after 4, 8, 12, 24, 36, 48, and 72 hours incubation. Observations on lesion numbers and conidia production allowed calculation of two dependent variables: spores/lesion and spores/cm twig length. The maximum amount of sporulation for both variables occurred at 15°C in 2007 and at 20°C in 2008. Quantitative models were developed for each dependent variable by fitting the Richards function to the temporal data. A range of values of the Richards shape parameter were examined iteratively to achieve the best possible fit across all temperatures. The models described 85 to 91% of the variation in sporulation when the asymptote and rate parameters were expressed as functions of temperature. Use of these models in conjunction with environmental data will allow prediction of inoculum availability for infection.

Genomics based diagnostic marker development for *Xanthomonas oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*
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Phytopathology 99:S69

Xanthomonas oryzae pv. *oryzae* and *X. oryzae* pv. *oryzicola* cause bacterial blight and leaf streak of rice, respectively. Although their symptomology is distinct, the two pathogens are difficult to differentiate from each other based on cultural or biochemical methods. Genomic analysis tools available in the Comprehensive Phytopathogen Genome Resource (http://cpgr.plantbiology.msu.edu) were used to identify over 150 primer pairs based on open reading frames with potential to distinguish these pathovars. Each primer set was screened against well-characterized isolates of each pathovar using conventional polymerase chain reaction (PCR). A subset of these primers was combined into a multiplex PCR set that accurately distinguished the two rice pathogens in a survey of a geographically and genetically diverse collection of *X. oryzae* pv. *oryzae*, *X. oryzae* pv. *oryzicola*, other xanthomonads, and diverse genera of plant pathogenic and plant associated bacteria. Bacteria previously isolated from rice plants in the United States in the 1980's, that were confirmed to be pathogenic to rice and identified as *X. campestris* pv. *oryzae*, amplified with the *X. oryzae* and *X. oryzae* pv. *oryzae* specific primers in the multiplex set. However, most *X. oryzae* pv. *oryzae* specific primers did not amplify the US strains. These results suggest that the weakly virulent US strains are *X. oryzae*, but are distinct from *X. oryzae* pv. *oryzae* or *X. oryzae* pv. *oryzicola*.

Evaluation of a new non-fumigant nematicide for vegetables
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Phytopathology 99:S69

Southern root-knot nematode, caused by Meloidogyne incognita is a primary pest of cucumbers in Georgia. Root knot nematode feeding results in stunting, wilting, chlorosis and yield loss. Thiazosulfene is a new, non-fumigant nematicide that may have less crop phytotoxicity potential as fumigant nematicides. Thiazosulfene applied as a pre-plant incorporated (PPI) spray alone or in combination with thiazosulfene drip applications was compared to labeled applications of oxamyl (Vydate), 1,3-dichloropropene (Telone II), and ethoprop (Mocap) for slicing cucumber (Cucumis sativus) grown on black plastic mulch. Significant root gall suppression compared to the check was observed in treatments receiving thiazosulfene applied at 1.33 lb a.i. pre-plant incorporated followed by thiazosulfene at 1.33 lb a.i. post-plant drip injected, thiazosulfene applied at 1.78 lb a.i. pre-plant incorporated alone, thiazosulfene applied at 1.78 lb a.i. pre-plant incorporated followed by thiazosulfene at 1.78 lb a.i. post-plant drip injected, thiazosulfene applied at 3.56 lb a.i. pre-plant incorporated alone, thiazosulfene applied at 3.56 lb a.i. pre-plant incorporated followed by thiazosulfene at 3.56 lb a.i. post-plant drip injected, Telone II applied alone, and Telone II followed by foliar Vydate sprays. No phytotoxicity was observed. These data indicate that thiazosulfene is an effective, non-fumigant option for reducing losses to root knot nematode in slicing cucumbers.

Cropping system effects on soilborne potato diseases and soil microbial communities
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Phytopathology 99:S69

Four different potato cropping systems, designed to address specific management goals of soil conservation (SC), soil improvement (SI), disease suppression (DS), and a status quo (SQ) standard rotation control, were evaluated for their effects on soilborne diseases of potato and soil microbial community characteristics (SMCC). SQ consisted of barley underseeded with red clover followed by potato (2-yr). SC featured an additional year of forage grass and reduced tillage (3-yr, barley/timothy-timothy), SI added yearly compost amendments, and DS featured diverse crops with known disease-suppressive capability (3-yr, mustard/rapeseed-sudangrass/rye). Each system was also compared to a continuous potato control (PP) and evaluated under irrigated and non-irrigated conditions. Data averaged over three potato seasons demonstrated that all rotations reduced stem canker (10–50%) relative to PP. All rotations reduced black scurf (28–58%) relative to PP, and scurf was lower in DS than all other systems. The SQ, SC, and DS systems also reduced common scab (15–35%), and scab was lower in DS than all other systems. Irrigation increased black scurf and common scab, but also resulted in higher yields for most rotations. SI had the greatest effects on soil microbial parameters, and produced the highest yields under rainfed conditions. DS produced high yields and low disease overall. Each rotation resulted in distinctive changes in SMCC as represented by microbial populations, substrate utilization, and FAME profiles.

Evidence that a QTL may be involved in a partial resistance response to *Pea enation mosaic virus* in pea (*Pisum sativum* L.)
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Phytopathology 99:S69

Pea enation mosaic virus (PEMV) is a serious disease of fresh market and dry pea in the Pacific Northwest region of the U.S. The *En* gene confers resistance to PEMV in pea, however a limited number of available cultivars contain the gene, and sources of tolerance have not been reported. In 2007, advanced pea breeding lines were screened for resistance to PEMV in naturally infected field trials in Corvalis, OR. Lines were considered resistant based on lack of PEMV symptoms such as vein clearing and translucent flecks on leaves. Field-resistant lines were further screened for resistance in repeated greenhouse experiments by mechanical inoculation with the virus. Four symptomless field-resistant lines exhibited symptoms of PEMV under greenhouse screening conditions, but the plant growth of these lines remained robust and was not significantly different than the buffer-treated controls (BTC). These lines demonstrated tolerance to PEMV since minor proliferations or bumps were expressed on pod surfaces but pod-fill and seed size was not significantly less than the BTC. RT-PCR primers specific to the coat protein gene of PEMV were used to assess symptomatic tissue from inoculated plants. Virus titer was not different from susceptible controls on three of the four lines but was significantly less in line PS08-41. Potential new QTLs with resistance to PEMV may be present in the tolerant lines that can be used to develop new cultivars with partial resistance to PEMV.

***Meloidogyne incognita* potential yield reduction and management options in corn in the Deep South**
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Phytopathology 99:S69

Corn acreage has increased in the southern USA and root-knot nematode, *Meloidogyne incognita* is emerging as an economic pest. No nematicides have been labeled on corn in many years, thus experimental nematicide tests were conducted in the greenhouse and in naturally infested fields in AL and MS to determine potential management options. Abamectin (AVICTA), terbufos (Counter 15 G), and several experimental seed treatments (A-D from DuPont) were evaluated in five field trials for nematode population effects and yield. Data were analyzed utilizing GLIMMIX in SAS 9.1. Residuals exhibited a normal distribution. Dunnetts test was used to compare nematicide treatments to the untreated control and differences are reported significant at $P < 0.10$. Abamectin and Experimental D reduced *M. incognita* numbers by 91% and 68% as compared to the untreated in repeated greenhouse evaluations. Although, root and shoot fresh weights and plant heights were not influenced by any seed treatment during the 45 day trials. In two concurrent field trials, corn yields were increased from 39% to 57% in all nematicide seed treatments as compared to the untreated control. Three further tests found yields were increased 23% by abamectin alone, 21% by the abamectin plus terbufos combination and 15% by terbufos alone. Nematode numbers were not consistently lower in the nematicide treatments in these field trials. Abamectin and terbufos offer growers options for root-knot nematode management in southern production systems.

Phylogenetic analysis and delineation of phytoplasmas based on *secY* gene sequence

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Phytopathology 99:S70

The *secY* gene, encoding for a protein translocase subunit, is a molecular marker useful for finer phylogenetic delineation and differentiation of closely related phytoplasma strains. Phylogenetic analysis based on nearly complete *secY* gene sequences from 54 phytoplasma strains representing 11 16Sr groups, delineated lineages which coincide with those based on ribosomal protein (rp) genes (*rps3* and *rpl22*) or 16S rRNA gene sequences. The average *secY* gene sequence similarity between two given 16Sr phytoplasma groups ranges from 57.4 to 76.0%. The *secY* gene sequence variability is similar to those of rp and *secA* gene sequences but much greater than that of 16S rRNA gene sequences. However, due to more informative characters in *secY* gene sequences, as evidenced by RFLP analysis, the resolving power of *secY* is slightly better than either rp or *secA* gene sequences for finer differentiation of phytoplasma strains in a given 16Sr group.

Current status of chestnut plantations and major diseases/pests in Korea

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Phytopathology 99:S70

Chestnut tree is one of the major fruiting tree in Korea. The planted area of chestnut is about 56,000 ha, and the export of produced nuts to other countires is a major income source for the residents in agricultural and forest areas. Total amount of nut export is approximately 19,000 M/T, which reaches upto 30% of the total production. Chestnut plantations had been rapidly increased in Korea since the 1970's, and the planted areas mainly located at southern and central parts of the Korea. The planted areas, however, are gradually diminished because of aging of chestnut trees and chestnut growers, soil acidification, and damages by diseases or pests. Three insects, chestnut curculio, peach pyralid moth, and chestnut moth, are known as major chestnut insects among 217 pests, which are reported as insects that feed on chestnut tree. Twenty pathogens are being reported as disease-inducing agent on chestnut tree. Among these, Cryphonectria parasitica, Botryosphaeria dothidea, Pseudovalsa modonia, and Melanconis microspora are known as the major blight fungus on twigs and trunks of chestnut. C. parasitica is the famous pathogen in chestnut plantations, and Phytophthora katsurae was first isolated from both the infected wood tissues and infested soils in 2006.

Sensitivity of *Phytophthora capsici* to mandipropamid and evaluation of its effectiveness against pepper Phytophthora blight

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Phytopathology 99:S70

Mandipropamid was evaluated for its effectiveness in controlling *Phytophthora capsici*, the causal agent of pepper Phytophthora blight *in vitro* and *in planta. In vitro* sensitivity of *P. capsici* was assessed using isolates of *P. capsici* that were collected from diseased pepper plants between 2005 and 2007 in Korea. All isolates were sensitive to mandipropamid, with EC_{50} values ranging between 0.01 µg/ml and 0.037 µg/ml. Fungicidal activity of mandipropamid was evaluated in the laboratory, greenhouse and field. In laboratory tests, at 0.16 µg/ml of mandipropamid, germination of zoosporangia and mycelial growth were inhibited by more than 90% compared with the untreated control. In greenhouse inoculation tests with 5 to 6 leaf stage plants, the application with 100 µg/ml 7 days before inoculation showed 100% of control value, whereas there was no control activity one day after inoculation. Furthermore, field trials with four-sprays of mandipropamid showed that the fungicide had good activity against pepper Phytophthora blight. The control value of a 14 day-interval application was as good as that of the 7 day-interval one. The application of mandipropamid after rainfall was not as effective as the application before rainfall. In summary, mandipropamid is an effective inhibitor of spore germination and mycelial growth of *P. capsici* and can potentially be used as a preventive fungicide against pepper Phytophthora blight.

Isolation, identification and growth characteristics of *Phytophthora katsurae*, causing chestnut ink disease in Korea

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Phytopathology 99:S70

Dead or dying chestnut trees showing inky ooze on necrotic trunks were found in southern parts of Korea. The causal fungus was isolated by placing infected tissues on selective medium or using rhododendron leaves as a bait for the soil. The isolates produced homothallic oogonia with protuberances on V-8 medium. Numerous sporangia were formed in creek water, and were papillate, ovoid to obpyriform in shape. The isolates had 100% similarity with *Phytophthora katsurae* isolates from Japan and New Zealand, and 99.6% with others in ITS sequences. All Korean isolates were completely identical in sequences. Numerous sporangia were formed in filtered and unfiltered creek water, but no sporangia formed in sterile distilled water for 10 days incubation. Unfiltered water was more effective than the filtered. Sterilization of unfiltered water did not give a difference in oospore and sporangia formations, but non-sterilization induced slightly more sporangia formation. Light induced sporangia formation at 500 and 1,000 lux, but did not induce oospore formation. No sporangia formed in sterilized unfiltered water in the dark, but light induced sporangia formation, but no influence on oospore formation. β-sitosterol slightly increased mycelial growth in both solid and liquid media, but the difference was not significant. In contrast, oospore and sporangia formation was stimulated by adding β-sitosterol. β-sitosterol increased oospore formation upto 1.3 to 14 times as the control depending on the culture media.

Plant propagative material as a pathway for the movement of exotic plant pests into and within the Greater Caribbean Region

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Phytopathology 99:S70

Plant propagative material is any plant material capable of and intended for propagation, including plants for planting. Reasons for importing plant propagative material include its use in commercial nursery and horticulture production, agriculture and forestry, "plant exploration" by botanical gardens or researchers, or planting by private collectors or homeowners. Plant propagative material may present a phytosanitary risk in two ways: 1) by introducing exotic plant pests, and 2) by becoming an invasive weed in the introduced range. We found that there is a high likelihood that pests, especially plant pathogens, are being spread between countries of the Greater Caribbean Region through both legal and illegal movement of plant propagative material s. Due to the relative ineffectiveness of port-of-entry inspections and the scarcity of diagnostic tests for pathogens, there is no easy solution to this problem. The plant propagative material pathway also facilitates the spread of invasive exotic plants in the Greater Caribbean Region, where they cause considerable economic and environmental damage. Most invasive exotic plant species in the GCR were introduced on purpose. There are few safeguards in place to prevent this from happening, as weediness assessments are not required for the importation of plant propagative materials.

Agrobacterium tumefaciens*-mediated transformation of the plant pathogenic fungus, *Cochliobolus sativus

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Phytopathology 99:S70

Agrobacterium tumefaciens-mediated transformation provides a powerful tool for targeted gene disruption and random mutagenesis in a number of plant pathogenic fungi, but it has not been reported and used for *Cochliobolus sativus* (*Bipolaris sorokiniana*), the causal agent of three important diseases (spot blotch, common root rot and black point) in barley and wheat. Here we report the successful transformation of *C. sativus* mediated by *A. tumefaciens*. A binary plasmid vector (pBIN19) containing the hygromycin B phosphotransferase (*hph*) under the control of the *cpc-1* promoter from *Neurospora crassa* was introduced into four strains (AGL-1, EHA105, LBA1100 and LBA4404) of *A. tumefaciens*. The bacterial cells of these strains were pre-treated with acetosyringone and then co-cultivated with the conidia of the *C. sativus* isolate ND93-1. Stable transformants of the fungus were produced from the co-cultivation with AGL-1, EHA105 and LBA1100. PCR and Southern hybridization showed that the T-DNA with *hph* was integrated into the genome of the transformants. All of the transformants tested remained mitotically stable after several generations of growth. We evaluated the effect of several major factors (number of bacterial cells used, acetosyringone treatment and duration of co-cultivation) on the transformation efficiency. The transformation system developed will be very useful for functional genomics of *C. sativus*.

Chemical alternatives to methyl bromide for control of *Fusarium* spp. in conifer nurseries

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Phytopathology 99:S71

Methyl bromide, a known ozone depleting agent, has long been used to control *Fusarium* spp. in conifer nurseries in the Pacific Northwest. The phase-out of methyl bromide as a result of the Montreal Protocol has led to reliance on other chemicals for pre-plant fumigation. The efficacy of Metam Sodium, Dimethyl Sulfide, Methyl Iodide, and Methyl Bromide against *Fusarium* spp. was tested at three conifer nurseries in western Washington and Oregon. In addition to testing the use of alternative fumigants, Methyl Iodide efficacy was examined under low and high permeability fumigation tarps: Virtually Impermeable Film (VIF) and the current industry standard High Density Polyethylene (HDPE), respectively. Rye seeds were inoculated with six *Fusarium* spp. isolates at each nursery, buried in fumigation plots 1–3 days before fumigation and removed 1 month after fumigation. Percent *Fusarium* spp. growth on PDA media after fumigation was measured. Soil samples from fumigation plots were collected 1–3 days before fumigation, 1 month after fumigation and 7 months after fumigation. CFU/g were measured on PDA media. Buried inoculum isolates and soil isolates were sequenced using mitochondrial rDNA (mtSSU) and elongation factor 1-alpha (EF-1α) to characterize *Fusarium* species, with specific emphasis placed on determining the efficacy of fumigation against *Fusarium oxysporum* and *Fusarium commune*, species that are non-pathogenic and pathogenic respectively.

Occurrence of the G143A mutation conferring resistance to QoI fungicides in Michigan populations of *Venturia inaequalis*

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Phytopathology 99:S71

Since their registration in Michigan in 1999, the quinine outside inhibiting (QoI) fungicides have been utilized for and represent a highly effective tool for apple scab control. However, in 2008, we visited several orchard locations in Kent County, MI with widespread scab symptoms following QoI fungicide applications. This prompted a field survey in which infected leaves were collected from 17 orchards located in western MI and in central/eastern MI and from an experimental orchard in E. Lansing, MI. Monoconidial isolates were established from foliar lesions for each orchard and then subjected to PCR analysis to detect the G143A mutation in the mitochondrial cytochrome *b* gene, that confers high-level resistance to QoI fungicides. This mutation was detected in 89.0% (170/191) of samples from western MI (8 of 8 orchards), 28.6% (32/112) of samples from central/eastern MI (4 of 8 orchards), and was not detected in E. Lansing (0/17). Spore germination was further examined on water agar amended with 0.1 µg/ml kresoxim-methyl and 100 µg/ml of salicylhydroxamic acid to also detect QoI resistance. Among the 188 isolates tested using both PCR and spore germination tests, resistance was identified in 87.1 and 90.6% of isolates from western MI, 24.0 and 22.9% in central/eastern MI and 0% of E. Lansing isolates. There was a 96.3% concordance between the tests. These results indicate the immediate need for altered strategies to manage strobilurin resistance in MI orchards.

Incidence and severity of daylily leaf streak caused by *Aureobasidium microstictum*

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Phytopathology 99:S71

Over 350 daylilies (*Hemerocallis* spp.) were evaluated for incidence and severity of leaf streak caused by *Aureobasidium microstictum*. Plants were established at the Waterman Agricultural and Natural Resources Laboratory, Columbus, Ohio in 2002. Symptoms varied from small spots to large elongate necrotic lesions along the mid-vein that extended from the base to the tip of the leaf. Leaf streak incidence was assessed in a single plant of each cultivar by estimating the percent of leaves with lesions. Disease severity was assessed by rating the amount of blighted foliage due to leaf streak in the entire plant. Incidence and severity were assessed in July 2008 by four independent raters. Standard deviations were calculated to estimate variation among raters and Z-scores were calculated for incidence and severity to estimate relative deviation from the population means. Incidence was positively correlated with severity, although some cultivars with high leaf streak incidence had relatively low severity due to small lesion size. *A. microstictum* was isolated from samples taken from several cultivars. We did not observe daylily rust (*Puccinia hemerocallidis*) in any plants.

Suitable tool disinfectants for *Tobacco mosaic virus* – look no further than the kitchen cupboard

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Phytopathology 99:S71

Disinfectant treatments were tested for the ability to prevent transmission of *Tobacco mosaic virus* (TMV) from virus-contaminated razor blades to *Petunia* × *hybrida* plants using a two-tiered screening strategy. A strain of TMV isolated from petunia was used for these studies. The coat protein (CP) open reading frame from this TMV strain was amplified by RT-PCR and sequenced and is 98% (nucleotide) and 99% (amino acid) identical to wild type TMV. Razor blades were contaminated by dipping in sap prepared from TMV-infected petunias. All eight treatments tested reduced the incidence of TMV infection of petunias relative to the water control. The four most effective treatments were tested in a subsequent experiment to simulate tool contamination during vegetative propagation. Each razor blade was used to make a single stem cut on a TMV-infected petunia, followed by a one minute disinfectant treatment and a single stem cut on a petunia plant. Plants were assayed for infection by symptom ratings and DAS-ELISA. Nonfat dry milk (NFDM), with or without Tween 20, Virkon S and household bleach significantly reduced the incidence of TMV infection ($P = 0.5$). There was zero incidence of infection from treatment of TMV-contaminated blades with 0.6% sodium hypochlorite or NFDM plus Tween 20 for one minute.

Assessing vegetable producers beliefs regarding food safety issues

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Foodborne disease outbreaks caused by fresh vegetables contaminated with *Salmonella* sp. and *Escherichia coli* O157:H7 continue to be a concern in the United States despite efforts by industry, academia and the government to reduce incidence. In 2007, researchers from food safety and plant pathology laboratories at the Ohio Agricultural and Research Development Center began a multi-state investigation to determine the perspectives of vegetable producers regarding pre- and post-harvest food safety. A mental model approach was used to assess experts in the field of vegetable food safety and vegetable producer's level of understanding and determine any critical gaps that might exist in their knowledge of food safety issues related to fresh vegetables. The expert and producer models were compared and a qualitative influence diagram (QIF) was developed. Key gaps and misconceptions identified in the QIF were then used to develop a comprehensive questionnaire so that a prevalence estimate of the beliefs identified by the experts and producers could be determined. The questionnaire was sent to 621 vegetable producers in Ohio, Michigan, Kentucky and Indiana with a 32% response rate. Responses to the questionnaire are presented.

***Zygophiala* spp. on apple fruit associated with flyspeck signs in China**

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Phytopathology 99:S71

A survey of sooty blotch and flyspeck (SBFS) blemishes on apple fruit was conducted in China from 2004 to 2007. Twenty seven *Zygophiala* isolates were obtained from flyspeck mycelial types from Shaanxi, Henan, Gansu and Liaoning Provinces. Six well-supported clades resulted from parsimony analysis of ITS sequences. The most widely distributed species, *Z. wisconsinensis*, was isolated from three provinces. The other previously reported species, *Z. cryptogama*, was isolated from a single province. The remaining four putative *Zygophiala* species have not been previously described. One of these species was isolated from two provinces and other three *Zygophiala* spp. were each isolated from a single province. *Schizothyrium pomi*, one of the most common members of the SBFS complex in the U.S. and Europe, was not recovered. This may suggest that *Zygophiala* spp. have different geographic ranges as well as differing environmental requirements. Anyhow, the recent increase in the number of *Zygophiala* species suggests that additional species in the genus remain to be discovered.

Characterization of *Tomato spotted wilt virus* NSm protein domains involved in tubule formation, movement and symptoms

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Phytopathology 99:S71

Absence of a reliable reverse genetics system for *Tomato spotted wilt virus* (TSWV) has impeded direct demonstration of gene function. We previously

used a *Tobacco mosaic virus* (TMV)-based expression system to demonstrate that the TSWV NSm protein is able to support cell-to-cell movement in the absence of any other TSWV proteins. The TMV-based expression system also facilitated demonstration that NSm induced tubule formation in protoplasts, supported long-distance movement and induced TSWV-like symptoms in plants. We have now identified the essential NSm domains required for tubule formation, movement and symptoms using deletion-mapping and alanine-substitution mutagenesis via the TMV-based system. Two regions of NSm were required for both tubule formation in protoplasts and cell-to-cell movement in plants, indicating a correlation between these activities. Results of our mutagenesis studies of conserved amino acids suggest that the function(s) predicted from domains common to tospovirus NSm proteins may be conserved across the genus. Further exploration of functions of this interesting protein from both additional TSWV isolates and other tospovirus species is merited.

A novel gene for resistance to stripe rust in wheat genotype PI 181434

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Phytopathology 99:S72

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (PST), is one of the most devastating diseases of wheat worldwide. Growing resistant cultivars is the most effective approach to control the disease, but only a few effective genes are available. The common spring wheat genotype 'PI 181434', originally from Afghanistan, was resistant in all greenhouse and field tests. To identify the resistance genes, PI 181434 was crossed with susceptible genotype 'Avocet S'. Adult plants of 103 F_2 progenies were tested in the field under the natural infection. Seedlings of the parents, F_2 and F_3 were tested with US races PST-100 and PST-127 under controlled greenhouse conditions. The genetic study showed that PI 181434 has a single dominant gene conferring all-stage resistance. The resistance gene analog polymorphism (RGAP) and simple sequence repeat (SSR) techniques were used to identify molecular markers linked to the gene. A linkage map of 8 RGAP markers and 2 SSR markers was constructed for the gene using the 103 F_2 plants. Amplification of a set of nulli-tetrasomic Chinese Spring lines and ditelosomic lines with an RGAP marker and the two SSR markers mapped the gene on the long arm of chromosome 3D. Polymorphism of the two closest flanking markers in 45 wheat genotypes was 82.2% and 73.3%, respectively, indicating that these markers are useful in incorporating the gene into wheat cultivars and pyramiding with other genes for durable resistance.

Cultivar-specific interactions between switchgrass and *Puccinia emaculata*

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Phytopathology 99:S72

Switchgrass (*Panicum virgatum* L.) has attracted increasing interest by the landscape and bioenergy industries because of its drought tolerance, rapid growth, minimal fertility requirements and colorful foliage. An outbreak of rust caused by *Puccinia emaculata* Schwein was observed on ornamental switchgrass in nurseries and landscapes, and agronomic switchgrass field plots in North Carolina and Tennessee in 2008. Leaf chlorosis was often the first disease symptom observed and occurred when uredia were forming under epidermal cells. Urediniospores were single-celled, globose or oval in shape. Urediniospores germinated 1 h after inoculation on 1% water agarose. Germ tubes elongated, branched several times, and differentiated appressoria. On leaf surfaces, appressoria formed over stomata and infected leaves. Cultivars of switchgrass varied in susceptibility to rust and variations in rust/cultivar interactions were detected as differences in pustule size, latent period and necrotic lesion formation. Isolates of rust from ornamental switchgrass were more virulent than isolates obtained from agronomic field plots. These results indicate that variations in pathogenicity exist within *P. emaculata* and epidemics may be more severe in some areas depending on the genotype of grass planted. Transportation of potted ornamental switchgrass could also pose a threat of introducing virulent rust strains into new geographic areas.

Anthracnose: A new disease of switchgrass

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Phytopathology 99:S72

Switchgrass (*Panicum virgatum* L.) is an important ornamental grass and bioenergy crop. Diseased leaves with anthracnose symptoms were observed in nursery grown switchgrass 'Prairie Sky' in North Carolina and Tennessee in the summer of 2008. Symptoms were fusiform lesions with red-brown edges. Acervuli formed in necrotic leaf and sheath tissues. Fungal isolates were grown on half-strength potato dextrose agar at room temperature with a 12 h photoperiod. Black conidiomata formed in rings on white vegetative mycelia at 2 days after inoculation. Conidia were fuisoid to sickle-shaped and range from 20.0 to 35.6 µm long × 3.1 to 5.9 µm wide. Setae were black acicular and 3 to 5 septate, ranging from 68.5 to 148.1 µm long. Conidia germinated and formed appressoria at the end of short germ tubes in drops of water on plastic cover slips 20 h after inoculation. Appressoria were oval or pear shaped and ranged from 7.76 to 11.69 µm long and 5.01 to 7.46 µm wide. These morphological characteristics are consistence with the description of *Colletotrichum graminicola* (Cesati) G.W. Wilson.

Evaluation of soybean cultivars for resistance to *Phomopsis longicolla*

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Phytopathology 99:S72

Phomopsis longicolla is the primarily cause of soybean Phomopsis seed decay (PSD). To identify soybean lines resistant to this pathogen, 50 soybean cultivars were selected based on the recommendation by Mississippi State University (MSU) Variety Test Program in 2007. Two lines that were previously reported to be resistant in Missouri also were included. Susceptible cultivars, Hill and Williams 82 were used as checks. A field experiment was conducted using a randomized complete block design with four replications at Stoneville, Mississippi in May 2007. Plants were inoculated at the R5 stage with a spore suspension prepared from a combination of 10 isolates of *P. longicolla* collected from Mississippi. The seeds of soybean lines obtained from MSU without inoculation were generally healthy. Of 50 lines tested, six lines had 100% germination, 30 lines had germination rates with range from 80% to 97%, and 12 lines ranged from 63 to 77%. In the seed plating assay, 37 lines had no *P. longicolla* infected seed, 10 and three lines had *P. longicolla* incidence of 3% and 7%, respectively. Incidence of *P. longicolla* in seeds from inoculated field plots differed significantly ($P \leq 0.05$) ranging from 6% to 50% among soybean lines. Several lines with low disease incidence were identified and will be confirmed for resistance in 2009 field trials. Collaborative research between USDA and university scientists on germplasm screening is underway to identify resistance sources to soybean PSD.

The 5' sequence of the *Tobacco necrosis virus* A^C coat protein gene is involved in local lesion symptoms in *Chenopodium amaranticolor*

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Phytopathology 99:S72

An RNA element at the 5' terminus of the *Tobacco necrosis virus* A^C (TNV-A^C) coat protein (CP) open reading frame (ORF) affects localized symptoms in *Chenopodium amaranticolor*. Deletion of 17 nts at the 5' end of the CP ORF resulted in greatly reduced lesion numbers without having substantial effects on the lesion phenotype. Mutations of the 17-nt region that did not affect the CP sequence resulted in significant reductions in lesions and viral RNA accumulation in inoculated leaves. However, large numbers of lesions appeared in leaves inoculated with mutants containing the 17 nt sequence, irrespective of deletions truncating the CP C-terminus. When transcripts containing β-glucuronidase (GUS) substitution for the entire CP ORF were inoculated, leaves failed to develop GUS foci, but when GUS was fused downstream of the 17 nt region, large numbers of GUS foci appeared. When the 17 nt element was inserted immediately down stream of the GUS ORF, stained foci were not evident. However, when an 81 nt TNV RNA spacer element encompassing 64 nt immediately upstream of the 17 nt sequence was inserted downstream of GUS, stained foci were evident. In contrast, foci failed to appear in leaves inoculated with mutants containing a 294 region of TNV sequence that included the 17 nt structure positioned downstream of the GUS ORF. Reduced local lesion numbers and RNA blots to evaluate the abundance of the TNV RNAs were consistent with GUS staining activity in the GUS fusion of 81 nt, nut not in that of 294 nt.

A PCR-based approach to characterizing resistance responses of soft red winter wheat cultivars to *Fusarium graminearum* infection

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Fusarium graminearum causes Fusarium head blight (FHB) in wheat, which leads grain contamination with the mycotoxin deoxynivalenol (DON).

Resistance is recommended for managing FHB and DON; however, resistance to FHB does not always parallel resistance to DON. Three field experiments were conducted between 2007 and 2008 to evaluate cultivar effects on relationships among visual symptoms, grain colonization, and DON accumulation. Plots of three cultivars with different levels of FHB resistance (Truman, moderately resistant; Hopewell, moderately susceptible; and Cooper, susceptible) were spray-inoculated at anthesis with *F. graminearum* spores and 20 spikes were tagged in different disease severity categories. Tagged spikes were hand-harvested, tested for DON, and *F. graminearum* biomass quantified by amplifying Tri5 DNA using quantitative RT-PCR. There were significant linear relationships between all pairs of variables for all cultivars, with significantly difference regression slopes among cultivars for the FHB/DON and FHB/biomass relationships. For a given level of FHB, Hopewell had significantly higher fungal biomass and accumulated significantly more DON than Cooper and Truman. The rates of DON and fungal biomass increase with increase in FHB were similar for Cooper and Truman in two of the three experiments, but significantly higher for Hopewell. However, the rate of DON increase with increase in fungal biomass was similar among the cultivars in all experiments.

Tomato spotted wilt and early leaf spot reactions in peanut genotypes from the U.S. and China

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Tomato spotted wilt, caused by Tomato spotted wilt virus (TSWV), and early leaf spot caused by *Cercospora arachidicola* are important diseases of peanut (*Arachis hypogaea*). As part of a study examining genotypic and phenotypic variation, disease reactions to these two diseases were evaluated in twenty-two genotypes from the U.S. and China in field trials at Tifton, GA in 2007–08. There was a continuous range of final incidence of spotted wilt from 20% to 80%, with genotypes UF NC 94022, Georganic, C 689-6-2, Georgia-01R, C724-19-25, C 209-6-13, and Tifguard being the most resistant genotypes for spotted wilt and GTC-20, PE-2, and GTC-9 the most are susceptible genotypes for spotted wilt, with the moderately resistant cultivar Georgia Green and several others with intermediate incidences of spotted wilt. Final percent defoliation by early leaf spot ranged from 10% to 97%. Genotypes C 689-2, Georgia-01R, C 12-3-114-58, C 11-154-6, Tifguard, and Georganic had the lowest levels of defoliation, whereas Georgia Green, NC-6, SunOleic-97R, Spancross, GTC-9 and GTC-20 had the highest levels of defoliation. Disease reactions will be used in conjunction with genetic characterization of these genotypes and populations developed from crosses of selected genotypes in efforts to develop markers for resistance to TSWV and *C. arachidicola*.

Behavior of the Triple Gene Block proteins of *Alternanthera mosaic virus* differs from those of *Potato Virus X*

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The host range of viruses is determined in part by their ability to move cell-to-cell and long distance. Potexvirus movement functions are provided by the Triple Gene Block (TGB) and Coat (CP) proteins; we have examined subcellular localization of these proteins of Alternanthera mosaic virus (AltMV) and observed differences between AltMV and PVX. When AltMV GFP:TGB2 and DsRed:TGB3 were co-expressed by agro-infiltration, no obvious interaction was detected; GFP:TGB2 was observed mainly in the epidermis, and DsRed:TGB3 accumulated primarily in the mesophyll. In contrast, the equivalent PVX proteins are reported to co-localize, and agro-infiltrated PVX DsRed:TGB3 localized to the periphery of epidermal cells. C-terminal deletions of AltMV DsRed:TGB3 still accumulated in the mesophyll, but N-terminal deletions of TGB3 localized to the periphery of epidermal cells. Infectious clones of AltMV with either TGB2 or CP expression disrupted were unable to spread beyond the initially infected cells, whereas a clone with TGB3 expression ablated spread to multiple epidermal cells, but not into the underlying mesophyll. Complementation with agro-infiltrated wild-type TGB3 restored the ability of TGB3-deleted AltMV to spread through the mesophyll to the opposite epidermis throughout the agro-infiltrated area. Over-expression of TGB3 from infectious AltMV caused veinal necrosis. These results suggest that TGB3 contributes to both cell-to-cell and long distance vascular movement.

Development of a polyprobe to detect simultaneously six viroids of pome and stone fruits

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A dot blot hybridization assay using a digoxigenin (DIG)-labeled polyprobe was developed for the simultaneous detection of six important viroids of pome and stone fruits on a single membrane. The polyprobe was designed to detect apple scar skin (ASSVd), apple dimple fruit (ADFVd), apple fruit crinkle (AFCVd), pear blister canker (PBCVd), hop stunt (HSVd) and peach latent mosaic (PLMVd) viroids. It was constructed by sequentially cloning nearly full-length sequences of each viroid into a single vector with runoff transcription of the complementary DIG probe driven by the T7 promoter. The sensitivity of the polyprobe was comparable to that of single hybridization probes by dot blot hybridization. The efficacy of the polyprobe was validated by testing total nucleic acid (CTAB procedure) extracts of pome and stone fruit samples from the U.S. National Plant Germplasm System repositories in Corvallis, OR, Davis, CA, and Geneva, NY. The viroid infections from these samples were confirmed by RT-PCR and sequences of the cloned RT-PCR products. The polyprobe offers effective simultaneous detection of all six viroids and greatly reduces the costs and labor involved when a large number of samples are tested. Any samples that are positive by the polyprobe can be further identified by using the individual viroid-specific probes. This procedure has the potential for routine use in diagnostic screening, certification, and quarantine programs for pome and stone fruits.

Draft genome sequence of potato 'Zebra Chip' associated bacterium '*Candidatus* Liberibacter solanacearum'

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A new species of *Candidatus* Liberibacter, '*Ca*. L. solanacearum' (Lso) was recently confirmed to be associated with a potato zebra chip (ZC) disorder. The bacterium belongs to gram negative, phloem-limited, α-Proteobacteria. Because Koch's postulates have not been fulfilled, information regarding the etiology of ZC disease and pathogenesis of Lso are lacking. To gain insight into this new pathosystem, we developed a novel strategy to successfully sequence the whole genome of Lso using Roche 454 sequencing technology. This sequencing process generated ~350,000 sequencing reads with an average of ~230 bp per read. *De novo* sequences were assembled into 110 contigs (1.0 to 85 Kbp) with ~16X redundancy, representing a genome size of ~1.26 Mbp with a GC content 34.8%. By comparative ortholog gene analysis, we predicted 1,126 coding sequences (CDS) with known and putative functions. Putative "toxin" proteins, secretion systems, transport and cell motility factors were also identified in the draft genome. Genes involving metabolite pathways were reconstructed and possible functional implications were assessed. Information from this genome sequencing data will improve our understanding of the biology of the bacterium and the etiology of ZC disease.

A pectate lyase homolog, *pel1*, from *Xanthomonas axonopodis* pv. *citri* is associated with the watersoaked margin formation of canker lesions

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The typical citrus canker lesions produced by *Xanthomonas axonopodis* pv. *citri* (*Xac*) are erumpent, callus-like, with watersoaked margins. Three novel atypical symptom-producing variants of *Xac* were described recently in Taiwan. Only the variant designated A^r type produces lesions without watersoaked margins on leaves of citrus species and does not possess pectolytic activities on polypectate media. Five PCR amplified pectolytic genes *pel1*, *pel2*, *pel3*, *peh1*, and *peh2* from strains XW19 (a typical canker lesion producing strain) and XW121 (a strain of A^r type) were cloned and characterized. Only a 1.2-kb *pel1* gene cloned from XW19 expressed the pectolytic activity. Sequence analyses revealed over 99% identities in nucleotide and deduced amino acid sequences among *pel1* genes from XW19 and XW121, and a *pel* homolog from strain 306 (GenBank accession no. NC_003919, XAC3562). The *pel1* from XW19 and XW121 encoded a Pel1 protein consisting of 377 amino acids. However, there was a stop codon located at the 350th amino acid residue of the *pel1* gene from XW121. The *pel1* gene from XW19 was transformed into XW121. The transformant exhibited the pectate lyase activity and also induced watersoaked margin

surrounding the restricted and raised lesions on grapefruit leaves. The results suggest that expression of the *pel1* gene of *Xac* is associated with the formation of watersoaked margin of the bacterial canker lesions.

Biological and molecular properties of *Potato virus* S from late blight resistant potato

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Late blight, caused by *Phytophthora infestans*, is an extremely devastating disease of potato (*Solanum tuberosum*) worldwide. Potato cv. Defender is the first and only commercially released potato cultivar with tubers and leaves that survive late blight. Severe symptoms including severe leaf mottling, stunting and malformation of plants were displayed in cv. Defender. Out of all known potato viruses, *Potato virus* S (PVS, family *Flexiviridae,* genus *Carlavirus*) was detected in these symptomatic plants. PVS usually induces inconspicuous symptoms in potato cultivars such as Russet Burbank, Norkotah, Gemstar, Ranger Russet and 6LS. Our recent studies further showed that LBR potato clone 4106 (also known as A95053-61) infected with PVS also resulted in similar severe symptoms. To better understand this phenomenon and to characterize PVS at biological and molecular levels, the complete nucleotide sequence of the PVS isolates from cv. Defender and LBR4106 were determined. Host response studies of PVS showed that Defender and other late blight resistant potatoes appear to be susceptible to PVS infection. Screening of LBR clones and the pedigree of LBR clones might provide important clues regarding the possible genetic linkage between late blight resistance and virus susceptibility.

Identification of grapevine xylem sap protein profiles in response to *Xylella fastidiosa* infection

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Pierce's Disease (PD) of grapevines is caused by the gram-negative, xylem-limited bacterium *Xylella fastidiosa* (*Xf*). All *Vitis vinifera*-based cultivars are highly susceptible to *Xf* infection. However, some grape species from the southern United States are resistant. Given that *Xf* is limited to xylem vessels, it has been speculated that chemical composition of host xylem sap could play an important role in *Xf* pathogenesis. To further investigate underlying molecular profiles and dissect possible molecular mechanisms involved, we identified protein profiles of xylem sap collected from PD-resistant and –susceptible genotypes segregating from *V. arizonica* × *V. rupestri* breeding population. Extracted peptides were analyzed by a LC-MS/MS Mass Sepctrometer and amino acid sequences were annotated by BLAST against NCBI non-redundant protein databases with a cut-off E value of 10^{-4}. We have identified protein involved cell wall metabolism such as cell wall degradation, lignification and cell death. We also identified pathogenesis-related proteins such as "thaumatin-like protein" (TLP), a protein reported to confer defense response to microbial infection. The identification of sap proteins involving phosphorylation modifications suggests the possible roles in regulation of development, biotic/abiotic stresses, and may be involved in the process of a long distance signal transduction pathway in xylem.

Development of SSR markers for detection, genotyping, and genetic diversity study of Citrus huanglongbing bacterium, '*Candidatus* Liberibacter asiaticus'

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Huanglongbing (HLB), previously known as citrus greening is a destructive disease of citrus. The causing pathogens are believed to be phloem-restricted bacteria, "*Candidatus* Liberibacters" which are naturally transmitted by citrus psyllid, *Diaphorina citri* in Asia and America, and *Trioza erytreae* in Africa. Due to the fastidious nature of the bacteria, till today, information regarding to the pathogen's genetic variation, population structure, epidemiological relationships in related to the evolutionary adaptation, and host selection is very limit. Using the recently sequenced whole genome of '*Can.* L. asiaticus' (Las), we conducted genome wide search to identify simple sequence repeat (SSR) sequencing loci and developed multi-locus SSR marker system for Las genotyping and genetic analyses. Thirty five loci were identified which contain various types of repeat motif potentially suitable for SSR primer design. Among them, 6 SSR primers showed good polymorphism after being validated against multiple Las strains from China, India, Brazil and Florida. Computational algorithms were conducted to design multiplex primer sets that allowed combining 2, 3 or up to four sets of primers each labeled with a different fluorescent dye (FAM, NED, PET and VIC) in a single tube of PCR reaction simultaneously. This multiplex detection platform will increase through-put of sample analysis and suitable for a large scale genotyping, genetic diversity and molecular epidemiological study of HLB.

Communities of *Pythium* and *Fusarium* in soils from Ct, NTand SC systems and their relationship with seed rot and damping-off of soybean

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Community analysis of soil *Pythium* and *Fusarium* is essential for understanding pathogen ecology and the impact of farming systems on pathogen communities and disease incidence. These pathogen communities were assessed in soils with long-term (10 yr) conventional tillage (CT), no-tillage (NT) and succession (SC; natural vegetation) using denaturing gradient gel electrophoresis (DGGE) and DNA sequence analysis. DGGE analysis demonstrated farming management systems dramatically impacted the *Pythium* and *Fusarium* communities. CT soils were dominated by *P. spinosum*, *P. irregulare* and *P. ultimum*, NT soils were dominated by *P. spinosum*, *P. irregulare* and *P. attrantheridium*, and the SC soils were mainly dominated by *P. attrantheridium*. *Fusarium* spp. were also influenced differentially by the three management practices. Some *Fusarium* spp. such as *F. oxysporum*, *F. solani*, *F. equiseti*, only existed in CT and NT soils; *F. moniliforme*, and *F. graminearum* only existed in NT soils. However, other species such as one genotype of *F. oxysporum*, *F. langsethiae* and *F. redolens* were not affected by the different management practices. This research also showed disease incidence of seed rot and damping-off was highest in NT soils (0.9747), followed by CT (0.9137) and SC soils (0.6667), which were due to the different composition of *Pythium* and *Fusarium* species in soils with different management systems.

The integrated disease management for red globe grape without chemical pesticides

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Red globe grape is one of main cultivar in Beijing, China. Some fungal diseases, such as grape downy mildew, white rot and gray mould, often caused a great decrease of fruit yield and quality. In order to explore an effective control approach without chemical pesticides, a research on the integrated disease management for Red Globe Grape was launched in Yanqing County, Beijing. The overall design was from the angle of ecology system of the grape garden, based on the suitable culture measures, by using a complex microbe agent EM and a activating protein from fungi to enhance the plant inverse-resistance at key stages of its growth and development, two mineral agents Bordeaux mixture and Lime Sulphur to prevent the disease at the susceptible stages, two microbe fungicides Polyoxin and A02-SL (a bioactive metabolite of *Streptomyces lydicus*) to control the disease at the early occurring stages. The two years result showed that the diseases could be controlled under the economic injury levels, in which the control effect to downy mildew reached 88.17%–95.70%, to white rot 69.81%, and the fruit yield increased by 5.10%–33.64%. In addition, the average weight of grape berry, the soluble solid content, total sugar, vitamin C and most of mineral elements, such as K, Na, Mg and P, were higher significantly by the treatment with EM. This study provided the basic data for the establishing of the higher effective, sustainable, pollution-free control strategy of grape diseases.

Screening antagonistic microbes and study on its controlling effect to Phytophthora blight of pepper

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Phytophthora blight of pepper is an important soil-borne disease. At present, this disease was controlled by chemicals fungicides, and now, the biological control technologies are considered to control this disease. In this research, 264 actinomycetes isolates and 382 bacterial were isolated from 13 soil samples from Nanjing, Yangzhou and other place in Jiangsu province, China. These isolates were assayed their antifungal activity against *Phytophthora capsic*. The result indicated that more actinomycetes isolates showed antifungal activities with 44.1% rate, bacterial isolates were with 8.5%. In this assay, an actinomycetes isolate, GD6-2, showed a high activities against

Phytophthora capsici, the diameter of antifungal area was 26.0 mm, inhibition rate was 57.8%, GD6-2 showed broad antifungal spectrum to other six plant pathogens. A bacterial isolate, YD4-3, showed high antifungal activity to *Phytophthora capsic*, the diameter of antifungal area was 25.5 mm, inhibition rate was 56.8%, YD4-3 showed broad antifungal spectrum to other seven plant pathogens. We used these two isolates to control phytophthora blight under the pot experiment condition, isolates NP6-7 and YD4-3 can effectively control phytophthora blight, their control effect were 67.4% and 63.8% respectively. The exhibition of two isolates had laid the foundation to control phytophthora blight of hot pepper, we will identify these two isolates and study on their controlling mechanism to explore their apply technology.

Adaptation of CANARY biosensors for rapid detection of plant pathogens

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The CANARY (Cellular Analysis and Notification of Antigen Risk and Yield) technology uses a recombinant B cell line expressing membrane-bound antibodies that are pathogen-specific, and at the same time expresses the calcium sensitive bioluminescent protein, aequorin. Upon crosslinking of antigens of a specific pathogen to the antibodies, B cells produce an elevated level of calcium. The process triggers an aequorin conformation change and leads to light emission through conversion of its prosthetic group coelenterazine into coelenteramide and CO_2. The emitted light can be easily detected by a luminometer. We adapted this technology for detection of regulated plant pathogens. In this report, we present the initial results on testing with a Ralstonia-specific B cell line. Two strains of *Ralstonia solanacearum* race 3 biovar 1 and race 1 biovar 1 were used. In the first six days of a 15-day assay period, the limit of detection of 3 CFU/CANARY test can be achieved in spiked geranium extract. We can still detect as low as 300 CFU/test at the end of the 15-day period. Comparable results were obtained with killed bacterial suspension. The procedure requires simple instrumentation and sample testing only takes a few minutes. Testing for cross-activity with related organisms and infected plant tissues are under way.

Preliminary study on the seed rot and physiological properties of chestnut in storage

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The incidence of disease and the content of water, starch, soluble saccharine, protein, hydroxybenzene, tannin and the activities of peroxides (POD), polyphenol oxidase (PPO), phenylalanine (PAL), catalase (CAT), chitinase and β-1, 3-glucanase in seed of chestnut were tested regularly during storage period. It showed that the content of water in chestnut seed were gradually decreased from 51.3% to 23.5%, the content of starch were decreased, the content of soluble saccharine were increased in the storage. The content of protein reached its highest value when stored for 90 days. The content of hydroxybenzene and tannin didn't show any change with the storage time went on. The activity of POD reached its highest value (267.53) when stored for 30 days. The activity of CAT was increased gradually. The activities of PPO and PAL displayed the same change trend and reached their highest value after stored for 90 days. However, the change range of PPO was greater than that of PAL. The activities of chitinase and β-1, 3-glucanase displayed the same change and reached their highest value after stored for 60 days. In addition, the incidence of chestnut seed rot was gradually increased, especially during late storage. Thanks to Beijing Municipal Education Commission (PXM2008-014207-055164, KM200710020003).

Development of quantitative PCR assays for *Podosphaera macularis* and *Podosphaera clandestina*, the causal agents of hop and cherry powdery mildew

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Powdery mildews on hop and sweet cherry, caused by *Podosphaera macularis* and *P. clandestina*, respectively, are economically important diseases in the Pacific Northwest of the United States. Our objectives were to develop specific and quantitative real-time PCR (qPCR) assays for better understanding the epidemiology of these diseases and improving their management. One set of specific primer per pathogen, based on the sequence polymorphisms within intergenic spacer region of rDNA, was selected to develop qPCR assays using SYBR Green chemistry. Under optimized conditions, each assay amplified only its corresponding target but not 27 other powdery mildews belonging to six genera. The PCR efficiency and detection limit were 85.6 – 91.4% and DNA extracted from 50 conidia, respectively, for *P. macularis*, and were 91.3 – 96.2% and DNA extracted from 5 conidia, respectively, for *P. clandestina*. A linear relationship was established between Cp values and log (number of conidia) for each pathogen. Similar trends were found for both pathogens for the dynamics of inoculum in the air with traditional spore count and data estimated with qPCR assays using DNA extracted from rotorod samplers collected weekly during growing seasons. These results indicate that these qPCR assays are reliable indicators of inoculum density in the air.

Gene sequencing reveals heterokaryotic variations in *Puccinia striiformis*

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Puccinia striiformis, the causal agent of stripe rust, is an obligate biotrophic fungus without known sexual reproduction. The objectives of this study were to identify polymorphic genes for determining the mechanisms of the pathogen variation. Primers were designed for seven important putative genes including elongation factor, beta-tubulin, TATA-box binding protein, serine/threonine kinase, conidiation protein, mitogen-activated protein kinase, and cell wall glucanase selected from the full-length cDNA library of *P. striiformis* f. sp. *tritici*, the wheat stripe rust pathogen. The full-length genomic sequences of the seven genes were obtained for 21 isolates to represent different race groups of *Pst* in the US and China and *P. striiformis* f. sp. *hordei*, the barley stripe rust pathogen. The TATA-box binding protein and conidiation protein genes had identical sequences among all tested isolates. The five remaining genes had various levels of polymorphism. Phylogenetic trees generated with each of the five genes showed different relationships among the isolates, but the consensus tree had a low, but clear association with the virulence patterns. We found that some of the isolates had clearly distinct base pair differences, providing the first evidence at the DNA level for heterokaryotic variations in the stripe rust pathogen. The heterokaryotic variations may help to understand the molecular mechanisms by which the asexual fungus evolves into various races.

Conventional and real-time PCR assays for diagnosis of Phacidiopycnis rot, gray mold, and Sphaeropsis rot in stored d'Anjou pear fruit

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D'Anjou pear fruit are subjected to inspection for Phacidiopycnis rot (*Potebniamyces pyri*) for export to some overseas markets, but it is difficult to distinguish it from gray mold (*Botrytis cinerea*) and Sphaeropsis rot (*Sphaeropsis pyriputrescens*) based on symptoms alone because of the similarity in symptoms among these three postharvest diseases. The objective of this study was to develop PCR-based assays for rapid diagnosis of these three diseases. Specific primers based on the internal transcribed spacer (ITS) region of rDNA that amplified only their corresponding target pathogens, but not nontarget fungi commonly associated with pear fruit, were selected for PCR assays. Under optimized conditions, detection limits were determined to be 10 to 50 pg and 5 pg of fungal DNA for specific conventional and real-time PCR assays, respectively. PCR assays, using specific ITS-based primers and size variations among EF-1α amplicons, were validated using decayed fruit resulting from artificial inoculations and natural infections in comparison with the pathogen isolation assay. For wound-inoculated fruit, pathogens identified using PCR assays were consistent with those used for fruit inoculation. Among 111 of 114 naturally infected fruit, causal agents identified using PCR assays were in agreement with results of the isolation-based assay. A combined procedure was proposed for identifying these three diseases in a timely and economical manner.

Profiling of secreted proteins involved in the white pine blister rust pathosystem: A case study of the *Pinus monticola* thaumatin-like protein family

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The protein content in the *Pinus monticola* needle apoplast was expected to change dramatically as a result of the host defense response upon infection with white pine blister rust (WPBR) caused by the pathogen *Cronartium ribicola*. This study demonstrates the feasibility of using proteomics for the identification of novel secreted proteins using two-dimensional (2-D) protein

electrophoresis followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Five *P. monticola* thaumatin-like proteins (PmTLP) were identified in the needle apoplast by MALDI-TOF-MS. Based on protein sequences seven *PmTLP* genes (*L1-L4, S1-S3*) were characterized during compatible and incompatible interactions of white pine with *C. ribicola*. Reverse transcription quantitative PCR (RT-qPCR) showed that each member of the *PmTLP* family had a different and characteristic pattern of mRNA expression. Among the seven *PmTLP* genes, *PmTLP-l2*, *-l3*, and *-s1* were up-regulated post rust infection but with different patterns between resistant (*Cr2/cr2*) and susceptible (*cr2/cr2*) seedlings during the early stages (0 h-96 h) post rust inoculation. These three *PmTLPs* were also regulated by canker development in diseased susceptible seedlings (*cr2/cr2*). Their transcripts showed very high accumulation levels (6~27 fold) locally in the infected needle and cankered stem. Expression profiling revealed that the members of the *PmTLP* family play different roles in host defense.

Monitoring microbial communities in vegetables grown in different management systems
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Soil management practices can significantly impact populations of soil microorganisms. To monitor changes in microbial communities in different agricultural management practices in carrot/potato cultivation in the San Joaquin Valley, we measured populations of fungi, bacteria, pseudomonads, nematodes, degree of fluorescein diacetate (FDA) hydrolysis, and profile changes in ECO Biolog plates. The experiment consisted of three treatments-organic production which included the incorporation of green manures for the primary source of nitrogen; a transitional treatment which included composted animal manures supplemented with synthetic sources of nitrogen; and conventional practices. The latter treatment included a fall application of metam sodium. Soil samples were collected at monthly intervals. Community reaction patterns on Ecolog plates were observed at 24, 48, 72 and 96 hrs using a Biolog Micro Station. The changes were analyzed via Principle Components Analysis (PCA) of Average Well Color Development (AWCD). In general, populations of pseudomonads and total bacteria and fungi were greater in the organic and transitional plots. Similarly, FDA levels were greatest in the plots that received green manure. In a bioassay, melon seeds were planted in small pots of the soil from each treatment amended with high inoculum concentrations of Pythium ultimum. Plots with highest levels of microbial activity were generally suppressive to damping-off.

Evaluation of four amendments as sources of available silicon to accumulator plants grown in soilless media
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As part of an on-going project to evaluate the potential of silicon to reduce biotic and abiotic stresses in floricultural crop production, four amendments were incorporated into a standard soilless growing medium (Sunshine Mix #2) prior to transplanting three-week-old seedling plugs of sunflower and zinnia. The same amendment-medium combinations were extracted in a ten-extraction series using the Saturated Media Extraction (SME) technique in the laboratory and analyzed with inductively coupled plasma atomic emission spectrometry (ICP-AES). The plugs were grown utilizing quarter-strength Hoagland's nutrient solution (equivalent to 50 ppm N) made with deionized water (18 uohm purity) to minimize exposure to silicon prior to transplant. Plants grown in unamended medium served to establish baseline levels of silicon accumulation for analytical comparisons of tissues utilizing scanning electron microscopy with energy dispersive x-ray analysis (SEM-EDXA) and ICP-AES. The amendments evaluated included: calcium silicate slag, parboiled rice hulls, chopped switchgrass straw, and coconut coir. Leaf tissue was harvested at 2, 5, and 8 weeks after transplant to determine a timeline for silicon uptake/accumulation. These accumulation rates were compared to the SME results to determine the predictive power of standard laboratory soil analysis methods for silicon accumulation potential. Together, these data describe approaches for reliable and effective delivery of silicon to silicon-accumulating crops.

Genomic characterization of a seed-borne caulimovirus associated with flower distortion in *Rudbeckia hirta*
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Mild to severe flower deformation and phyllody in *Rudbeckia hirta* were associated with infection by a previously undescribed caulimovirus that was named Rudbeckia flower distortion virus (RuFDV). The virus has spherical 47–50 nm virions containing a circular 8222 bp dsDNA genome (GenBank Accession No. NC_011920) containing 5 open reading frames (ORFs) similar in size and arrangement, and having significant sequence similarity only to the corresponding coding regions of known caulimoviruses. Two additional RuFDV ORFs capable of encoding proteins of 169 and 121 amino acids respectively had no identity to known viral genomic sequences. In the annual *R. hirta* varieties 'Hot Chocolate' and 'Indian Summer' RuFDV was identified at flowering in approximately 50% of plants grown from seed. RuFDV was not transmitted by mechanical innoculation or by aphids from *R. hirta* to *R. fulgida* or *R. occidentalis*, and no integrated RuFDV sequences were detected in *R. hirta* genomic DNA by southern hybridization using a full-length RuFDV genomic clone as probe.

Influence of glyphosate on Fusarium wilt, Cercospora leaf spot, and Rhizoctonia root and crown rot diseases of sugar beets
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Roundup Ready® technology is now widely used in U.S. sugarbeet production systems. Glyphosate (Roundup, Monsanto, St. Louis, MO) has been shown to have effects on several pathogenic fungi by inhibiting 5-enolpyruvyl-3-shikimate phosphate synthase, having direct effects on disease development, inducing systemic resistance, or enhancing plant health. Field, greenhouse, and *in vitro* experiments were done to test effects of glyphosate application on the severity and incidence of Cercospora leaf spot (*Cercospora beticola*), Fusarium wilt (*Fusarium oxysporum* f. sp. *betae*), and Rhizoctonia crown rot (*Rhizoctonia solani* AG 2-2) and direct effects on mycelial growth of these fungi. No significant effects ($P \leq 0.05$) of glyphosate treatment (3 applications of Roundup WeatherMAX®) on Fusarium wilt, Rhizoctonia crown rot, or Cercospora leaf spot incidence or severity were observed in the field in 2008. In greenhouse experiments, 3 applications of Roundup WeatherMAX® significantly reduced ($P \leq 0.05$) the severity and incidence of Fusarium wilt, but showed no effect on Rhizoctonia crown rot or Cercospora leaf spot development. In *in vitro* experiments, technical glyphosate incorporated into water agar at 0.00, 0.05, 0.10, and 0.20 µl /ml water agar, had no effect on mycelial growth of the three fungi, while 0.50 µl/l, which represents 4x rate of the highest label rate, significantly ($P \leq 0.05$) reduced mycelial growth of all three fungi. Effects on spore germination will be discussed.

A method to assess infection of soybean roots by soybean cyst nematode with quantitative polymerase chain reaction
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Heterodera glycines, the soybean cyst nematode, is the major pathogen of *Glycine max* (soybean). Effective management of this pathogen is contingent on the use of resistant cultivars, thus screening for resistance is essential. The purpose of this research was to develop a method to assess infection of soybean roots by *H. glycines* with real-time quantitative PCR (QPCR), a prelude to differentiation of resistance levels in soybean cultivars. Two-day-old soybean roots were infested with eight levels of second-stage juveniles (J2)/mL: 0, 10, 100, 400, 500, 600, 700, and 1000. Twenty-four hours after infestation, the roots were surface sterilized and DNA was extracted with the DNA FastKit (MP Biomedicals, Solon, Ohio). For the QPCR assay, primers for four genes were tested, with *Hg*SNO, which is involved in the production of vitamin B6, selected as the best choice for *H. glycines* DNA amplification within soybean roots and corroboration of the tenfold increase in infestation levels (10, 100, and 1000 J2). Quantification of *H. glycines* infection by traditional means (numbers of females produced in 30 days) is a time-consuming practice; the QPCR method can replace the traditional one and improve precision in determining infection levels. The next phase of this research is to quantify differences in infection of soybean cultivars with different resistance genotypes.

Characterization of the *occT* gene located in the *occ* gene cluster associated with production of occidiofungin in *Burkholderia contaminans* MS14
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Burkholderia contaminans strain MS14 isolated from a disease-suppressive soil has a broad spectrum of antifungal activities against plant fungal pathogens, which is attributed to the production of the oligopeptide occidiofungin. Mutagenesis analyses revealed the 56-kb *occ* gene cluster is involved in the production of occidiofungin, which harbors 17 open-reading frames (ORFs) including LuxR-type regulatory genes and nonribosomal

peptide synthetase genes. ORF2, named *occT* and 1,701 bp in size, was predicted to code for a protein of 63 kDa. Database search showed that the putative protein OccT shared significant identities to the members of the ATP-binding cassette superfamily, which are responsible for secretion of cyclic peptides, including an uncharacterized ORF (Bamb_6469; 90%) of *B. ambifaria* AMMD and SyrD (57%) of *Pseudomonas syringae* pv. *syringae* B301D. Further analysis revealed the presence of a transmembrane domain at N terminus (Position: 24-301; E-value: 7.1e-20) and an ATP binding cassette at C terminus (Position: 343-566; E-value: 3.1e-8). Quantitative real-time PCR analysis demonstrated transcription of *occT* was positively regulated by both *ambR1* and *ambR2*, regulatory genes for production of occidiofungin. Based on these preliminary results, the *occT* gene was predicted to be involved in the secretion of occidiofungin. Mutagenesis analysis is underway to investigate the role of the *occT* gene in the production of occidiofungin.

Characterization of spontaneous mutants of *Phytophthora capsici* resistant to iprovalicarb

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The capacity of spontaneous mutation of *Phytophthora capsici* strains resistant to iprovalicarb, a carboxylic acid amide fungicide, was evaluated *in vitro*. Six mutants were directly screened from parent strain PCAS2 on iprovalicarb-amended media. The mutation frequency was approximately 6×10^{-12}. No mutants were obtained from parent strain PCAS1, but nine were obtained from its 38 self-crossed progenies. The mutation frequency of sexual progeny is higher than that of asexual progeny. It implies that the possibility of emergence of resistant mutants will be higher if both mating types are present in the same field. All of the mutants exhibited a high level of fungicide resistance with factors of more than 100 folds. The resistance of all mutants was stable after sequential subcultures of hypha for 10 transfers or zoospores for 5 generations on fungicide-free media. Fitness studies showed that the capacity of hyphal growth, sporulation, cystospore germination and pathogenicity had not been significantly changed in comparison with those of the parent strains, except the sporulation capacity of few mutants. Iprovalicarb exhibited a cross-resistance with dimethomorph, mandipropamid and flumorph, but not with metalaxyl, cymoxanil, zoxamide, etridiazole, azoxystrobin and chlorothalonil. These results suggest that there is a potential resistance risk for *P. capsici* to iprovalicarb in field.

Genetic diversity of polyketide synthase/nonribosomal peptide synthetase genes in isolates of the barley net blotch fungus *Pyrenophora teres* f. *teres*

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Polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) are multifunctional enzymes responsible for biosynthesis of diverse small molecules (e.g., mycotoxins and phytotoxins) in filamentous ascomycetes. Both *PKS* and *NRPS* genes are present in fungal genomes as large gene families but only a few have been shown to be involved in pathogenesis. We have identified three *PKS* genes and six *NRPS* genes from the barley net blotch fungus *Pyrenophora teres* f. *teres* (*Ptt*). Each *PttPKS/NRPS* gene has been amplified by PCR from a total of twenty-three *Ptt* isolates collected from different geographic regions including Japan, Brazil and the USA. DNA sequencing indicates that *PttPKS1*, *2*, *NRPS1, 2* and *3*, which are highly conserved (>90% similarity) in the closely related tan spot fungus *P. tritici-repentis* (*Ptr*), have little variation among different *Ptt* isolates. In contrast, *PttPKS3*, *NRPS4*, *5* and *6*, which are only weakly or moderately conserved (50–68% similarity) in *Ptr*, appear to be significantly divergent in *Ptt* populations. Differential PCR primers have revealed polymorphic patterns in *PttPKS3* (from one Japanese isolate), *NRPS4* (from three North Dakota isolates) and *NRPS6* (from one Japanese, one Brazilian and two ND isolates), which is likely paralogous to *NRPS5*. These polymorphic *PttPKS/NRPS* genes will be further investigated through genetic and functional analyses for potential roles in mediating pathogen-host interactions in the *Ptt*-barley pathosystem.

Mycelium pigmentation in relation to melanin-inhibiting compounds and pathogenicity of *Sclerotinia sclerotiorum* on Valencia peanut

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Sclerotinia sclerotiorum is a fungal pathogen of a wide range of economically important crops. While in the literature *S. sclerotiorum* is described as a fungus with white mycelium on growth media, darkly-pigmented mycelial variants have been reported on peanut in New Mexico and Texas. This study was conducted to determine the effect of melanin-inhibiting compounds on mycelium pigmentation in *S. sclerotiorum*, and to assess whether mycelium pigmentation affects pathogenicity of *S. sclerotiorum* on peanut. Two melanin-inhibiting compounds, kojic acid and phthalide, which interfere with melanin biosynthesis via L-3,4-dihydroxyphenylalanine (DOPA) and 1,8-dihydroxynapthalene (DHN) pathways, respectively, were used to evaluate pigmentation and growth in a darkly-pigmented variant and to produce a stable non-pigmented isolate of *S. sclerotiorum*. Mycelial growth was similar for both darkly-pigmented and non-pigmented variants over a range of temperature from 10 to 35°C. Peanut plants were inoculated with either the darkly-pigmented or non-pigmented isolate to test for pathogenicity and virulence. The darkly-pigmented isolate caused disease and death in peanut plants within two weeks of inoculation. In contrast, the non-pigmented isolate was unable to cause death of peanut plants within a two-week timeframe. Collectively, our data suggest that melanin-based pigmentation in mycelium may play a significant role in the pathogenicity and virulence of *S. sclerotiorum*.

Effect of relative humidity on infection of almond kernels by *Aspergillus flavus* and *A. parasiticus* and levels of aflatoxin contamination

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Aspergillus flavus and *A. parasiticus* can contaminate almond nuts with aflatoxins. Humid conditions during the drying process of the nuts on the orchard floor or storing in stockpiles may affect aflatoxin levels in almonds. Almond kernels were inoculated with the toxigenic isolates of A. flavus A224 and A. parasiticus A408, wound-inoculated, incubated at 30°C under relative humidity (RH) of 70, 75, 80, 85, 90, 95, and 100%, respectively, and sampled 1 week later. A real-time PCR assay was used to quantify the number of spores per kernel. The aflatoxin level for each sample was quantified by high performance liquid chromatography (HPLC). A positive linear relationship was observed between the number of spores per kernel and RH for each species. Toxigenicity of A408 was about 10-times higher than that of A224. Aflatoxin levels were lower under 70, 75 and 80% RH with A224, but were higher under 85, 90, 95, and 100% RH. The A408 produced aflatoxins under all RH treatments. For this isolate, no quantitative relationship between RH and aflatoxin levels was observed, but aflatoxins were significantly higher under 100% RH than under the other RHs. No correlation was observed between the detected levels of spores and aflatoxins for either species, but in a few cases, significantly higher numbers of spores per kernel were associated with high aflatoxin levels. Drying the nuts quickly on the orchard floor should reduce aflatoxin contamination in almonds.

***Phytophthora cinnamomi* on Atlantic Rain Forest soil in Bahia, Brazil**

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The Atlantic Rain Forest still harbours a significative part of Brazil biodiversity, mainly in the cacao growing area of Bahia which concentrates quite a number of endemic trees. *Phytophthora* spp. from the rizosphere of three endemic plants of Southeastern Bahia Atlantic Rain Forest were studied in three conservation units. Selective agar media PARPH was used to isolate *Phytophthora* spp. and soil samples collected from December 2007 to July 2008 yielded 67 *Phytophthora* spp. isolates. Eleven of the heterothalic isolates presented: coralloid or spherical hyphal swellings; sympodial sporangiophores; non papillated, non caducous, terrminal, ovoid, obpiriform, ellipsoid or ellongated sporangia, 26.3 – 54.3 × 19.3 – 38.5 µm (42.3 × 27.8 µm), with sporangial proliferation; chlamydospores 37.6 – 55.3 µm (44 µm) of diameter. Based on morphological and physiological characteristics and sequence of nuclear genes ITS and β-tubulina these isolates were identified as *P. cinnamomi*. All 11 isolates were pathogenic to the original hosts *Parinari alvimii*, *Manilkara máxima*, *Harleyodendrum unifoliolatum* and to some cultivated plants. This is the first report of *P. cinnamomi* on Atlantic Rain Forest soil.

Glyceollin and lignin limit the growth of *Phakopsora pachyrhizi*

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Soybean rust, caused by *Phakopsora pachyrhizi,* is a devastating foliar disease of soybean. Understanding the biochemistry of the plant defense mechanism to this disease will assist in development of cultivars resistant to soybean rust. In this study, differences in phenolic metabolism were analyzed between inoculated and non-inoculated, two susceptible and three resistant soybean

lines with known resistance genes. Accumulation of isoflavonoids and flavonoids in soybean leaves was greatly increased in response to rust infection in all genotypes tested. While the soybean phytoalexin glyceollin was not detected in leaves of uninfected plants, accumulation of this compound at marked levels occurred in rust infected leaves. T here was a correlation between glyceollin concentration in soybean leaves and rust resistance. In addition, there was inhibition of *P. pachyrhizi* spore germination by glyceollin measured on agar plates. Lignin synthesis also increased in all inoculated soybean lines while there was no significant difference in all non-inoculated soybean lines. Cell wall lignification was markedly higher in inoculated resistant lines compared to inoculated susceptible lines indicating possible protective role of lignin in rust infection development.

Ralstonia solanacearum* Phc confinement-sensing system is required for slow-killing of the nematode *Caenorhabditis elegans
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Many bacteria regulate expression of some genes using quorum-sensing strategies that detect accumulation of extracellular autoinducers. In *Ralstonia solanacearum* the unique Phc system, which responds to 3-OH PAME, controls a typical acyl-homoserine lactone (AHL) system that regulates expression of one gene (*aidA*) but otherwise has no known function. Recently, the *aidA* ortholog in *Burkholderia ceonocepacia* was shown to be required for slow-killing of *Caenorhabditis elegans*. We consequently evaluated diverse *R. solanacearum* wild-type strains and mutants to evaluate their ability to kill *C. elegans*. Half of the 25 wild-type strains evaluated supported growth of axenic L1 larvae into fertile adults. The other strains, which included AW1, K60 and GMI1000, supported little or no growth and the larvae died. Surprisingly, neither *aidA* nor the AHL system was essential for toxicity of AW1. The extracellular polysaccharide and the type II and type III protein secretion systems also were nonessential. In contrast, inactivation of *phcA*, the Phc system global transcriptional regulator, reduced the toxicity of all strains tested. Microscopy revealed that an AW1 variant producing green fluorescent protein colonized the larvae's digestive tract to a much greater extent than did a comparable *phcA* mutant. We concluded that PhcA is essential, but not in itself sufficient, for production of unknown factors that improve the ability of *R. solanacearum* to colonize and slowly kill *C. elegans*.

Constructing physical and genomic maps for *Puccinia striiformis* by comparing EST sequences to the genomic sequence of *P. graminis*
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Phytopathology 99:S78

The stripe rust fungus, *Puccinia striiformis* (*Ps*), does not have a known alternate host for sexual reproduction, which makes it impossible to study gene linkages through the classic genetic approach. The objective of this study was to determine if the genomic sequence of *P. graminis* (*Pg*), the stem rust fungus, can be used to establish linkage relationships for *Ps* genes. A total of 4,219 *Ps* expression sequence tags (ESTs) were compared to the *Ps* genomic sequence database using BLAST searches. Of the genes, 1,432 (34%) had significant homology (e-value < $1e^{-5}$) with the *Pg* sequence. On the hypothesis that many *Ps* genes retain a colinear, syntenic relationship with the *Pg* genes, physical maps were constructed for the 1,432 *Ps* genes into 242 supercontigs corresponding to the *Pg* supercontigs. To validate the linkage relationships of *Ps* genes, 21 pairs of genes were selected to screen the *Ps* BAC library. The pairs of genes were those within 50 Kb in the *Pg* genome. Primers for the first gene in a pair were used in PCR amplification to screen the BAC library using a three-dimensional pooling approach. Identified individual positive clones were amplified with the primers for the second gene in a pair. Genes in 12 pairs (57%) were successfully identified in same clones, supporting their linkage relationships. These results show that the *Pg* genome sequence is useful in constructing physical maps for *Ps* genes and in study important genes in the two rust fungi.

***Fusarium* comparative genomics reveals genetic plasticity and pathogenicity development**
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Phytopathology 99:S78

The genus *Fusarium* collectively represents the most important group of fungal plant pathogens. We will present the results of comparative genomics analysis among three economic important and closely related *Fusarium* species: *F. graminearum, F. verticillioides* and *F. oxysporum*. More than 90% of the *F. verticillioides* genome can be unambiguously aligned to the syntenic regions in *F. oxysporum* with an average 90% sequence identity. Specifically, all eleven chromosomes in *F. verticillioides* have corresponding chromosomes in *F. oxysporum* and *F. graminearum*. In contract, over 15 Mb sequences, including 4 of the 15 *F. oxysporum* chromosomes, lack significant orthologous sequence in the other two genomes, defining the *F. oxysporum* lineage specific regions (*Fol* LS regions). These *Fol* LS regions are enriched for secreted proteins, transcription factors, and genes involved in signaling transduction regulation. The secreted proteins encoded in the *Fol* LS regions include known virulence factors such as, *SIX* (Secreted in Xylem) proteins, Necrosis and ethylene-inducing proteins, Peroxidases, and plant/fungal cell wall degrading enzymes. Gene families important in lipid metabolism and generation of lipid-derived second messengers are expanded through the gene encoded in these *Fol* LS regions. The evolutionary mechanisms underlying the acquisition and diversification of such genetic material and their potential impact on the pathogenicity will be discussed.

Non-citrus strains of *Colletotrichum acutatum* can colonize citrus leaves and produce conidia in response to citrus flower extracts
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Phytopathology 99:S78

A single *Colletotrichum acutatum* strain causes epidemics of postbloom fruit drop (PFD) of citrus throughout the Americas. This strain infects flowers, where it produces necrotic lesions on petals and premature abscission of fruit. It survives on vegetative tissues during non-flowering periods and conidiation from colonized tissue is stimulated by flower extracts. Genetically distinct strains from other hosts can cause PFD symptoms, but it is not clear if they can survive on vegetative tissue and sporulate in response to flower extracts. In the present study, isolates from anthracnose-affected blueberry, leatherleaf fern, strawberry, and Key lime and PFD-affected citrus were evaluated for their ability to survive surface sterilization of tangelo leaves up to 27 days after inoculation and to produce conidia in response to flower extracts. Colonies were recovered from tissue inoculated with all non-PFD strains after surface sterilization, with some non-PFD isolates recovered at incidences equal to or greater than that of the PFD isolate. Conidia production from leaves increased for all strains after flower extract treatment. Conidia numbers were comparable across isolates and there was no evidence that the multiplicative increase differed between isolates. These results indicate that the non-PDF isolates can colonize vegetative tissues of citrus and respond to flower extracts as does the PFD strain.

Characterization of *CHT1*, a putative C_2H_2 transcription factor involved in fumonisin biosynthesis and conidiation in *Fusarium verticillioides*
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Phytopathology 99:S78

Fusarium verticillioides causes ear rot and stalk rot in maize worldwide. It produces mycotoxin Fumonisin B1 (FB1), which is linked to human and animal cancers. Previous microarray study showed that *CHT1* gene, which encodes a putative C_2H_2 transcription factor, is up-regulated during FB1 biosynthesis and conidiation. Thus, we hypothesized that functional *CHT1* is required for FB1 biosynthesis and conidiation in *F. verticillioides*. Our initial approach to generate a *cht1* gene-deletion mutant was not successful, and it is conceivable that *cht1* mutation is lethal. As an alternative approach, we generated *CHT1* over-expression mutant strains, which were designated Ale1 and Ale16. The mutants and wild type showed different colony morphologies when grown on select media. On V8 agar, we observed dense mycelial growth and 40% increase in conidiation in mutants. In contrast, Ale strains produced significantly lower number (< 90%) of conidia when grown on corn kernels. When strains were grown on defined medium with $NaNO_3$ as the nitrogen source or sorbitol as the carbon source, the wild type colonies showed tan-brown pigmentation whereas the mutants showed white. Significantly, the mutants produced lower levels of FB1 when grown in cracked-corn medium. Gene expression analysis and the detailed physiological study of Ale mutants will be discussed. We will also examine virulence of Ale mutants on corn ears and stalks.

Brown stem rot caused by types A and B of *Phialophora gregata* reduces yield and growth of soybean
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Phytopathology 99:S78

Brown stem rot (BSR), a common disease of soybean, is caused by two types of *Phialophora gregata* in North America. Type A produces internal stem

browning and leaf necrosis, while type B typically produces internal stem symptoms and limited leaf symptoms. Type A has been considered to be more aggressive than B. The objective of this study was to determine if both types reduce yield and growth of soybean. Studies were conducted in Illinois and Minnesota in replicated field and greenhouse studies with multiple soybean cultivars either resistant or susceptible to BSR. At the V1 growth stage plant stems were injected at the soil line with types A or B. Stem symptoms of BSR developed in all studies. BSR reduced yields in all field studies over four seasons in multiple varieties, but differences compared to noninoculated controls were often not significant ($P > 0.1$). Yield in field studies was significantly reduced at least 17% in Minnesota by types A and B and 12% in Illinois by type B. In greenhouse studies, the number of pods per plant were reduced up to 40% and fresh biomass of stems and leaves was reduced up to 41% with types A and B relative to controls. Effects on plant height were inconsistent. Types A and B of *P. gregata* are both significant pathogens that can reduce yield and growth of soybean.

***Tomato bushy stunt virus* inoculation of roots versus leaves reveals differential effects by the coat protein and the P19 silencing suppressor**
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A traditional laboratory method for introducing *Tomato bushy stunt virus* (TBSV) into plants employs mechanical rub-inoculation of the leaf surface. However, under natural conditions transmission of TBSV occurs via the root system. To test root inoculation under laboratory conditions, a controlled method was adapted for inoculating virus RNA transcripts through gentle wounding of *Nicotiana benthamiana* and tomato roots. Inocula consisted of wild type TBSV, or a derivative in which the coat protein (CP) gene is replaced by the green fluorescent protein gene to give T-GFP, or a mutant not expressing the silencing suppressor P19. GFP expression was examined by UV illumination; confirmation of protein expression was determined by western blot analyses. It was found that systemic infections were established upon root inoculation of TBSV and T-GFP whereas after similar inoculations of leaves, T-GFP expression was restricted to the site of introduction. Moreover, even though inoculation of leaves with TBSV not expressing P19 resulted in initial systemic infection of *N. benthamiana*, this did not occur upon root inoculation. Thus, these preliminary studies suggest that the contribution of TBSV CP or P19 in establishing a systemic infection depends on the point of entry of the virus in the plants.

Oleic acid levels modulate defense signaling by regulating expression of resistance genes
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Phytopathology 99:S79

Oleic acid (18:1) is one of the major monounsaturated fatty acids (FA) in plants and its biosynthesis is catalyzed by the SSI2 encoded soluble stearoyl-acyl-carrier-protein-desaturase (SACPD). We have previously shown that reduction in the 18:1 levels, via a mutation in ssi2 or silencing of SACPD genes, results in constitutive activation of plant defense pathways in Arabidopsis and soybean, respectively (1). More recently, we have provided evidence linking 18:1 levels with resistance (R) gene expression and pathogen resistance (2). Lowering the levels of 18:1, via genetic mutations in SSI2, or by exogenous application of glycerol, induces the expression of several R genes. Genome-wide analysis showed that at least 25 R genes (and R proteins) were upregulated in the ssi2 plants in an salicylic acid-independent manner. The altered defense-related phenotypes in ssi2 plants can be rescued by restoring the 18:1 levels via second site mutations in genes encoding a glycerol-3-phosphate (G3P) acyltransferase (3), a G3P dehydrogenase (4), and an acyl carrier protein (5). We have also identified additional suppressors that restore defense signaling without affecting 18:1 levels in ssi2 plants. Detailed molecular and biochemical characterization of these suggests that 18:1 modulates their activities by binding to them.

Chromosomal polymorphism in *Fusarium virguliforme*, the causal agent of Sudden Death Syndrome in soybeans
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Phytopathology 99:S79

Fusarium virguliforme, also known as *Fusarium solani* f. sp. *glycines*, is a soil-borne pathogen that causes Sudden Death Syndrome (SDS) in soybeans. Despite the importance of the disease, the pathogenicity determinants in the fungus are mostly unknown. Fungal isolates are known to have varied levels of virulence as evidenced by greenhouse and field experiments. Little is known about the genome structure of the pathogen or the degree of chromosomal polymorphism in *F. virguliforme* isolates. Chromosome length polymorphism (CLP) was assessed on six different isolates of *F. virguliforme* using contour-clamped homogeneous electric field (CHEF) gel electrophoresis. The isolates, collected from soybean fields in Illinois and Iowa, showed different levels of virulence on soybeans. CLP was detected among the different isolates and two distinct karyotypic patterns were observed. This is the first report of CLP among *F. virguliforme* isolates. Additional studies are in progress to establish the relationships between CLP and geographical origin and aggressiveness of *F. virguliforme* isolates on soybean.

Biocontrol of sclerotinia stem rot of canola using *Pseudomonas fluorescens* and *Bacillus subtilis*
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Phytopathology 99:S79

To study biocontrol of *Sclerotinia sclerotiorum,* 120 bacteria recovered from canola phyllosphere and rhizosphere were screened for their antagonistic ability *in vitro*. Of these, two strains including a *Pseudomonas fluorescens* (Pf1) and *Bacillus subtilis* (Bs1) were selected based on several assays. In antibiotic production assay, Pf1 caused 90% and Bs1 caused 85.1% reduction in mycelial growth. Culture filtrate assay indicated that Pf1 and Bs1 inhibited mycelial growth up to 84% and 100%, respectively. In volatile assay Pf1 and Bs1 reduced mycelial growth up to 83% and 34%, respectively. Antibiotic resistant mutants of these two strains were selected on Nalidixic acid and Rifampicin containing medium. *In vitro* tests showed that antagonistic properties of the wild type and mutants were not altered. Further studies using specific primers indicated that Pf1 has pyrrolnitrin and pyoluteorin and Bs1 has 2,4-Diacetyl phloroglucinol producing genes. Greenhouse experiments indicated that Bs1 caused 74% and Pf1 caused 83% reduction of the area of lesion, Bs1 caused 3.3 times and Pf1 caused 3.7 times of fresh weight of canopy and Bs1 caused 4.8 times And Pf1 caused 5.9 times of dry weight of canopy in comparison with positive control. The results indicated that Bs1 and Pf1 reduced the lesion up to 74 and 83 percent, respectively. The Bs1 and Pf1 increased the fresh weight of canopy up to 3.3 and 3.7 times, respectively. The dry weight of canopy was increased up to 4.8 and 5.9 times in Bs1 and Pf1, respectively.

Characterization of benomyl-resistant isolates of the fungal pathogen of banana *Mycosphaerella fijiensis,* collected in Mexico
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Phytopathology 99:S79

The fungus *Mycosphaerella fijiensis* cause black leaf streak disease (BLSD) of banana is the most economically important pathogen in the world. The fungus affects leaf tissues causing a marked reduction of the photosynthetic area, which leads to premature fruit ripening, and loss of production. The control of BLSD is based on intensive application of chemical fungicides including triazoles, strobirulins and benzimidazoles. The objective of this study was evaluated the sensibility to benomyl of 11 colonies monosporic of *M. fijiensis* collected of different localities of the states of Chiapas, Tabasco, Michoacán and Colima, Mexico. The concentrations of benomyl to 0.3, 1, 5 and 10 ppm were used. Nine colonies showed resistant to benomyl, because this growth to 10 ppm of benomyl. The sensible isolates were of the localities Cerro de Ortega, Colima and Pascuales, Colima. Actually benomyl is applied for the control of BLSD in the majority of the regions producers of banana in Mexico. Is important determiner the levels of sensibilities of *M. fijiensis* to benomyl for prevent the development of resistance of this important pathogen.

Phylogeography of the cotton root rot fungus *Phymatotrichopsis omnivora*
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Phytopathology 99:S79

The soilborne fungus *Phymatotrichopsis omnivora* (Duggar) Hennebert causes an important root rot of numerous dicot crops, including cotton, alfalfa, grape, and fruit and nut trees. The disease is variously named Phymatotrichum, cotton, Texas or Ozonium root rot and is limited in occurrence to alkaline calcareous soils of the southwestern United States and northern Mexico. To better understand the population biology of *P. omnivora*, 125 isolates were collected from cotton and alfalfa fields in Arizona, Texas and Oklahoma. The internal transcribed spacer regions of the nuclear ribosomal DNA (ITS-rDNA) from all isolates were amplified, sequenced and aligned with additional ITS-rDNA sequences from GenBank. ITS sequences possessed numerous indels which varied with the geographic source. Using a

probalistic alignment program, ITS-rDNA haplotypes clustered phylogeographically. Arizona isolates possessed the most diverse ITS haplotypes, while most isolates collected in north central to southeastern Texas and southern Oklahoma consisted of just two haplotypes. No host specialization was observed among ITS haplotypes. Also, partial gene sequences of the RNA polymerase II subunit 2 (rpb2, regions 5 to 7) of 70 of the 125 isolates were sequenced and aligned and found to be nearly identical, supporting the probability that the isolates represent a single species. This study provides an initial framework for further population biology studies of *P. omnivora*.

The *NLP1* and *NLP2* genes of *Sclerotinia sclerotiorum* (Lib.) de Bary exhibit different expression patterns in axenic cultures and infected soybean plants

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Phytopathology 99:S80

Sclerotinia sclerotiorum is a cosmopolitan fungal pathogen causing severe damage to more than 400 crop species around the world. We identified two necrosis and ethylene inducing-like proteins, NLP1 and NLP2, in the *S. sclerotiorum* genome database. Expression analysis of the *NLP1* and *NLP2* genes was performed for cells grown on several different agar media or *in planta* (soybean hypocotyls; 0, 6, 12, 24 and 48 hours post inoculation). Northern blots and quantitative (q)RT-PCR analysis revealed that these genes differ in their expression patterns. The *NLP1* gene was expressed on water agar (WA) and glycerol–containing minimal medium, and repressed on complete medium (CM), potato dextrose agar (PDA), minimal medium (MM), and MM containing olive oil or ethanol as a carbon source. The *NLP2* gene was expressed on all seven media. During infection of soybean hypocotyls *NLP1* was not expressed during the first 48 hours after inoculation, at which time the necrotic lesion is well established. In contrast, *NLP2* gene was showing strong expression by early infection (6 hours) and that expression level maintained until 48 hours after inoculation. These data suggest different roles for NLP1 and NLP2 in pathogenesis of *S. sclerotiorum*. Their specific function during infection is currently being investigated using gene knock-out mutants obtained through *Agrobacterium tumefaciens*-mediated transformation (ATMT).

Relationships between *Dickeya* species and strains from heart rot of pineapple based on sequence comparison of *dnaJ*, *gyrB*, *dnaA*, and *recN* genes

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Phytopathology 99:S80

The broad host range pathogen *Dickeya* (formerly *Erwinia chrysanthemi*) causes diseases on agriculturally important crops in tropical and subtropical regions. In Hawaii, *Dickeya* sp. was identified as the causal agent of pineapple heart rot disease, which first occurred in 2003. The relationships of *Dickeya* reference strains to strains collected in Hawaii between 2003–2006 were investigated by the comparison of partial nucleotide sequences of *gyrB*, *dnaJ*, *recN* and *dnaA*. *Dickeya* strains isolated in Hawaii from pineapple clustered separately from strains isolated from irrigation water. Two *Dickeya* sp. reference strains isolated from pineapple in Malaysia grouped within the cluster containing strains isolated from Hawaiian pineapple. A single irrigation water strain from 2003 clustered with *D. dadantii*, whereas, strains isolated from irrigation water in 2006 clustered with *D. zeae*. *Dickeya* sp. from pineapple and irrigation water show similar genetic separation and clustering with BOX-PCR and the nucleotide sequences of the four loci. The phylogenetic relationships among the Hawaiian *Dickeya* strains further suggest that strains from pineapple may represent a new species of *Dickeya* or subspecies of *D. zeae*, and did not arise from a local *D. zeae* or *D. dadantii* population.

A new potent bio-fungicide for the control of Banana Black Sigatoka

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Phytopathology 99:S80

Black Sigatock (BLS) caused by *Mycosphaerella fijiensis* is a major concern for the Banana production. The disease spreads rapidly around the globe and causes vast economic damages. The intensive use of fungicides (up to 70 sprays/year), due to resistance development, is a major concern for the environment and human health. A new organic formulation Timorex Gold (TG) containing 23.8% tea tree oil (TTO) was recently introduced and demonstrated high efficacy against BLS both in organic and conventional productions. In vitro tests showed that TG at inhibited spore germination and mycelial growth of the fungus. A single leaf test showed that it effectively controlled BLS. TG exhibited translaminar activity against BLS in banana, in which it also limited the expansion of lesions up to stage III. Whole plants tests revealed that TG at 0.4 lit/ha was as effective as spiroxamine, difenoconazole and azoxystrobin as standards. Consequtive foliar applications in large organic and conventional plots in central and south America showed that TG at 0.4–0.5 lit/ha provided the best protection and was as effective as or better than commercial standards. In general, TG-treated plants exhibited greater number of healthy leaves than standard treatments. It has a prophylactic and curative activity. TG is an effective and attractive residue free and environmental friendly product against BLS in organic and conventional growth and a tool for anti-resisrance program.

Irrigation water composition affects rapid blight of perennial ryegrass

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Phytopathology 99:S80

Rapid blight, caused by *Labyrinthula terrestris* (D.W. Bigelow, M.W. Olsen, and Gilbertson.), increases with increasing irrigation water salinity. Irrigation water from Western (WW) and Eastern (WE) coastal regions of the United States, where rapid blight occurs, have different levels of mineral constituents contributing to salinity. WW primarily differs from EW salinity in having higher Ca and slightly lower Na. Isolates of *L. terrestris* from each region have different salinity optima *in vitro* as well. Several experiments were conducted in a greenhouse environment to evaluate differences in isolate pathogenicity and irrigation water composition on the occurrence of rapid blight in perennial ryegrass. At nearly equivalent salinity levels, measured by electrical conductivities, irrigation water with lower levels of Ca but higher levels of Na indicative of EW sources resulted in substantially more rapid blight than higher levels of Ca indicative of WW sources. Ryegrass shoot moisture content and shoot dry weight decreased with inoculation and concentration of 2.4 and 4.6 dSm^{-1} and was reduced more with EW irrigation than with WE. Perennial ryegrass plants irrigated with EW and infected with rapid blight also accumulated more Na and less K in leaves compared to infected plants that were irrigated with WW. Differences in pathogenicity among isolates from the two environments were minimal.

Towards a more fitting spatial analysis of microbial community composition

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Phytopathology 99:S80

Rapid determination of the etiology of newly introduced diseases is hampered by fast-growing opportunists present in samples along with the primary disease agent. We are seeking to overcome these difficulties using culture independent methods based in a spatial analysis of all phyllosphere microorganisms on transects from more healthy to more diseased areas in suspect fields. The transition through space into diseased areas simulates a disease time-course so that the primary agent can be identified at a distance from the focus. In order to do this, we must first characterize the background microbial community as the context against which the presence of the pathogen must be distinguished. We are working with a data set developed from automated rRNA intergenic spacer analysis (ARISA) of fungal communities represented in DNA extracts of above-ground peanut and cotton tissues collected in western Florida. We will present our evaluation of the relative effectiveness of geospatial, cluster and principal component analysis as multivariate approaches to identifying spatially coherent patches of similar communities. We have discovered patches of high diversity communities, interspersed with simple communities mostly dominated by single species. These patches span many meters and are repeated along the crop row, indicating that a fragmented background community may be present against which an invading pathogen could be detected.

Downy mildew quarantine diseases of grape vines in Uzbekistan

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Phytopathology 99:S80

Grape vine downy mildew was discovered for the first time in different soil-climatic conditions of the Tashkent region of Uzbekistan in 2003. The pathogenicity of the disease and varietal resistance was studied. Based on these studies, the recommended control of the disease involves the use of Alto-Super, 33% c.e. (0.3 liter/ha).

Analysis of molecular variability and PCR amplification of race 1-specific fragment in *Verticillium dahliae* isolates

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Phytopathology 99:S81

Verticillium dahliae Kleb. is a soilborne fungal pathogen that causes Verticillium wilt with severe yield and quality losses in hundreds of economically important crops worldwide. In California, race-specific resistance is available in lettuce and tomato cultivars. Two races of *V. dahliae* pathogenic on tomato and lettuce have been identified but race identification through virulence assay on host differentials is labor-intensive. To further characterize these populations and develop molecular detection of races, 90 isolates were collected from different hosts, including 55 from tomato, and analyzed for DNA polymorphisms in internal transcribed spacers (ITS), intergenic spacer region (IGS) and microsatellites (Simple Sequences Repeats, SSRs) sequences. The complete ITS and IGS regions of 560 bp and ~1.8 kb were sequenced for all 90 isolates. The pair-wise sequence identity for the ITS region ranged from 96.3 to 100% and 78.8 to 100% for IGS sequences. Analyses of the 22 SSR markers showed a total of 92 alleles (each locus producing between 2 and 7 alleles), which showed polymorphisms among isolates of *V. dahliae*. Using a PCR assay, a race 1-specific fragment was amplified in isolates of *V. dahliae*. The primer pair can be used to differentiate the two races described in lettuce and tomato. Virulence phenotypes on both crops validate the molecular race diagnosis.

Genetic diversity of *Tilletia caries* isolates from wheat in Washington State

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Phytopathology 99:S81

With the growth of the organic sector, there is a critical need for research on seedborne diseases under organic cropping systems, specifically common bunt of wheat, caused by *Tilletia caries.* To develop bunt resistant cultivars, it is imperative to know the races and the extent of genetic diversity of *Tilletia caries* isolates in Washington State. From 2007 to 2008, wheat spikes naturally infected with common bunt were collected. Race identification tests were conducted by inoculating the teliospores of 11 isolates on 15 bunt differential lines. Genomic DNA from teliospores was extracted, and the genetic diversity of five isolates was assayed using amplified fragment length polymorphism (AFLP). The five most informative primer combinations were chosen in the AFLP analysis. Only polymorphic fragments with high resolution, and between 80 and 550 bp, were included in the analysis. Results suggest the possibility of two new races of *Tilletia caries* from Washington State. Three isolates had similar AFLP fingerprints but had different virulence combinations. Also, two sets of isolates showed AFLP fingerprint divergence but had the same virulence combinations. Moreover, two isolates showed distinct molecular types when DNA from single sori were assayed, suggesting either mixed infection or high genetic variability within one infected head. These findings have significant implications on breeding cultivars with durable bunt resistance under organic cropping systems.

Comparison of products and application methods for control of Sclerotinia drop of lettuce

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Phytopathology 99:S81

In Arizona, Sclerotinia drop of lettuce is caused by *Sclerotinia minor* and *S. sclerotiorum*. Field trials were conducted during the 2007 and 2008 growing seasons to compare disease control with different products and methods of application. Lettuce was seeded on raised beds in double rows 30 cm apart. After thinning to a 30-cm spacing within rows, approximately 2,100 sclerotia of *S. minor* or 800 sclerotia of *S. sclerotiorum* were distributed on the surface of each 7.6-m-long plot between the rows of lettuce, then incorporated into the top 5-cm layer of soil. Compared to nontreated plots, the mean number of diseased plants for the two trials in plots containing *S. minor* was reduced 79% by one application of fluazinam to the soil surface, 68% by two applications of boscalid incorporated into soil to a depth of 5 cm, and 61% after two applications of boscalid applied to the soil surface without incorporation. With *S. sclerotiorum*, disease reduction was 72% for one application of fluazinam, 66 and 62% after two applications of boscalid and iprodione, respectively, incorporated into soil, and 55 and 50% after two respective applications of boscalid and iprodione to the soil surface. When applied to the soil surface and incorporated into the soil with water, *Coniothyrium minitans* reduced the number of plants infected by *S. minor* and *S. sclerotiorum* by 43 and 76%, respectively.

Identification of networks and pathways in the *Magnaporthe oryzae* transcriptome during stress conditions

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Rice blast disease is caused by the ascomycete fungus *Magnaporthe oryzae* and is the most important disease of cultivated rice, destroying about 30% of the cultivated rice every year. Understanding how it is able to invade plant cells and overcome stressful conditions can provide useful information for disease control. The focus of our research is to investigate compatible interactions in which *M. oryzae* faces stressful conditions in the plant cells and is able to establish the disease. We studied the transcriptome of *M. oryzae* during *in planta* and *in vitro* oxidative and nutritional stress experiments using microarrays. We are currently identifying canonical pathways and gene networks from our transcriptome data set that are likely to be the most important for the conditions faced by the fungus. Many cellulose, hemicellulose, and sugar degrading enzymes were up-regulated *in planta* at 72 hours post-inoculation as well as reactive oxygen species (ROS) scavenger genes, such as superoxide dismutase, catalase and glutathione. The increase in gene expression of these genes in stress conditions compared to their expression in the control conditions suggests that the dataset generated was able to capture changes in gene expression that are important to *M. oryzae* when subjected to adverse conditions. Further molecular characterization of specific genes and pathways will reveal gene functions and their effects on pathogenesis.

Identification and pathogenicity of *Botryosphaeria* species associated with avocado branch dieback and trunk canker in California

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Phytopathology 99:S81

Species of *Botryosphaeria* are well known pathogens which cause canker and dieback in woody hosts. The objective of this study was to survey species diversity of *Botryosphaeria* associated with branch and trunk canker of avocado in California and to determine the pathogenicity of the species. Five symptomatic trees in each of six groves were chosen for study. Fungi were isolated from diseased tissues using potato dextrose agar amended with tetracycline (0.01%) (PDA-tet) from an average of 50 symptomatic branch and trunk canker samples per tree (250 samples per grove). Percent recovery of Botryosphaeria spp. based on morphological characters ranged from 40–100% in Riverside county, 42–53% inVentura county, 33% in Santa Barbara county and 60% in San Diego county. Molecular methods were used to identify species based on the analysis of the internal transcribed spacer region (ITS) of rDNA, and a partial sequence of the β-tubulin gene. Four different species of Botryosphaeria were identified: *B. dothidea, B. lutea, B. parva* and *B. australis.* Pathogenicity tests were performed by stem wound inoculations on 1-year-old cv. Hass seedlings. Plants were maintained in the greenhouse and, after six months, symptoms included internal vascular lesions extending from the wound site. Each species was consistently reisolated from inoculated plants. The results show that multiple species of Botryosphaeria are involved in the branch and trunk canker disease of California avocado.

Etiology and management of sour rot in vineyards in Ontario, Canada

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Phytopathology 99:S81

Late season bunch breakdown sour rot has been a recurring problem in Niagara vineyards for the past few years, particularly in thin-skinned varieties. Many organisms, including yeasts, bacteria and fungi, have been implicated in this disease in Europe but the etiology in Ontario is unknown. Samples of fruit with sour rot symptoms were collected from a commercial vineyard, cv. Riesling, in St. Catharines, ON in 2007. The most frequent pathogenic organisms were identified as *Hanseniaspora uvarum* and *Gluconobacter cerinus.* Foliar-applied Ca, kelp and Serenade Max, alone and in rotation with a commercial standard, applied at bunch close, veraison and 2 wk post-veraison controlled incidence and severity of both bunch rot and sour rot as well as the commercial standard rotation. In another trial, a plant growth regulator applied at mid-bloom reduced cluster compactness and incidence and severity of bunch rot and sour rot. In a third trial, potassium metabisulfite (KMS), chlorine dioxide, MilStop, copper hydroxide, Pristine and Serenade Max were applied to clusters infected with sour rot in a commercial vineyard of Riesling. Two sprays were applied and fruit were sampled the day following each spray and analyzed for volatile acidity. Only KMS consistently reduced VA compared to the untreated check. KMS applied as little as 1 day

pre-harvest did not affect vinification and SO_2 residues were comparable to the untreated check.

Evaluation of the antibiotic kasugamycin for control of fire blight
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Phytopathology 99:S82

The emergence and spread of streptomycin-resistant (Sm^R) strains of *Erwinia amylovora* in Michigan has necessitated the discovery and evaluation of new compounds effective for fire blight control. Kasugamycin (Ks) targets the bacterial ribosome and is particularly active against *E. amylovora*. The efficacy of Ks (Kasumin, Arysta Corp.) for control of fire blight was evaluated in five experiments conducted over three field seasons in our experimental orchards in East Lansing, MI. Blossom blight control was statistically equivalent to streptomycin in all experiments. The occurrence of shoot blight in Kasumin-treated trees was also statistically equivalent to streptomycin in six of seven comparable experimental treatments. In replicated lab experiments, the development of spontaneous resistance in *E. amylovora* to 250 or 500-ppm Ks was not observed. However, exposure to increasing concentrations of Ks in media (initial conc. 50 ppm) resulted in the selection of Ks tolerance (at 150–200 ppm) in three strains with corresponding mutations in the *ksgA* gene. The possible occurrence of a reservoir of Ks^R genes in orchard environments was also examined. Environmental bacteria from five Michigan apple orchards were surveyed; a total of 72 Ks^R isolates (20 different species) were recovered from soil samples, and only 7 Ks^R isolates (5 species) were recovered from aerial apple tissue. Examination of the Ks^R mechanism in these isolates is ongoing.

Oxytetracycline- and copper-resistance in *Xanthomonas arboricola* pv. *pruni* isolates from Michigan orchards
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Phytopathology 99:S82

An intensive copper (Cu) and oxytetracycline (oxyTc) spray program has been used for many years to control bacterial spot, caused by *Xanthomonas arboricola* pv. *pruni* (Xap), on susceptible peach and nectarine cultivars in Michigan. The observation of possible reductions in control of these compounds has increased the need for current assessments of resistance. To begin to determine the extent of this resistance, diseased leaves were sampled once from 15 Michigan orchards in 2004. In 2008, three orchards were sampled on 9 July, and two of these orchards were sampled again on 6 August. Two additional sites were sampled on 13 August 2008. Xap isolates were recovered from lesions onto nutrient agar (NA), and then patched to NA amended with 25 µg/ml oxyTc, and NA amended with 300 µg/ml $CuSO_4$. The occurrence of copper resistance was variable (30.2% and 14.5% in 2004 and 2008, respectively). No isolates collected in 2004 grew on NA + oxyTc. In 2008, 34 of 330 isolates (10.3%) grew on NA + oxyTc with the percentage as high as 26.7% (16/60 isolates) in one orchard. Initial screening of the tetracycline resistant isolates by PCR for known tetracycline resistance genes has identified the *tet*B resistance gene in 77.8% of the oxyTc-resistant isolates. We are currently determining the genetic location and mobility of *tetB* in the Xap isolates.

Fungicide sensitivity and resistance of the cucurbit powdery mildew pathogen in New York, Pennsylvania, Ohio, and Indiana in 2008
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Phytopathology 99:S82

To obtain information to guide fungicide recommendations, sensitivity of *Podosphaera xanthii* to registered fungicides at risk for resistance was examined with a seedling bioassay. Seedlings were treated with fungicides, put for several hours in a cucurbit field where powdery mildew was developing, incubated in a greenhouse for 7 to 13 days, then proportion of the pathogen population tolerating each fungicide concentration was estimated by comparing severity on treated and non-treated leaves. Resistance to FRAC Code 1 (MBC) fungicides, which is qualitative, was estimated to be 63%, 94%, 83%, and 49% of the sampled population in NY, PA, OH, and IN, respectively. Resistance to FRAC Code 11 (QoI) fungicides, which is qualitative, was estimated to be 64%, 76%, 91%, and 100% of the populations, respectively. Resistance is quantitative for other mobile fungicides used to manage cucurbit powdery mildew. Proportion of the pathogen population tolerating 120 ppm myclobutanil (FRAC Code 3 fungicide that is the active ingredient in Nova) was estimated to be 9%, 25%, 4%, and 4% in NY, PA, OH, and IN, respectively. Proportion tolerating 50 ppm boscalid (FRAC Code 7 fungicide that is an a.i. in Pristine) was estimated to be 8%, 10%, 62%, and 12%, respectively. Pathogen strains tolerating 175 ppm boscalid were detected in NY and PA but not IN. Proportion tolerating 10 ppm quinoxyfen (FRAC Code 13 fungicide that is the a.i. in Quintec) was estimated to be 0.5%, 0.7%, 0.4%, and 0%, respectively.

Spatial distribution of *Phytophthora cinnamomi* in forest soils of the Carolinas
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Phytopathology 99:S82

Phytophthora cinnamomi is an important plant pathogen in the forests of the southeastern United States. It is widespread in forest soils and known to attack many plant species. In recent studies, thinning and prescribed burning had little to no effect on the incidence of this pathogen in soil. Therefore, we investigated the vertical and horizontal distribution of *P. cinnamomi* in soil in three hardwood forests in the western parts of North and South Carolina. At each study site, horizontal distribution was determined in three square plots (210 cm per side) with 64 grid-points at 30-cm intervals. A soil core (2 cm diameter, 20 cm deep) was collected at each grid point, and these were assayed for *P. cinnamomi* using a baiting bioassay. Vertical distribution was studied in 13 soil cores (5 cm diameter, 50 to 74 cm deep) taken from all three sites, and subsamples of soil were assayed for *P. cinnamomi* at six standard depths. *P. cinnamomi* was present in 7 of 9 square plots and was detected in 14 to 97% of the soil cores from each of these plots. Horizontal distribution was not aggregated based on spatial distribution analysis. *P. cinnamomi* was present in 10 of 13 vertical cores. It was detected at the soil surface in 6 cores and at all five depths below the surface: 6 cm (3 cores), 23 cm (1 core), 40 cm (3 cores), 57 cm (2 cores), and 74 cm (1 core). In most cores, vertical distribution of *P. cinnamomi* was discontinuous—i.e., it was not found in contiguous subsamples; in three cores, it only was found at 40 to 74 cm deep.

Investigation of the southern green stink bug (*Nezara viridula* L.) vector potential using bacterial and fungal cotton pathogens
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Recently, we reported the capacity of the southern green stink bug (SGSB) to act as a vector of an opportunistic *Pantoea agglomerans* strain into green cotton bolls. Here, we tested the hypothesis that the SGSB is a vector for certain pathogens by incorporating two identified cotton bacterial opportunists (*P. ananatis* and *Klebsiella pneumoniae*) and the yeast pathogen *Nematospora coryli* into our model. Sequentially, lab-reared SGSB were provided a pathogen contaminated food source (2 days), sterile food (5 days), caged with a green boll (2 days), and then the fruit was harvest 2 weeks later. Variants of *P. ananatis* (Pa-1R) or *K. pneumoniae* (Kp 5-1R) with rifampicin resistance were the bacterial opportunist representatives, and *N. coryli* was only detected from stink bugs exposed to the fungus. Both Pa-1R and Kp 5-1R were recovered from SGSB provided contaminated food, and then caged with bolls at levels reaching 10^3 and 10^4 CFU per insect, respectively. However, bolls with evidence of feeding by insects infested with Pa-1R or Kp 5-1 had a normal locule appearance and neither strain was detected from seed and lint tissue. Insects infested with *N. coryli* transmitted the pathogen, resulting in diseased bolls and yeast concentrations that reached 10^6 CFU/g locule tissue. These results indicated that the SGSB potential to act as a disease vector is not solely based on acquisition of an opportunist, and at least a transient colonization of the pathogen in the insect mouthparts is necessary for transmission.

Competitive interactions among isolates of *Aspergillus flavus* during maize infection
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Phytopathology 99:S82

Aflatoxin contamination of maize results from crop infection by mixtures of *Aspergillus flavus* that vary in several characteristics including vegetative compatibility and aflatoxin-producing ability. Aflatoxin content is dictated, in part, by intraspecific interactions. To better understand these interactions, competitive differences among *A. flavus* isolates were examined. Living maize kernels were inoculated with either one isolate followed by another an hour later or a mixture of the two isolates. In a second experiment, seed were similarly inoculated but with 0, 1, 8, 24, 48, or 72 hours between the addition of isolates. After 7 days at 31°C, isolate-specific SNPs were quantified by

pyrosequencing. In both experiments, competitive advantage was conferred by inoculation order. Seed colonization varied significantly with inoculation interval, but there were no differences between 0, 1, and 8 hours. If the second isolate was added at least 24 hours after the first isolate, it comprised less than 5 percent of the total DNA at the end of the experiment but was not entirely excluded. The first isolate to reach the seed had an advantage even when spore germination did not occur prior to the arrival of another isolate. Improved understanding of intraspecific competition among *A. flavus* during crop infection will provide insights into the infection process and facilitate efforts to reduce aflatoxin contamination through competitive exclusion.

Current status of grapevine viruses in Washington State vineyards

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Phytopathology 99:S83

The wine grape industry in Washington State contributes $3 billion plus to the state's economy and has a national economic impact of $4.7 billion per year. Grapevine leafroll disease (GLD) is a significant constraint to sustainable growth of the wine grape industry in the State. We have initiated studies to document viruses associated with GLD. Leaf samples from grapevines showing symptoms of (or suspected to be infected with) GLD in red grape varieties and random samples from white grape varieties (which are asymptomatic) were collected from commercial vineyards during the growing season. Extracts from petiole samples were tested for different viruses by one tube-single step RT-PCR technique using virus-specific primers. The sequences of DNA fragments amplified from RT-PCR assays were compared with corresponding sequences available in the GenBank. The results from a three year study showed the presence of *Grapevine leafroll-associated virus* (GLRaV) -1, -2, -3, -4, -5, and -9 in different wine grape varieties showing or suspected for GLD symptoms. Mixed infections of these viruses in different combinations were frequently detected in individual grapevines. GLRaV-3 was found to be the most prevalent among the six GLRaVs documented. The results also revealed the presence of *Rupestris stem pitting-associated virus*, *Grapevine Virus A*, *Grapevine Virus B*, *Grapevine fanleaf virus* and *Grapevine fleck virus* as mixed infections with GLRaVs.

Insect transmission and genotypic variation of pecan pathogenic *Xylella fastidiosa* strains in Louisiana

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Phytopathology 99:S83

Xylella fastidiosa causes disease in many economically important crops including grape, peach, coffee, citrus, and pecan. Symptoms of pathogen infection may include leaf scorching and defoliation, yield reduction and plant death. Pecan bacterial leaf scorch (PBLS) caused by *X. fastidiosa* can reduce nut production by as much as 16% in susceptible cultivars. In nature, insect transmission is the primary means of pathogen spread. Several species of Cicadellidae known to transmit *X. fastidiosa* in other crops, such as grape, have recently been found to transmit to pecan, suggesting that common vectors may transmit the pathogen in different plant hosts. Despite the possibility of inter-species transmission of *X. fastidiosa* due to shared vectors, the influence of other hosts on the epidemiology of PBLS has not been studied. In addition, the relationship between *X. fastidiosa* strains isolated from pecan and other known *X. fastidiosa* subspecies is not clearly understood. In an attempt to determine the phylogenetic stature of the *X. fastidiosa* strains isolated from pecan and other hosts in Louisiana among known subspecies of *X. fastidiosa*, we are currently analyzing DNA sequence variation in a known virulence gene, *pglA*, encoding polygalacturonase. Additional genomic elements, such as the internal transcribed spacer (ITS) and 16S rDNA sequences, will also be analyzed for the same purpose.

The capsid protein of *Cowpea chlorotic mottle virus* is a determinant for vector transmission by a beetle

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Phytopathology 99:S83

The bromovirus *Cowpea chlorotic mottle virus* (CCMV) is transmitted by chrysomelid beetles and infects *Nicotiana clevelandii* in an asymptomatic manner. The related *Cucumber mosaic virus* (CMV) is transmitted in a nonpersistent manner by aphids and induces severe stunting and leaf deformation in *N. clevelandii*. The capsid protein is known to be responsible for vector transmission specificity in many plant virus systems. In this study we investigate the ability of a hybrid CMV expressing the capsid protein of CCMV to be transmitted by the spotted cucumber beetle (*Diabrotica undecimpunctata*). *N. clevelandii* plants were mechanically inoculated with CCMV, CMV and hybrid virus. Teneral beetles were fed for 24 hours on detached leaves from infected plants. Beetles were then transferred to uninfected *N. clevelandii* plants, fed another 24 hours and removed. Seven to ten days after beetle-inoculation, hybrid virus-infected plants were observed with symptoms resembling CMV infection. CCMV was transmitted less efficiently than the hybrid virus and infected plants were asymptomatic. No beetle transmission of CMV was observed. Our findings suggest that CMV is not transmitted by beetles and the replacement of its capsid protein by that from a beetle transmitted virus enables beetle transmission.

Transmission efficiency of Potato virus Y strains PVY^{O} and $PVY^{N\text{-}Wi}$ by five aphid species

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Phytopathology 99:S83

Potato virus Y (PVY) is a re-emerging problem in potato production in North America. While the 'ordinary' strain, PVY^{O}, is still the dominant isolate in U.S. seed potatoes, the recombinant strain of the virus $PVY^{N\text{-}Wi}$ (=$PVY^{N:O}$) has become widespread. An increase in the prevalence of a PVY strain could be due to differences in the efficiency of transmission by aphid vectors. To test transmission efficiencies of PVY^{O} and $PVY^{N\text{-}Wi}$, five isolates of each strain were tested for transmission by a clone of the aphid *Myzus persicae*. No apparent differences in transmission by *M. persicae* were observed. Other aphid species frequent but do not colonize potato, and they may play a role in the nonpersistent transmission of PVY. A single isolate of each of PVY^{O} and $PVY^{N\text{-}Wi}$ were tested for their ability to be transmitted from potato to potato by five aphid species: *Aphis glycines*, *A. gossypii*, *A. nasturtii*, *M. persicae*, and *Rhopalosiphum padi*. Both PVY strains showed a similar transmission phenotype in being transmitted efficiently by *M. persicae*, but very poorly or not at all by *A. glycines*, *A. gossypii*, and *R. padi*. The aphid *A. nasturtii* transmitted both strains with an intermediate level of efficiency. The data does not support a model for a differential aphid transmissibility being responsible for the increase in the prevalence of $PVY^{N\text{-}Wi}$.

Impact of application of endophytic *Bacillus* spp. for biocontrol of cacao diseases on native microbial communities

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Phytopathology 99:S83

Interest in ecologically-based management of cacao diseases has lead to research on biocontrol. Sixty-nine endophytic *Bacillus* spp. were isolated from cacao trees escaping disease near Quevedo, Ecuador and screened as potential biological control agents. Four elite *Bacillus* spp. are currently being field evaluated to determine their ability to suppress witches' broom disease, caused by *Moniliophthora perniciosa*. Research was conducted to determine how applications of biocontrol agents impacts diversity and abundance of native microbial communities. Since most endophytes are likely neutral in terms of plant health, it is hypothesized that application of beneficial bacteria will displace neutral endophytes to positively impact overall plant health. Each isolate was applied to runoff at log 8.0 CFU/ml with 0.20% Silwet L-77 adjuvant to 4 branches each of 3 replicate nacional trees per treatment. Controls consisted of application of adjuvant alone. Three months after both the application and reapplication of the bacteria, leaf discs were excised from 2 sprayed leaves per branch. Leaf discs from one tree were combined and placed in RNALater for preservation during shipment. Genomic DNA was extracted from the sample and Automated Ribosomal Intergenic Spacer Analysis (ARISA) was utilized to determine ecological shifts in both bacterial and fungal communities. Data will be presented on effects of application of these biocontrol agents on native microbial communities.

Biological control of *Theobroma cacao* pod diseases with endophytic *Bacillus* spp.

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Phytopathology 99:S83

Cacao farmers suffer extensive pod losses from the condition known as cherelle wilt and the diseases black pod, frosty pod, and witches' broom. Desire for ecologically-based farming coupled with the prohibitively high cost of pesticides has lead to research on biological control options for pod diseases. Endophytic *Bacillus* spp. were isolated from superior cacao trees near Quevedo, Ecuador and screened as potential biological control agents

(BCAs) for cacao diseases. Four *Bacillus* spp. are being evaluated as potential BCAs in field experiments conducted at two sites near Quevedo, Ecuador. Applications at site one occurred on 1-month-old hand pollinated nacional pods on a low input research farm. Application at site two occurred on 1.5-month-old open pollinated CCN-51 pods on a commercially operated farm. For both sites, bacterial solutions of log 8.0 CFU/ml with 0.20% Silwet L-77 adjuvant were applied to individual pods using a handheld pump aerosol sprayer. All treatments within one replication were sprayed onto pods on a single tree. Treatments were replicated 80 times at each location. Sampling 24-hours after application indicated that applied bacteria colonized both the epiphytic and endophytic environment, despite a strong rain event 8 hours after application. Pods were rated monthly for cherelle wilt and damage from the three diseases. Data will be presented on the impact of applications of BCAs on disease suppression and overall pod health.

Characterization of a naturally occurred suppressive soil to potato common scab in Michigan

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Phytopathology 99:S84

A field SS with two decades of consecutive potato crops and located in East Lansing, Michigan, has shown a decline of potato common scab. To confirm that the disease decline was due to suppressive soil and characterize properties of the soil that may be related to suppressiveness, two types of greenhouse evaluations were conducted. In the first evaluation, SS soil was treated at various temperatures: room temperature, 30, 45, 60, 75, 90, and 121°C for 30 min. For the other evaluation, 0, 25, 50, 75, 100% of SS soil was mixed with autoclaved SS soil. Each treatment was replicated 3 or 4 times. *Streptomyces scabies* was introduced into the soil at final concentration of 10^8 colony forming units/g soil. In the trials, either radish (CV "Cherry Belle") or potato (CV "Atlantic") was planted in the soil. At the end of each trial, radish roots and potato tubers were removed from the pots, disease severity and the root size (radish only) were measured. The results showed that the size and weight of radish was positively, but disease severity (radish and potato) was negatively correlated with the percentage of suppressive soil (SS) in the final soil mixture. The size and weight of radish decreased, but disease severity (radish and potato) increased when the temperature for soil treatment increased. Therefore, the soil suspected of having suppressive properties has biological factors that suppress potato common scab; and these factors can be removed by higher temperature.

Identification of Xanthomonas leaf blight from umbelliferous seed crops grown in Oregon

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Phytopathology 99:S84

Xanthomonas leaf blight of umbelliferous seed crops caused by *Xanthomonas campestris* is an important seedborne disease that causes significant economic losses in Oregon. It was first reported in 2004 that unique pathogenic strains of *X. campestris* that differed molecularly from *X. campestris* pvs. *carotae* and *coriandri* were isolated from a coriander seed lot grown in Oregon. This finding raised regulatory concern about the export of Oregon seed crops. To determine if these new strains were present on umbelliferous seed crops grown in Oregon, we visually inspected 662 fields of umbelliferous seed crops during the 2004 to 2008 growing seasons and assayed 27 carrot and coriander seed lots for *X. campestris*. Xanthomonas leaf blight was observed in 153 carrot seed fields. A total of 569 *Xanthomonas* strains were isolated from diseased plants and seeds and identified as *X. campestris* pv. *carotae* (*Xcc*) by PCR with the specific 3S primer pair and rep-PCR. Of four *Xcc* genotypes (1a, 1bH, 1bL, and 2) identified by ERIC-PCR DNA fingerprinting, genotype 2 was dominant (53%), and genotype 1bL showed the lowest frequency (7%). The data show that Xanthomonas leaf blight of umbelliferous seed crops grown in Oregon was caused by *Xcc* and was only found on carrot seed crops in our surveys. The previously reported *X. campestris* strains pathogenic on coriander were not found in our surveys. We will continue to monitor Xanthomonas leaf blight on umbelliferous seed crops grown in Oregon.

Distribution, morphological description and molecular characterization of *Pratylenchus* spp. associated with biofuel crops

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Phytopathology 99:S84

The distribution and diversity of *Pratylenchus* species associated with biofuel crops was investigated in Midwestern USA. The populations were identified based on morphology and morphometrics, and further characterized based on the rDNA D3A/B region of the large subunit (LSU). The root lesion nematode was present in 30 of the 73 Miscanthus and switchgrass fields sampled, with population ranging from 45-486/100 cc soil. A total of 4 *Pratylenchus* species were detected belonging to *Pratylenchus penetrans, P. hexincisus, P. scribneri, P. brachyurus*. The D3A/B rDNA region revealed large sequence variation between the different species and low sequence divergence within the same species supporting the morphometric analysis. The identification of different species of root lesion nematode associated with biofuel crops suggests that these nematodes might have significant effect on biomass yield. However, nothing is known about the effect of *Pratylenchus* species on biofuel crops. Therefore, it is essential to determine the relative level of pathogenicity to recommend effective control measures and to distinguish between species in diagnostic samples.

Regulatory control of the *Fusarium graminearum* transcriptome in wheat and rice

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Phytopathology 99:S84

Infection of wheat with *Fusarium graminearum* causes spreading necrosis and accumulation of high levels of trichothecene mycotoxins. In contrast, inoculation of rice with the same strain of the fungus causes only localized plant infection and negligible trichothecene accumulation. To identify differential fungal gene expression patterns that could be responsible for differences in toxin accumulation and symptom onset and development, analyses of gene expression were conducted during infection of rice or wheat using the *F. graminearum* Affymetrix GeneChip. Expression profiles were generated for time points 48, 96, and 192 hours after inoculation of plants. Differences in temporal patterns of global fungal gene expression were observed during infection of the different hosts. In wheat, transcript levels of all fungal genes increased over time, whereas transcript levels of fungal genes in rice remained relatively constant after 48 h. These results were well-correlated with symptom onset and fungal colonization observed on both plants. Profile analyses revealed subsets of genes expressed only in wheat (188), rice (34), wheat or rice (324) or wheat and rice (102). Chromosomal locations of genes expressed exclusively *in planta* revealed enrichment of these genes in regions of high SNP density and recombination frequency ($P < 0.0005$). Three novel clusters composed of genes expressed exclusively in planta were discovered. Select genes from these clusters have been targeted for reverse genetic analysis.

Prevalence of fungicide resistance in *Botrytis cinerea* isolates from strawberry fields in California

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Phytopathology 99:S84

The resistance to four fungicides used for Botrytis rot control in strawberry was evaluated in 65 isolates of *Botrytis cinerea* from coastal California. The isolates were obtained from infected strawberries collected in the Oxnard and Watsonville districts. Fungicide inhibition was tested by agar diffusion assay: fungicide solutions were applied to wells in Czapek-Dox agar plates inoculated with conidial suspensions of the pathogen. Isolates not showing inhibition zones around wells were considered resistant. Threshold concentrations of active ingredients used for detecting resistance were 50 ppm for thiophanate methyl (Topsin), 10 ppm for fenhexamid (Elevate), 50 ppm (combined a.i.) for cyprodinil/fludioxonil (Switch) and 100 ppm (combined a.i.) for bocalid/pyroclostrobin (Pristine). Most isolates (92%) in all fields surveyed were resistant to thiophanate methyl. Resistance to the more recent products was also widespread and occurred in nearly all fields. Overall, resistance incidence among isolates was 38% for fenhexamid, 28% for cyprodinil/fludioxonil and 66% for bocalid/pyroclostrobin. Over 89% of *B. cinerea* isolates cumulated resistance to at least two products and four isolates exhibited resistance to all four fungicides. This widespread occurrence of resistance to single-site active fungicides suggests that their efficacy against Botrytis rot might be impaired and that more aggressive resistance management is needed.

***Beet black scorch virus* in Iran is more diverse than anywhere**

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Phytopathology 99:S84

The diversity of iranian *Beet black scorch virus* and its satellite was studied by sequence analysis of selected samples. The presence of the BBSV was systematically associated with the presence of the *Beet necrotic yellow vein virus*, the *Beet soil-borne virus* or the *Beet virus Q*. The complete genome sequences of seven Iranian BBSV were determined : three BBSV associated

with a satellite and four BBSV without. They showed identities ranging from 99% to 88% nucleotide identities. Amino acid identity in the predicted genes ranged from 59% in the *p10* gene only evidenced in the BBSV-CO US isolate to 100% in the *p7a* gene of the 'Ningxia' isolate from China. The level of diversity at nucleotide level is higher in Iran than observed for the previously reported BBSV from China, U.S.A. or Europe, raising the hypothesis of multiple contaminations of the sugar beet in Iran, possibly from a *Brassicaceae*. Such diversity, including additional putative genes, will be discussed. The Iranian BBSV satellite comprises 617 nucleotides rather than 615 for BBSV-X satellite from China, with an identity of 86% between both sequences. By mechanical infection, the virus gave a localized infection on *Chenopodium quinoa* and *Beta macrocarpa*. The 3′ UTR of some sources of BBSV showed significant changes at nucleotide level, but with no structural changes in the secondary structure of this region of major importance for the viral replication.

The state of ironwood (*Casuarina equisetifolia* subsp. *equisetifolia*) decline on the Pacific island of Guam

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Despite the myriad of utilities and merits of the ironwood tree (*C. equisetifolia* subsp. *equisetifolia*) to the Pacific island of Guam, its future is in doubt as its health and survival rate has been deteriorating since 2003. With the award in 2008 of a three-year Western Region Sustainable Agriculture Research and Education grant, research began in earnest September 2008. An international team of researchers participated in an "Ironwood Tree Decline Conference" on Guam in January 2009. As a result of the working conference, it was concluded that a complex of biotic and abiotic factors are most likely responsible for the decline. Possible biotic factors include fungi of the genera *Ganoderma*, *Pestalotia*, *Botryosphaeria*, and *Fusarium* and several yet unidentified fungi and bacteria. Insects that may play a role in decline are termites and a newly discovered eulophid wasp which forms galls in branchlet tips. Among the abiotic factors are the major typhoons Chata'an (July, 2002) and Pongsona (December, 2002), the intervening severe drought, and proximity to urban development. Decline prevalence was highest on plantations (wind breaks, beaches, parks, and golf courses). The healthiest ironwood trees are located on Cocos Island, a *Casuarina* dominated island just 1.6 miles off the southern tip of Guam, and at Ritidian Point, a National Wildlife Refuge located on the northern tip of Guam.

Exposure of soil-dwelling nematodes to diacetylphloroglucinol (DAPG)

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Phytopathology 99:S85

Some isolates of the bacterium *Pseudomonas fluorescens* produce the antibiotic diacetylphloroglucinol (DAPG). DAPG is toxic to various organisms, including plants, fungi, viruses, and bacteria. In addition, crop yield increases have been reported after application of DAPG-producing isolates of *P. fluorescens*. The goal of this study was to determine whether DAPG is toxic to selected soil-dwelling nematodes, including free-living nematodes and plant parasites. In laboratory assays, the nematodes *Caenorhabditis elegans*, *Heterodera glycines*, *Meloidogyne incognita*, *Pratylenchus scribneri*, *Pristionchus pacificus*, *Rhabditis rainai*, and *Xiphinema americanum* were immersed in DAPG at concentrations ranging from 1 to 75 or 100 µg/ml. There were no observable effects on some species, but DAPG inhibited *M. incognita* egg hatch, tended to stimulate *C. elegans* egg hatch, and was toxic to *X. americanum* adults. The results indicate that DAPG produced by bacteria in the soil would not directly suppress population numbers of every nematode species exposed to the compound; effects would vary with nematode taxon and with life stage of the nematode.

Application of abscisic acid increases curing of Pierce's disease-affected potted grapevines

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Phytopathology 99:S85

Xylella fastidiosa is a xylem-limited, gram-negative bacterium that causes Pierce's Disease (PD) in grapevines. One year-old *Vitis vinifera* 'Pinot Noir' and 'Cabernet Sauvignon' grapevines growing in one gallon containers were mechanically inoculated with *Xylella fastidiosa* 'Stag's Leap' in the spring. In August, vines were confirmed to be infected with *X. fastidiosa* by PCR. In November, as the vines were going dormant, *X. fastidiosa*-infected vines were soil-drenched or sprayed with solutions of a naturally occurring or a synthetic abscisic acid (ABA). Vines were rated for symptoms of PD and tested for *X. fastidiosa* the following summer. ABA-treated vines had higher curing rates than untreated controls and some of the ABA-treated 'Pinot Noir' grapevines treatments had curing rates between 94–100%. The most effective curing treatment was the synthetic ABA applied as a drench. If *X. fastidiosa*-infected field-grown vines respond to ABA application as well as potted vines, growers may have a new tool to manage Pierce's disease.

Accounting for host resistance in Stevens' forecast of Stewart's wilt caused by *Pantoea stewartii*

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Phytopathology 99:S85

Observations by Stevens have been used for 75 years to forecast early-season Stewart's wilt on corn. If the average temperature from Dec. through Feb. is above freezing, Stewart's wilt may be severe. If this average is below –1.1°C, Stewart's wilt is unlikely. This relationship was developed from reactions of susceptible (S) cultivars. Stevens' forecast does not consider that hybrids may have moderate (M) or resistant (R) reactions. In order to account for host reactions, incidence of Stewart's wilt on sweet corn hybrids with different levels of resistance was monitored in 70 field trials over the past 10 years. Three replicates of 9 to 12 hybrids with S, M, and R reactions to *P. stewartii* were planted from April 14 to May 25 in RCBs at 19 locations. Incidence of naturally-occurring Stewart's wilt was assessed at the five-leaf stage from a sample of nearly 450 plants per hybrid. Mean winter temperature was calculated for each location. When mean winter temperature was >0.6°C, the relative frequency of >1% incidence of Stewart's wilt was 0.30, 0.26, and 0.56, and the relative frequency of >5% Stewart's wilt was 0.08, 0.11, and 0.27 for R, M, and S hybrids, respectively. When mean winter temperature was below <–2.8°C, the relative frequency of >1% Stewart's wilt was 0.03, 0.06, and 0.27 for R, M, and S hybrids, respectively, and >5% Stewart's wilt was not observed. This information may influence Stewart's wilt control decisions made prior to planting.

Levels of *Aspergillus flavus* and *A. parasiticus* in soils of almond orchards

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Aflatoxins, produced by *Aspergillus flavus* and *A. parasiticus*, are potent liver carcinogens, and aflatoxin contamination of food and feed is strictly regulated by governments who have set very low tolerances. Numerous Rapid Alerts issued by the EU in 2007 for aflatoxin contamination of almonds has puzzled the California almond industry. Soil samples were collected from 28 almond orchards in three geographic areas in California. Soil was plated on semi-selective media and colonies of *Aspergillus* spp. were quantified. *A. flavus* was common in all three regions while *A. parasiticus* was more common in orchards of the northern region. The range of propagules densities among orchards was greater for *A. tamarii >A. parasiticus>A. flavus.* The range of densities of *A. flavus/A. parasiticus* in California almond orchards was greater (2–219 cfu/g soil) than pistachio orchards (2–36 cfu/g soil) and fig orchards (0.1–9 cfu/g soil). Interestingly, the atoxigenic strain AF36, which is registered and used to reduce aflatoxins in cottonseed in AZ and CA, was found at 3.4, 7.2, and 12.3% of the atoxigenic isolates in northern, central, and southern regions. Three strains of *A. flavus* based on the size of sclerotia were recovered: the S (small), the L (large), and a strain with intermediate size sclerotia. Sclerotia were also found in infected almonds on trees. The results suggest that propagules of aflatoxigenic fungi are very widespread in all three major almond producing regions.

Identification and molecular characterization of *Allium virus X*, a new potexvirus infecting ornamental allium

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Phytopathology 99:S85

A survey to identify virus diseases affecting ornamental allium spp. in the Netherlands was conducted during the summers of 2007–2008. ELISA and PCR assays showed the presence of a complex of different viruses consisting of potyviruses, *Tobacco rattle virus*, *Tobacco necrosis virus* and an unexpected potexvirus. The potexvirus had a ssRNA genome of 7,217 bp (excluding the poly(A) tail). The genome organization was found to be typical of members of the genus *Potexvirus* and consisted of five open

reading frames (ORF). Nucleotide and amino acid sequence comparisons with those of known potexviruses showed that this virus was related to *Hosta virus X* and *Hydrangea ringspot virus*. Amino acid identity and phylogenetic relationships suggested that this allium virus is a new member of the *Potexvirus* genus and the name, *Allium virus X* (AlVX) is proposed. An RT-PCR based assay for rapid and sensitive detection of this virus was developed and surveys are being carried out to determine the extent of its incidence.

Comprehensive QTL linkage map for resistance to Sclerotinia white mold in common bean

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Phytopathology 99:S86

Sources of resistance to white mold in common bean (*Phaseolus vulgaris*) are limited. We sought to characterize resistance in two novel dry bean sources and integrate the QTL with previously mapped resistance. Recombinant inbred line populations (Raven/I9365-31 and Benton/VA19) combined with bulked-segregant analysis was used for QTL analyses of partial resistance to white mold. Two major- and two minor-effect QTL derived from I9365-31 and one major- and one minor-effect QTL derived from VA19 were anchored to the core map. The location of these seven QTL relevant to the position of 30 previously mapped QTL showed that three of the six QTL mapped to new regions. Overall, we integrated 37 QTL for partial resistance onto a single linkage map. These 37 QTL coalesced into 16 regions across nine linkage groups. Seven QTL were identified in more than one population and detected by more than one test. This comprehensive map will facilitate marker-assisted breeding and provide a framework for integrating and interpreting future QTL.

First report of grape root rot caused by *Roesleria subterranea* in Michigan

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Phytopathology 99:S86

In September 2008, declining vines were observed in a vineyard (cv. Muscat) in Fennville, MI. Several symptomatic vines were dug up and showed signs of root decay. On one of the vines, distinctive fruiting bodies (apothecia) were apparent on the roots below the soil line and resembled those of *Roesleria subterranea*. The fungus was cultured on PDA directly from ascospores. DNA was extracted from the culture, and the identity of the fungus was confirmed by comparing ITS sequences obtained by PCR to known sequences using BLASTn. An aqueous hyphal suspension was prepared by blending mycelium with sterile deionized water (SDW) in a food processor at a concentration of 0.1 g/ml. Five two-node, rooted 'Chardonnay' cuttings (45 days old) were placed in the suspension. Five other cuttings were placed in SDW (control). After 25 days, inoculated plants were significantly smaller with lower fresh and dry root weights, and fewer fine roots than control plants. Necrotic root sections from the inoculated plants and control roots were surface disinfested with bleach and ground in SDW using a mortar and pestle, then placed on PDA. The pathogen was recovered from roots of all inoculated plants but not from the control. To the authors' knowledge, this is the first report of *R. subterranea* on grapes in Michigan and the Midwest. While this disease appears to be rare, it should be recognized as a potential cause of vine decline in Midwestern vineyards.

Evaluation of different inoculation techniques for resistance screening of blueberry fruit to anthracnose fruit rot

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Phytopathology 99:S86

Anthracnose fruit rot, caused by the fungus *Colletotrichum acutatum*, is an important disease of blueberries. In this study, five inoculation techniques were compared for evaluation of resistance to *C. acutatum* on detached blueberry fruit of the resistant cultivar Elliott and susceptible cultivar Jersey. A conidial suspension (10^6 conidia/ml) was used for each inoculation method, including spraying the berries until runoff, a syringe injection of 50 µl into the interior of the fruit, and applying a 10-µl droplet in the calyx cup, the side of the fruit and the open surface of cut fruit, using 4 replicates of 10 berries. Fruit rot incidence and spore production occurred faster in the injection (8 days post inoculation [dpi]) and the cut surface method (3 dpi) as opposed to the other methods (10 dpi), but final results were similar across inoculation methods. Using the cut surface method, 24 blueberry cultivars were evaluated for their resistance to a single strain of *C. acutatum*. At 3 dpi, fruit were evaluated for incidence and quantity of conidium production. Using a log-log regression, data correlated well ($R^2 = 0.83$) with a previous study (Polashock *et al*., 2005) on anthracnose resistance of blueberry cultivars. This suggests that anthracnose fruit rot resistance is expressed not only in the skin but also the flesh of blueberry fruit. In addition, the cut fruit screening method is rapid, and fewer fruit are required than for the other methods.

Appressorium formation and growth of *Colletotrichum acutatum* at different temperatures and stages of blueberry fruit development

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Phytopathology 99:S86

Anthracnose fruit rot, caused by the fungus *Colletotrichum acutatum*, is a major disease of blueberries and responsible for substantial pre- and postharvest losses worldwide. It is important to understand the environmental factors that promote infection. The effect of temperature on the mycelial growth rate of *C. acutatum* was studied on ¼-strength PDA for a period of 10 days, and 26°C was found to be optimal. The effect of temperature on appressorium formation was investigated by placing 10-µl droplets of a spore suspension (10^5 conidia/ml) on parafilm and monitoring the germination process every 4 h over a period of 24 h at various temperatures. The optimum temperature for appressorium formation occurred between 20 and 30°C. Melanized appressoria were formed within 8 h at 20, 25, and 30°C. In addition, the infection process was studied on resistant ('Elliott') and susceptible ('Jersey') blueberry fruit at different stages of development, ranging from immature to fully ripe. Berries on detached twigs were inoculated with a 10-µl droplet (10^5 conidia/ml) each. Fruit epidermal tissues were examined microscopically. The rate of appressorium formation was similar on both cultivars at all fruit developmental stages, except at ripening when the rate of appressorium formation increased on the susceptible cultivar. These results will be used to improve weather-based disease forecasting models and will improve our understanding of the latent infection strategies of *C. acutatum*.

Impact of application method on the efficacy of preventive DMI fungicide applications for fairy ring control on golf putting greens

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Phytopathology 99:S86

The objective of this study was to determine the impact of post-application irrigation timing and soil surfactant tank-mixtures on the effectiveness of two spring applications of triadimefon (0.15 g m^{-2}) and triticonazole (0.08 g m^{-2}) for preventive fairy ring control. Plots were 1.5 m × 3 m and arranged in a randomized complete block with four replications. The experimental design was a split-plot with fungicide as the main plot and application method as subplots. Fungicides were applied with or without Revolution (19.1 L ha^{-1}) and irrigated either immediately or 10 hr post application with 6 mm irrigation. Data were subjected to factorial analysis of variance with pooled error and means separation with LSD (=0.05). Fairy ring severity was significantly lower in fungicide-treated plots on all rating dates. No significant differences were detected among plots receiving irrigation immediately compared to10 hr post-application. Plots treated with Revolution alone had significantly reduced fairy ring severity compared to plots receiving no fungicide or wetting agent. Although not statistically significant, fungicide + Revolution plots tended to have higher fairy ring severity later in the season than fungicide – Revolution plots. Although irrigation timing did not impact the efficacy of preventive fairy ring control, tank-mixing a soil surfactant may have a negative impact on the length of fairy ring suppression provided by preventive DMI fungicide applications.

Disruption of the BR-signaling pathway by Geminivirus C4 transgene is partially responsible for the deprograming of *Arabidopsis thaliana* development

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Phytopathology 99:S86

The C4 gene of *Beet curly top virus* (BCTV; Curtovirus, Geminiviridae) induces hyperplasia in infected tissues and leads to cell division when expressed ectopically. Transgenic *Arabidopsis thaliana* plants in which the BCTV C4 transgene was expressed under the control of an inducible promoter (line IPC4-28) were used to further investigate the role the C4 gene has on plant development. Line IPC4-28 seedlings expressing C4 arrested early in development, and showed abnormal tissue organization in cotyledons, hypocotyls, and shoot and root apical meristems. Cell division was extensive on the cotyledons and hypocotyls of C4-expressing seedlings. C4-expressing seedlings failed to develop a vascular system. The exogenous application of brassinolide partially rescued the C4-induced phenotype, and the expression profiles of brassinosteroid (BR)-signaling target genes were altered in C4-expressing seedlings. In addition, the C4 protein interacts *in vitro* with all

subgroup II Arabidopsis Shaggy-like kinases, which are involved in BR-signaling. Taken together these results indicate that the expression of the BCTV C4 protein in *A. thaliana* deprograms plant development, and that this is due in part to the disruption of the BR signaling pathway.

Addressing the relationship between *Pseudoperonospora cubensis* and *P. humuli* by multigenic characterization and host specificity
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Phytopathology 99:S87

The pathogens *Pseudoperonospora cubensis* and *P. humuli*, the causal agents of the downy mildews on cucurbits and hop respectively, have been shown to be very closely related sister species. A recent study that examined sequence data from the internal transcribed spacer (ITS) and morphological characteristics of both pathogens suggested that the species are synonymous. As nomenclature has implications for pathogen identification, disease management tactics, and plant quarantine regulations, it would be beneficial to resolve if the synonymy of *P. cubensis* and *P. humuli* is accurate. To find better phylogenetic resolution, the mitochondrial cytochrome oxidase (COX) gene cluster, and two nuclear loci, ITS and β-tubulin, were extensively sequenced. Conserved single nucleotide polymorphisms (SNPs) were found that consistently differentiating *P. cubensis* and *P. humuli*. These SNPs include one in the second sub-unit of COX, two in ITS, and four in β-tubulin. Host specificity experiments performed on universally susceptible cucurbit hosts and hop cultivars with four isolates of each pathogen indicated that *P. cubensis* may infect hop at a low level whereas *P. humuli* never infected cucurbits. These data suggest that pertinent biological differences may exist between the pathogens that could be overlooked if *P. humuli* were reduced to a taxonomic synonym of *P. cubensis*.

Cover crop effects on root rot of sweet corn in Western Oregon
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Phytopathology 99:S87

Root rot of sweet corn is caused by several pathogens including *Drechslera* sp., *Phoma terrestris*, and *Pythium arrhenomanes*. Select cover crop species were planted and incorporated at 2 (late previous summer) and 7 (over winter) months after planting. The following June, susceptible sweet corn was planted into the treatment plots. Soils were sampled at 1, 2, 3 and 4 months after planting and corn root health (via greenhouse bioassay) and soil microbial activity (via rate of hydrolysis of fluorescein diacetate) were evaluated. Field grown corn roots were harvested at V6 stage and maturity and root disease severity was assessed. Oat 'Saia' was most consistently suppressive to root rot; mustard mix 'Caliente' and other Brassica species were not. Cover crop aboveground dry matter, ranging from 4.2 to 12.2 Mg ha^{-1}, was inversely correlated with root rot severity in greenhouse bioassays. Microbial activity was inversely correlated with disease severity when both were measured within several months of cover crop incorporation. Of the cover crop species tested, oat 'Saia' demonstrated the greatest potential for use as a root rot suppressive cover crop. However, oat residues have a high C:N ratio and immobilize nitrogen for several months after incorporation, and oats are susceptible to barley yellow dwarf virus, a significant disease of grass seed crops grown in rotation with sweet corn.

Leaf spots and Leaf blight disease complex in *Hydrangea macrophylla*
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Phytopathology 99:S87

Foliage diseases can impact the appearance, health and market value of on garden hydrangea. Pathogens Isolated from different disease symptoms were evaluated for pathogenicity by inoculating healthy leaves using detached leaves in-vitro assay technique. Sterile PDA agar plugs with no mycelia were used as controls. Pathogenic isolates that reproduced symptoms were re-isolated from lesions to complete Koch's Postulate. Five fungi were isolated from necrotic lesions and all five fungi were pathogenic on hydrangea and reproduced disease symptoms. Based on morphological characterization and DNA sequence analysis of the ITS region, the five pathogenic fungi were identified as *Phoma exigua*, *Myrothecium roridium*, *Corynespora cassiicola*, *Alternaria* sp. and *Botrytis elliptica*. *C. cassiicola*, *P. exigua*, and *M. roridium*, were isolated from symptoms associated with *Cercospora hydrangeae* and *Colletotrichum gloerisporoide* in extension publications. *C. cassiicola* was highly virulent on all 26 cultivars tested. *M. roridium* and *B. elliptica* were also highly virulent but on fewer cultivars. *P. exigua* has been reported as a pathogen of hydrangea in Italy where it caused severe defoliations and impacted the aesthetic value of the infected plants; *M. roridium* has been reported in Eastern Europe. To the best of our knowledge, *P. exigua*, *M. roridium* and *B. elliptica* have not been reported in hydrangea in the United States.

Isolates of *Fusarium* spp. are a potential hazard to dogwood production system
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Phytopathology 99:S87

A survey for Phytophthora diseases in Tennessee commercial nurseries resulted in the isolation of *Fusarium* species from dogwood stem and root tissues, and rhizosphere soil. Based on morphological features and DNA sequence analysis of the cultures, *Fusarium oxysporum* and *Fusarium solani* were identified. While *Fusarium* spp. are common in the soil, some species are important pathogens; *F. oxysporum* causes root rots and vascular wilt and *F. solani* causes root rots and stem cankers, but studies on tree plants are few. Eleven isolates of *F. oxysporum* and ten of *F. solani* were randomly selected from the collection and tested for pathogenicity in a greenhouse environment using 2-year-old dogwood (*Cornus florida*). Plants were arranged in a randomized complete block design with three replicates per isolate. Inoculum was prepared on rice substrate and applied on the root system (1×10^6 spores per ml). Four weeks after inoculation, disease symptoms started as leaf yellowing and in eight weeks, all plants inoculated with *F. oxysporum* died. Plants inoculated with *F. solani* had severe stem die back and surviving leaves became dull-green and developed large lesions; plants eventually died in about 12 weeks. Plant mortality in dogwood is often attributed to *Phytophthora* spp., but *F. oxysporum* and *F. solani* may impact dogwood production either individually or in a disease complex.

***Erwinia amylovora* strains transformed with the near-ubiquitous pEA29 plasmid exhibit enhanced colonization and virulence on pear and apple**
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Phytopathology 99:S87

Three plasmid-free strains of *Erwinia amylovora*, the causal agent of fire blight disease of pome trees, one from Iran, one from Egypt and one from Spain were transformed with the near-ubiquitous nonconjugative pEA29 plasmid from a wild-type strain and characterized. The plasmid deficient strains were levan and slime positive, motile, chemotaxis positive, induced HR on *Nicotiana tabacum* var. *xanthi* but produced several fold less amylovoran and were weakly virulent on pear slices and apple seedlings compared to plasmid-bearing wild-type strains. When inoculated on the wounded proliferating apple (cv. Royal Gala) leaves, the plasmid-free strains labeled with the green fluorescent protein (*gfp*) were mainly restricted to the inoculation site at the leaf tips in contrast to the plasmid-carrying wild-type strains that moved into the midrib xylem vessel and colonized the adjacent parenchyma cells. Upon introduction of the transposan-labelled pEA29 plasmid, amylovoran production, degree of oozing and tissue necrosis on pear slices were significantly elevated in all three strains. On the other hand, the levels of levan and levansucrase declined in the transformed strains. Only the strains from Iran and Egypt gained the ability to invade and colonize the proliferating apple leaves following the introduction of pEA29. It is concluded that gain of the nonconjugative ubiquitous plasmid may not necessarily confer a fitness increasing effect in all bacterial strains.

Genome characterization and transmission of Rose yellow vein virus, a new caulimovirus occurring in garden rose
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Phytopathology 99:S87

A previously undescribed caulimovirus was identified as the causal agent of a vein yellowing disease of rose. The virus has spherical particles 48–50 nm diameter containing a circular dsDNA genome approximately 8.6 kb in size. The virus was named rose yellow vein virus (RYVV) and occurred in an unnamed Damask rose in Minnesota and in the varieties 'Belle Poitevine', 'Madame Pierre Oget', 'Mozart', 'Prosperity', and 'Schnezzwerg' in New York. The virus was not transmitted by mechanical inoculation or by *Macrosiphum euphorbiae*, but was graft transmitted to healthy plants of the variety 'George Vancouver' in which characteristic vein yellowing symptoms developed and the presence of RYVV verified by PCR using virus specific primers. The genome organization of RYVV was similar to that of known caulimoviruses but the degree of sequence identity between homologous genomic regions was low (22–51%).

Resistance to strobilurin fungicides in a population of *Alternaria alternata* causing Alternaria brown spot of citrus

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Phytopathology 99:S88

Alternaria brown spot (ABS), caused by *A. alternata,* is a serious disease on tangerines and their hybrids. The main control method of ABS is multiple applications of fungicides from March to July. Strobilurin and copper fungicides are the most effective control options. Following a report of control failure after repeated use of strobilurin fungicides in August 2008, monoconidial isolates were collected from the grove and 3 isolates collected before strobilurin use in citrus were employed as sensitive controls. The isolates were tested for pathogenicity on Minneola tangelo leaves and were highly pathogenic. Percent spore germination was evaluated 3 times per isolate on PDA and on PDA amended with 100 ppm SHAM, 10 ppm pyrachlostrobin (PYR), 100 ppm PYR, 10 ppm PYR and 100 ppm SHAM or 100 ppm PYR and 100 ppm SHAM. Conidia germination of all isolates on PDA was >80%. The majority of isolates had >60% germination on 10 and 100 ppm PYR and the control isolates had <20% germination. Germination on SHAM-amended PDA approached 100% for isolates tolerant to PYR, but only 20% for sensitive isolates. When SHAM was added with to the PYR, there was no germination of sensitive isolates and overall germination of tolerant isolates was reduced by 10% on average. All factors tested; isolate, presence of SHAM, PYR concentration and interactions, except repetition, had highly significant effects on percent conidia germination ($P < 0.0001$).

Characterization of new races of *Phytophthora capsici* foliar blight syndrome in *Capsicum annuum* L.

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Phytopathology 99:S88

Phytophthora foliar blight caused by *Phytophthora capsici* L. is a serious problem in pepper production worldwide, especially in areas of high humidity. The pathogen is a heterothallic organism which, when both mating types are present, promotes new genetic diversity in the organism. The genetic recombination leads to the presence of new races. To successfully breed for resistance, knowledge of the races is important. In order to characterize the pathogen into races a host differential analysis was accomplished. The New Mexican Recombinant Inbred Lines (NMRILs) have successfully differentiated races for Phytophthora root rot. In this study, the NMRILs were used to differentiate races for Phytophhtora foliar blight. Isolates from different locations such as Turkey, Netherlands, Argentina, and the United States were used in this study. Several different races of Phytophthora foliar blight were identified in this study using the NMRILs as the host differential.

Expression of the nucleocapsid protein and glycoprotein G_N of *Tomato spotted wilt virus* in plants

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Phytopathology 99:S88

Tomato spotted wilt virus (TSWV) and its thrips vector, *Frankliniella occidentalis*, cause damage on a wide range of host plants worldwide. Previously, an exogenously-applied soluble form of the TSWV G_N protein was shown to bind thrips midguts and inhibit virus transmission. Our research goal is to determine if the G_N protein expressed in plants inhibits transmission by thrips. As a first step, we constructed vectors to express the nucleocapsid protein (N) and G_N protein *in planta* using the Gateway system. The viral proteins were expressed as fusion proteins with the green fluorescent protein (GFP) at the carboxy terminus. The recombinant proteins were transiently expressed in *Nicotiana benthamiana* plants by agroinfiltration. Western blot assays using antibodies to detect N, G_N and GFP were used to confirm the presence and size of the proteins, and we detected protein bands of approximately 62 kDa and 82 kDa for N:GFP and G_N:GFP, respectively. Using fluorescent microscopy, we determined that the N:GFP fusion protein localized to a single discrete spot within the epidermal cells, which is consistent with reports that the N protein localizes to the perinuclear region of plant cells. The G_N:GFP fusion protein displayed a particulate pattern consistent with Golgi localization. In future experiments, we will use the GFP-tagged proteins to study virus-vector interactions and to produce transgenic plants to evaluate their use as a control option for TSWV.

Sexual reproduction influences aflatoxin chemotype diversity in worldwide populations of *Aspergillus flavus* and *A. parasiticus*

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Phytopathology 99:S88

Aflatoxins are toxic polyketides produced by several *Aspergillus* species that contaminate food crops worldwide. *Aspergillus flavus* and *A. parasiticus* are the most common agents of aflatoxin contamination of oil-rich crops. The genes involved in aflatoxin biosynthesis are clustered and convert acetate and malonate to aflatoxins B_1, B_2, G_1, and G_2. We determined the frequency of the *MAT1-1* and *MAT1-2* mating-type genes in *A. parasiticus* and *A. flavus* sampled from single peanut fields in the United States (Georgia), Africa (Benin), Argentina, Australia, and India. To determine whether sexual reproduction influences aflatoxin chemotype diversity, we tested the null hypothesis of an equal number of *MAT1-1* and *MAT1-2* in populations sampled from each locality/species using a two-sided binomial test. For both *A. flavus* and *A. parasiticus,* when the number of *MAT1-1* and *MAT1-2* was significantly different in both uncorrected and clone-corrected samples, isolates grouped into specific chemotypes, either the nonaflatoxigenic class in *A. flavus* or the B_1-dominant and G_1-dominant classes in *A. parasiticus*. In *A. flavus*, sexual reproduction suggested by a 1:1 distribution of *MAT* genes reduces the frequency of nonaflatoxigenic strains and increases the resolution of recombination blocks. In *A. parasiticus*, sexual reproduction and recombination reduces the frequency of B_1-dominant and G_1-dominant chemotypes, and isolate G_1/B_1 ratios show a continuous distribution in the population.

Infection of olive seeds by *Colletotrichum acutatum* and its effect on germination

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Phytopathology 99:S88

Anthracnose of olive (*Olea europaea*) caused by *Colletotrichum acuatum* and *C. gloeosporioides* is the most serious fruit disease of this crop. Species of *Colletotrichum* can affect the seed germination on various crops. However, there has never been studied in olive. When detached and ripeness olive fruit from two cultivars, Hojiblanca (susceptible) and Frantoio (resistant), were inoculated with isolates of *C. acutatum*, the pathogen was able to infect all drupe tissues (epidermis, mesocarp, endocarp, and seed) of both cultivars. Nevertheless, infection progressed faster in the susceptible cultivar than in the resistant one. Under field conditions, the pathogen was isolated from most of endocarps and seeds from affected 'Hojiblanca' fruit. Even, 10% of endocarps and seeds from asymptomatic drupes were infected by the pathogen. In germination trials using seeds from affected fruit, 11% of seeds showed acervuli and conidial masses and did not germinate. In addition, 3.3% of germinated seeds from affected fruit developed a radicle necrosis and preemergence damping-off. *C. acutatum* was consistently isolated from the necrotic tissue. To our knowledge, this is the first report of this pathogen affecting olive seeds. Seed infection by *Colletotrichum* spp. should be considered in breeding and plant production programs that use olive seedlings.

Etiology and life cycle of cedar-quince rust in southern Spain

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Phytopathology 99:S88

Spermogonial and aecial stages of a rust fungus infecting quince (*Cydonia oblonga*) and other rosaceous species (*Crataegus monogyna*, *Mespilus germanica*, and *Pyrus communis*) were observed in southern Spain. The telial stage was surveyed on cedar (*Juniperus oxycedrus*) in the mountains 5–15 km from the rosaceous hosts. Two species of *Gymnosporangium* were identified infecting cedar: *G. clavariiforme*, causing witches' brooms and *G. confusum*, causing fusiform galls and cankers of branches. Besides symptoms on cedar, both species were well differentiated by their characteristic telia and teliospores. Species differentiation on the rosaceous host was more difficult but both species could be diagnosed in the aecial stage by morphology of the peridium cells and aeciospores. Fungal morphology and molecular analysis of nuclear DNA region ITS2-28S confirmed the identification of *G. clavariiforme* on *C. oblonga*, *C. monogyna*, *P. communis*, and *J. oxycedrus* while *G. confusum* was identified on *C. monogyna*, *M. germanica* and *J. oxycedrus*. Cross inoculations are being carried out on a larger collection of fungal isolates to determine host specialization of the two *Gymnosporangium* species.

Role of oxidative stress and salicylic acid during the interaction of *Pepper golden mosaic virus* and habanero pepper

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The application of Salicylic acid (SA) to plants induces several defense-related genes that typically accompany HR and SAR. These enzymes include the ROS-scavenging enzymes catalases (CAT) and peroxidases (POX). Therefore, many studies have demonstrated that SA is a critical signal for the activation of HR and SAR during plant-virus interaction, all reports focused on the defense against RNA viruses; in the case of DNA viruses, no work has been reported. In this work we infected habanero pepper *in vitro* plants by bombardment with infective clones of *Pepper golden mosaic virus* (PepGMV), a begomovirus. Symptoms development was observed nine days after inoculation (dai). CAT activity increased during the first hours after bombardment and the maximum activity was observed four hours after infection (hai). In contrast, POX activity increases during all the time course of the experiment, in relation to mock plants. Major peak of activity was observed 15 dai. SA increased during the first hours, and the maximum peak was observed 12 hai. This was correlated with the decrease of CAT activity, suggesting a regulatory role of SA over the CAT activity. We are in process of evaluating the H_2O_2 concentration in the infected plants and Alternative Oxidase (AOX) activity in order to study their role in defense response of pepper plants to begomoviruses. Gene expression of CAT and AOX will be analyzed.

Evaluation of two disease warning systems for Botryosphaeria panicle and shoot blight of California pistachios and control with early season sprays

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Phytopathology 99:S89

Two empirical models to predict infection events were evaluated for Botryosphaeria panicle and shoot blight, caused by a *Fusicoccum* sp., as well as the effectiveness of early season fungicide sprays on the control of this disease of pistachios. The model incorporating wetness duration was superior to one based solely on duration of rains ≥ 11°C and rain ≥ 1 mm per hour for ≥ 4 hours. The wetness duration threshold (W) for rain events ≥ 4 mm at a given temperature (T) for high risk infection events was W = –7.8+397/T and the threshold for moderate risk events was W = –6.9+220/T. Wetness durations interrupted by ≤12 hours were added together to calculate W. In two orchards with high levels of inoculum, one high risk event resulted in < 20% blighted fruit at harvest and 2 high risk events resulted in < 50% blighted fruit. Latent infections were 0 to 1% in instances where only low risk events (one to two events) occurred prior to collection of pistachio fruit for determination of latent infections and were 17 to 31% with one or two high risk events. Early season fungicide sprays in April to May effectively controlled panicle and shoot blight when applied close to infection events.

Effect of jasmonic acid on foliar diseases of American ginseng

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Phytopathology 99:S89

Alternaria panax and *Botrytis cinerea* cause blighting of American ginseng foliage. These foliar blights may negatively impact root yield and quality, which reduces their monetary value. Jasmonic acid (JA) has been studied for its ability to elicit plant defenses against necrotrophic fungi. The objective of this study was to determine the effect of JA on *A. panax* and *B. cinerea* when exogenously applied to foliage. Four-year-old American ginseng plants in individual humidity chambers were sprayed with a 1 mM JA solution or water (negative control) using a hand-held sprayer in a glass greenhouse on the campus of Michigan State University. Seven days following JA application, plants were inoculated with spore suspension of *A. panax* (1×10^5 spore/mL), *B. cinerea* (1×10^5 spore/mL), or water as the negative control. Plants inoculated with *A. panax* were rated 8 days post inoculation (dpi) and *B. cinerea* inoculated plants were rated 10 dpi. Plant health was assessed on a 1 to 5 scale, where infection was 1) <10% 2) 11–30% 3) 31–50% 4) 51–70% 5) >71%. Lesion incidence and diameter were assessed on inoculated foliage. The same two parameters were also used to assess overall disease pressure on treated and untreated foliage. Results to date indicate that the application of JA to the foliage did not significantly reduce disease severity, lesion incidence, or lesion diameter in this study.

Detection of different PVY strains from potato in Iran

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Phytopathology 99:S89

Potato virus Y (PVY), the type member of the genus *Potyvirus* is one of the most important virus infecting potato in Iran causing considerable yield and damages. Based on the reaction on potato and tobacco three main strains were distinguished: PVY^N, PVY^O and PVY^C. During the years 2007 and 2008 a survey was conducted throughout the potato fields in Eastern Azarbaijan province of Iran. A total of 294 symptomatic potato samples were collected and tested for the presence of PVY infection by DAS-ELISA methods. Results showed that 26.8% of the tested leaf samples were infected with the PVY. Using indicator tobacco plant (*Nicotiana tobaccum* cv. Samsun) different infected potato samples were biologically purified and used for further molecular diagnosis. Positive samples were inoculated in three replication and were assessed for strain specific symptoms of the virus. Using strain specific primers three different strains PVY including : PVY^C, PVY^O and PVY^N were detected by RT-PCR molecular method. According to the previous studies PVY strains have distributed throughout different potato field of Iran however this is the first report of the presence of three strains of PVY in Eastern Azerbaijan province of Iran. It should be taken into considerations for managment and control of this viral disease. Further studies on the distribution of these strains in the province is, now, undertaken.

Determining distribution and prevalence of Fusarium crown rot and common root rot in Montana wheat using real-time qPCR

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Phytopathology 99:S89

The distribution and prevalence of wheat Fusarium crown rot (FCR) and common root rot (CRR) in Montana was studied through a survey of 40 commercial fields during the summer of 2008. Detection and quantification of FCR pathogens (*Fusarium culmorum*, *F. graminearum*, *F. pseudograminearum*) and the CRR pathogen (*Cochliobolus sativus*) was determined using quantitative real-time Polymerase Chain Reaction (qPCR) and by plating on PDA and CLA media. Using qPCR, FCR pathogens were detected in low levels (0–80,000 copies) in 33 fields and in high levels (127,000–297,000 copies) in seven fields while CRR was found in variable levels in all fields. A negative correlation existed between FCR pathogens and CRR populations in stem tissues (r = –0.74). In four fields intensively sampled for yield, FCR populations correlated with yield –0.21, 0.19, –0.48, and 0.23 while CRR populations correlated with yield –0.03, –0.25, –0.24, and –0.69 for the respective fields. FCR pathogens populations were higher in irrigated fields whereas CRR populations were higher in dryland fields. Culture plate analysis showed 49.2% of stems were infected with FCR or CRR pathogens. FCR pathogens and the CRR pathogen were isolated from 35.4% and 13.8% of stems, respectively. *Fusarium culmorum* and *F. pseudograminearum* were the most prevalent FCR pathogens isolated. Correlations between cultured FCR and CRR pathogen populations and qPCR data were 0.71 and 0.08 respectively.

Effects of low temperature events on host susceptibility and on infection, colony development and survival of *Erysiphe necator*

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Phytopathology 99:S89

Disease progress for *Erysiphe necator* prior to grapevine bloom in temperate regions is often slower than what might be expected, based on the known effects of several environmental parameters on pathogen growth and development. During the first six weeks after budbreak, observed latent periods often exceed predicted values by 50%, and colonies exhibit sparse hyphal growth and reduced sporulation associated with host epidermal necrosis. This phenomenon diminishes as the season progresses and overnight low temperatures remain above 10°C. When we subjected existing powdery mildew colonies to 4°C for as little as three hours, individual hyphal segments and epidermal cells near appressoria died, and latent periods increased compared to controls. Pretreatment of *Vitis vinifera* leaves for two hours at 1°C also produced a host response upon inoculation that mimicked ontogenic resistance: reduced rates of haustorium and secondary hypha formation, and

reduced colony size. Moreover, cold pretreatment resulted in up-regulation of pathogen-defense genes in the absence of the pathogen. Our results suggest that pathogen growth is not merely slowed below previously-reported minimums for development, but that commonly-occurring cold events early in the growing season can induce host resistance and ultimately debilitate or kill existing powdery mildew colonies.

Prevalence and severity of *Tomato ringspot nepovirus* in a commercial apple orchard in York County, Pennsylvania

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Phytopathology 99:S90

Tomato ringspot virus (ToRSV) was confirmed on apple trees in a commercial orchard in York County, Pennsylvania in 2007. In the present study, the prevalence and severity of the disease in the orchard were assessed by rating trees on a scale of 1 to 5 (1 = no visible symptoms, 2: ≤ 25% of tree is dead, 3: 25≤50% of tree is dead, 4: 50≤75% of tree is dead, 5: 75≤100% of tree is dead), and analyzing representative samples from all 5 categories by the double-antibody enzyme-linked immunosorbent assay (ELISA) and the reverse transcriptase polymerase chain reaction (RT-PCR). Of the 8 cultivars ('Cameo', 'Gala', 'Golden glory', 'Honeycrisp', 'Jonagold', 'Nittany', 'Red Delicious', and 'York') in the orchard, 'Golden glory' had highest percentage of dead trees (37%), followed by 'York' (27.3%), whereas 'Jonagold' had no dead trees. Three commercially available ELISA kits were tested for their ability to detect ToRSV in samples. Of the three kits tested, two did not detect ToRSV in any of the samples, whereas one kit detected the virus in only 6 of the 8 cultivars: 'Golden Glory' (4.6%), 'Honeycrisp' (3.2%), Jonagold (3.0%), 'Gala', (2.9%), 'Nittany' (2.0%), and 'Red Delicious' (1.9%). Using RT-PCR however, ToRSV was detected in all 8 cultivars such that detection frequency varied significantly among cultivars, and ranging from 92% in 'Golden glory' to 28% in 'Honeycrisp'. The study highlights the importance of choice of technique in accurate detection of ToRSV in apple cultivars.

Prevalence of *Prune dwarf virus*, *Prunus necrotic ringspot virus* and *Tomato ringspot virus* in commercial orchards and nurseries in Pennsylvania

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As part of the certification program, surveys were conducted in summer 2007 and 2008 of *Prunus* species to determine the prevalence of *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV) and *Tomato ringspot virus* (ToRSV) in orchards and nurseries within Pennsylvania and grower outreach operations in Delaware and Maryland. Young shoots or leaves were obtained from each quadrant of the tree and pooled for analysis by the double antibody enzyme-linked immunosorbent assay (DAS-ELISA). Nursery blocks were sampled similarly from plants selected randomly in two diagonals cutting across a given field. Samples were processed and analyzed for the presence of the three viruses following ELISA kit manufacturer's recommendations. All three viruses were detected such that prevalence depended on *Prunus* species, the origin of samples, the year survey was conducted, and virus disease tested. PNRSV was detected at frequency ranging from 0.7 to 10% of samples in 2007, and declined to 0 to 6% in 2008. PDV was detected at a lower frequency, ranging from 0 to 6% in 2007 and 0 to 4.8% of samples in 2008. ToRSV was detected at lowest frequencies: 0 to 2.2% in 2007 and 0 to 1% in 2008. The implication of the detection frequencies of three viruses will be discussed with a view to developing a robust *Prunus* species certification program.

Genotypic variability and identification of sources of resistance to Fusarium root rot in common bean in Uganda

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Phytopathology 99:S90

Trials to identify sources of resistance to Fusarium root rot (*Fusarium solani* f. sp. *phaseoli*) among 147 common bean cultivars were conducted in Uganda. The cultivars included 27 local landraces, 31 from South Africa, 46 from CIAT Africa (resistant to Pythium root rot and Fusarium wilt), six Fusarium wilt disease differentials, 34 sources of resistance to FRR and three local commercial varieties. Screenhouse evaluations using a pathogenic isolate FSP-3 identified 46 moderately resistant cultivars. These were evaluated in a bean root rot infested field to confirm the resistance in the presence of other soil-borne pathogens and assess yield performance at Kawanda Agricultural Research Institute (KARI). Field and screenhouse disease severity data were highly correlated with cultivars differing significantly (P = 0.05). Disease severity at 28 and 56 days after planting (dap) were highly correlated. Fifteen and four cultivars at 28 and 56 dap, respectively, were moderately resistant, showing evidence of partial dominance of resistance to FRR. The identified resistant cultivars had previously been selected for resistance to Fusarium wilt and Pythium root rot. Root weight and root: shoot weight ratios was not significantly (P = 0.05) correlated to FRR severity. Small-seeded varieties and varieties with purple hypocotyls tended to be more resistant. The identified lines were used as resistance donors to improve resistance to FRR in commercial varieties in Uganda.

Characterization of Fsr1-mediated maize stalk rot virulence in *Fusarium verticillioides*

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Phytopathology 99:S90

Fusarium verticillioides is one of the key fungal pathogens causing maize stalk rot. However, our understanding of the maize-*Fusarium* interaction is limited. *F. verticillioides FSR1* gene plays an important role in stalk rot virulence. The predicted Fsr1 protein contains multiple protein-binding domains, and the coiled-coil (CC) domain in the N-terminus was determined essential for virulence. CC domain is known to mediate protein-protein interactions, and our premise is that this interaction triggers downstream gene signaling associated with stalk rot virulence. In this study, we investigated the subcellular localization of a fsrA::GFP fusion in *Aspergillus nidulans* and determined that Fsr1 localization is consistent with the endoplasmic reticulum and the nuclear envelope. To identify putative proteins interacting with the Fsr1 N-terminus, we used yeast two-hybrid (Y2H) approach with two bait constructs, a Fsr1 cDNA encoding the N-terminus and a Fsr1 cDNA encoding the N-terminus without the CC region, to screen a *F. verticillioides* cDNA prey library. We identified yeast clones showing positive interactions, and subsequent sequencing of prey cDNA inserts revealed *F. verticillioides* genes encoding putative proteins with domains that mediate protein interactions as well as those encoding 'common' false positives. Validation of Y2H result is needed before further characterizing the role of Fsr1-interacting proteins in fungal virulence.

Development of a transformation system in the swainsonine-producing fungi, *Undifilum oxytropis*

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Phytopathology 99:S90

Locoweeds are perennial flowering legumes, that when consumed by grazing animals induce a neurological disorder called locoism. Locoism is caused by an alkaloid, swainsonine, which is produced by the slow growing endophytic fungi, *Undifilum oxytropis* (Order: *Pleosporales*) residing within the locoweeds. Little is known about the biosynthetic pathway of swainsonine in *Undifilum*, but previous studies on other Ascomycete fungi indicate that saccharopine reductase may be a key enzyme in swainsonine biosynthesis. Genetic manipulation of *Undifilum* is important to elucidate the alkaloid biosynthesis pathway, however no transformation system has been available. In this study, we report the development of a protoplast, regeneration, and transformation system for *Undifilum*. Fungal mycelia required for generating protoplasts was grown in liquid culture, harvested, and enzymaticaly digested to generate protoplasts. The protoplasts were transformed with a fungal-specific vector driving the expression of green fluorescent protein (GFP). The quality of transformed protoplasts and transformation efficiency were monitored by DAPI staining and GFP expression during the process. After one week, the mycelia showed expression of GFP, demonstrating stable integration of the GFP marker. This demonstrates a reliable procedure for generating protoplasts and an efficient transformation system for *Undifilum* that will be used to study the role of saccharopine reductase in swainsonine biosynthesis.

White pine blister rust on new telial hosts (*Castilleja* and *Pedicularis*) in whitebark pine ecosystems at Mt. Rainier and Crater Lake National Parks

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Phytopathology 99:S90

Whitebark pine (*Pinus albicaulis*) is facing precipitous decline in high-elevation ecosystems across the western United States. White pine blister rust (WPBR), caused by the macrocyclic, heteroecious rust fungus Cronartium ribicola, is one of the primary agents of whitebark pine mortality. Until recently, it was believed that strains of WPBR in North America were only able to complete the telial stage of their lifecycle on currants and gooseberries in the genus *Ribes*. However, in 2006 McDonald et al. confirmed white pine blister rust infection on species within the genera *Castilleja* and *Pedicularis*

(family Orobanchaceae) in the northern Rocky Mountains. Forms of *C. ribicola* in its native Asia are known to utilize hosts in these genera, as are other closely related *Cronartium* species. The objective of this research was to determine whether *Castilleja* spp. and *Pedicularis* spp. play a role in the WPBR disease cycle in whitebark pine ecosystems in the Cascade Range, and whether or not this occurs under field conditions. Field observations in 2008 at Mt. Rainier National Park detected rust infection on *P. racemosa* growing in close proximity to WPBR-infected whitebark pine. Wild inoculations resulted in successful infection of *P. groenlandica* at Crater Lake NP, and *P. racemosa* at Mt. Rainier NP. PCR analysis will verify whether specimens are infected with *C. ribicola, C. coleosporioides*, or a hybrid species.

Association of specific variables with severity of Asian soybean rust as assessed by GIS analysis at the field level

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Phytopathology 99:S91

Since the discovery of soybean rust in the continental United States in 2004, there has been considerable interest in epidemiological details related to long distance and local spread of the disease. In this study, a geographical information systems (GIS) analysis was used to investigate within-field disease distribution. Research was focused on determining the extent of spatial and temporal variability of soybean rust with regard to soil nutrients, soil compaction, leaf nutrients, percent canopy coverage, soil moisture, and plant height and their interactions. The study areas included three soybean fields with a total area of about 15 acres. The fields were located close to Baton Rouge, LA. Using a grid pattern, each field was divided into 50 sample sites, and GPS coordinates were recorded at each site. The sites were rated during the 2007 and 2008 growing seasons to quantitatively assess disease severity. ArcMap was used to perform exploratory data analysis, including correlations among variables, variogram analysis of the spatial structure of each variable, and surface interpolation, with the results being displayed graphically. These results will be presented and discussed.

Long-term impacts of de-icing salts on roadside trees in the Lake Tahoe Basin

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Phytopathology 99:S91

World renown for its natural beauty, Lake Tahoe is a popular location for winter-sporting activities. Deicing compounds are used to maintain safe winter driving conditions. There is a concern that salts may accumulate in soils, and hence past use of deicing salts may continue to have adverse effects of roadside vegetation. Our objective was to quantify the long-term impacts of deicing salts on roadside vegetation in Tahoe Basin. In 2006–2007 we surveyed 79 plots in NV and in 2007–2008 we surveyed 58 plots in CA originally surveyed in 1990. We established additional control plots >300 m from roads. Chemical analyses of soil and vegetation samples were performed. The proportion of salt damaged trees per plot in 2008 (26%) and 2007 (24%) was not significantly ($p > 0.05$) different than in 1990 (19%). However, the proportion salt damaged trees in 2006 (55%) was greater ($p < 0.05$) than in 1990. In the current study the severity of salt damage was confined to a third of the crown whereas in 1990 it extended to more than a third of the crown. No salt damage was found in control plots. In both 1990 and in the current study, sodium and chloride concentrations of salt symptomatic foliage exceeded ($p < 0.05$) that of salt-asymptomatic foliage. Electroconductivity of soil samples (<0.27 dS/m) was below the threshold for any plant damage (>4 dS/m). We conclude that although a clear roadside effect does exist, we have not found evidence that salt is accumulating in the soil.

Evaluation of *Capsicum annuum* L. 'Avelar' as a broad spectrum source of resistance to *Potyviruses*

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Avelar plants were inoculated with *Pepper mottle virus*-FL (PepMoV-FL) and evaluated for their level of resistance. Virus was restricted to lower portions of the plant; however, the extent of the restricted movement varied from plant to plant. Five plants were selected that had the greatest degree of restricted PepMoV-FL movement and allowed to self for several generations. Avelar plants were then evaluated (comparatively with the susceptible pepper cv. Calwonder) for their response to PepMoV strains FL and CA, *Potato virus Y*-NN (PVY-NN), and *Tobacco etch virus* (TEV) strains HAT, Mex21, N and NW. The level of resistance varied with virus ranging from no detectable amounts of virus in inoculated leaves (TEV-HAT and PVY-NN), limited amounts of virus in inoculated leaves with no systemic infection (PepMoV-CA and FL) to varied amounts of systemic infection (TEV-Mex21, N and NW). F_1 populations of Avelar x Jupiter and Jupiter x Avelar F_1 plants were susceptible to each of the Potyviruses suggesting a single recessive gene conferring resistance. F_2 populations from each series of crosses were tested for their response to PepMoV-FL revealing a 1:3 ratio of resistant:susceptible. F_3 populations generated from selected F_2 plants were challenged with each of the Potyviruses. Preliminary results indicate that resistance to PepMoV-FL, PepMoV-CA and PVY-NN cosegregate which contradicts earlier studies, whereas TEV resistance correlated with PepMoV resistance.

Fluopyram for the control of diseases of horticultural crops

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Fluopyram is a new fungicide active ingredient in development worldwide by Bayer CropScience. It has demonstrated excellent crop safety and outstanding control of ascomycetes in horticultural crops at relatively low use rates of 60 to 250 g/Ha. Major foliar and fruit diseases controlled include: powdery mildews (*Uncinula necator* and *Leveillula taurica*), brown rot blossom blight (*Monilinia* spp.), early blight (*Alternaria solani*), gray mold (*Botrytis* spp.), and scab (*Venturia* spp.). Fluopyram has been formulated into and tested in premixes with other fungicidal active ingredients. Multiyear trial results in horticultural crops and pending label directions will be presented.

Combining resistance to Septoria leaf spot, Late blight and Early blight in tomato for joint control of defoliating diseases

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Phytopathology 99:S91

Late blight (LB), Early blight (EB), and Septoria leaf spot (SLS), are the 3 major foliar fungal tomato diseases in temperate regions. Growers currently rely upon fungicides for their control. Hybrid tomato varieties with EB tolerance and/or LB resistance are becoming available. However, these hybrids do not possess SLS resistance; therefore growers would continue to rely on fungicides to control foliar diseases. The objectives of this research were: to transfer SLS resistance to tomato, to test the relative levels of SLS resistance in homozygous and heterozygous genotypes, and to combine SLS, LB and EB resistances in tomato. After screening potential SLS resistant materials and their progenies for 2 generations, two sibling lines fixed for strong SLS resistance were obtained. Lesion size and number of pycnidia per lesion of the resistant lines were only 36.5 and 2.3% of those of the susceptible controls. The F1 of the LB/EB resistant parent with the SLS lines produced F1 hybrids with SLS resistance nearly as strong as the SLS parent, and segregation for SLS in the F2 lines fits a 3R:1S ratio indicating control by one nearly dominant gene. Mist chamber screens identified F3 populations homozygous for SLS resistance. Additional F3 plants from these progenies were selected using EB screen and LB markers to select plants homozygous resistant for SLS and LB and at least heterozygous for EB. Lines fixed for SLS/LB/EB resistance should be confirmed by September of 2009.

Benefits of early integration of interdisciplinary work for tomato improvement

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True interdisciplinary interaction, from the earliest stages of a program, provides greater efficiency and more opportunities for success than is possible in separate uni-disciplinary programs for control of plant disease. In this project we integrated the control of three important foliar tomato diseases by the use of genetic resistance and supported by reduced levels of mild control treatments. Early blight (EB), late blight (LB) and Septoria leaf spot (SLS) are typically controlled with the use of 4 to 8 fungicide sprays applied on a weekly basis in conventional fields. With the trialing of lines with EB tolerance and LB resistance we determine that the genes for the resistances for each of these pathogens needed to be homozygous in order to control disease under the severe pressure common to the Northeast. This provided vital information to commercial companies as they use these resistances in variety development. Continued integrated trials in 2007 in a grower's fields without fungicide sprays demonstrated that SLS was a severe issue as well for EB/LB resistant tomato lines. In 2007 we began the recovery of genetic SLS resistance, and its combination with the EB and LB genetic resistances already available. By working together, this process has progressed to the point that the first triple-resistant (EB/LB/SLS) tomato lines for integrated control of foliar disease will be field tested in summer of 2009.

Pre-emergence damping off of *Beta vulgaris* by *Rhizopus stolonifer*

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Phytopathology 99:S92

Rhizopus stolonifer (*Rs*), a cool temperature zygomycete that can cause a post-harvest rot on sugarbeet (*Beta vulgaris*), also causes pre-emergence damping off in other crops. We are interested in its potential pre-emergence damping off activity in sugarbeet. Sugarbeets are quite susceptible to seedling diseases during the first few weeks of development and in particular during germination. Germination averages 60% in fields in Michigan, ranging from 0 to 100%. Lower emergence often occurs in cool, water-saturated soils. Current practices do not include management for zygomycetes and there are no known sources of genetic resistance in seedlings. This study investigated the effect of *Rs* on beet germination and emergence. Germination of seeds on plates and in liquid media containing spore cultures of either *Rs* or *Phoma* (used as a positive control) or a negative media control was compared after 3 and 4 days, respectively. Results indicated that *Rs* spores consistently reduced or inhibited germination when compared to controls. In the greenhouse, surface disinfested seeds were planted and inoculated with either sterile millet or *Rs* infested millet. Two germplasms were used, SP6822, a putative susceptible, and EL51, a possible resistant. Germinated seedlings were counted ca. every 2 days for four weeks. Seedlings were surface disinfested for fungal isolation to confirm the presence of inoculates. *Rhizopus* caused an average reduction in sugarbeet germination of 40%, and varietal differences were observed.

Understanding GAFP: A unique lectin with broad-spectrum inhibitory activity

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Phytopathology 99:S92

The *Gastrodia* anti-fungal protein (GAFP) is an orchid-derived lectin which may have potential for agricultural application. In transgenic tobacco and plum, expression of the GAFP lectin provided increased tolerance to an endo-parasitic root-knot nematode (RKN), as well as oomycete pathogens causing Phytophthora Root Rot. Our goals are to confirm the direct action of GAFP against nematodes and oomycete pathogens *in vitro* and explore cellular localization of the lectin. We have isolated and purified the protein from transgenic tobacco by a combination of ion exchange, affinity, and size exclusion chromatography. The sensitivity of *Phytophthora nicotianae, P. cinnamomi,* and the root-knot nematode *Meloidogyne incognita* to GAFP will be determined. *Phytophthora* species are cultured on V8-agar amended with 100 µg/mL ampicillin. GAFP solution (5 µl) is then spotted at the periphery of the growing mycelium. Solutions of GAFP are prepared by diluting the purified lectin in 50 mM K_2PO_4. Preliminary results show visible inhibition of *P. cinnamomi* mycelium when as little as 5.0 µg (1.0 mg/ml) of GAFP was applied to the mycelium. Extracts purified from non-transformed tobacco in an identical manner lacked inhibitory activity on this pathogen. Immunomicroscopy will be employed to predict the site of action of GAFP in *Phytophthora* species and *M. incognita*.

Does phloem phenolic chemistry contribute to coast live oak resistance to *Phytophthora ramorum*?

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Sudden oak death, caused by *Phytophthora ramorum*, has resulted in high levels of coast live oak (CLO) mortality. However, apparently resistant CLO individuals have survived in areas with high disease pressure for over nine years. We tested the hypothesis that resistant (R) trees contain constitutively higher levels of phenolics than susceptible (S) trees. Branch phloem from trees rated as R or S in previous work was sampled thrice over two years. Branches from the same trees were also inoculated with *P. ramorum* to confirm resistance groupings. In a second study of trees inoculated in 2002, phloem was sampled from the trunks of three groups of trees categorized as R (no symptoms), healed (H)(showed symptoms but then recovered), and S (symptomatic over several years). Individual and total phenolics from trees with different resistance ratings were then quantified. In the first study, lesion lengths did not consistently confirm previous R and S ratings, rendering phenolic profile comparisons meaningless. However, lesion length at one of the dates was negatively correlated with the concentration of tyrosol. In the second study, R and H trees accumulated higher levels of catechin, ellagic acid, a tyrosol derivative, and total phenolics than S trees. Antimicrobial activity in catechin, tyrosol, ellagic acid and some of their derivatives is documented, suggesting that these compounds may be developed into biomarkers for resistance. Further studies are planned to confirm these preliminary results.

Characterization of Zmcup1, a protein involved in maize resistance to *A. flavus*

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The filamentous fungus *Aspergillus flavus* is a ubiquitous pathogen of several plant species including cotton, peanut and most notably maize. In maize, *A. flavus* causes an ear rot and produces aflatoxins. Aflatoxins are carcinogens that constitute a threat to human and animal health. Several studies were conducted to identify and characterize genes in maize responsible for resistance to *A. flavus* by using maize lines tolerant to the fungus. Previous proteome-based studies revealed the accumulation of several proteins exclusively in maize lines that exhibit relative tolerance to *A. flavus*. One of these proteins contains a cupin domain. Proteins with cupin domains are associated with several enzymatic activities such as isomerase, epimerase, and dismutase activities. Moreover, some cupins act as transcription factors. To characterize and study this protein, Zmcup1, for its role in resistance to *A. flavus*, cDNA coding for the protein was cloned and expressed in *E. Coli* and the anti-fungal properties of the expressed protein were tested on several maize pathogens. A hypothetical maize protein-protein interaction map was also constructed to predict some of the possible interactions of Zmcup1.

Effect of soil texture and fluctuating soil moisture in carpogenic germination of *Sclerotinia sclerotiorum* sclerotia

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The effect of soil texture and moisture on carpogenic germination (CG) of *Sclerotinia sclerotiorum* sclerotia was studied under controlled conditions. Samples of a Fargo Silty clay soil (44% clay) and an Aylmer-Bantry fine Sand soil (92% sand) were mixed in proportions of 1:0, 1:2, 1:1, 2:1, and 0:1 v/v to create different textures. Soil matric potential was calculated for each soil mixture; sclerotia were buried in samples from each texture set at constant 100%, 75%, 50% or 25% saturation; or to conditions fluctuating back and forth between 100 and 75%, 100 and 50%, 100 and 25%; 75 and 50%, 75 and 25% or 50 and 25% saturation. Samples were incubated at 14 to 18°C for 82 days. CG and time to first apothecia were recorded. Moisture fluctuations reduced CG compared to constant moisture (P = 0.05). Under constant conditions CG decreased with increased moisture; sclerotia in soils at 25% saturation yielded highest (67%) and earliest (22 days) CG while no CG was observed in soils at constant 100% saturation. Under fluctuating conditions, highest CG was observed when moisture remained between 75 and 50%. This information will be used to develop a predictive model for carpogenic germination.

***Cronartium ribicola* on wild *Ribes* hosts: Rust severity in local woodland sites shows a site by year interaction**

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White pine blister rust severely impacts some white pine industries and ecosystems. The basidiospores that infect white pines are released while the rust is on *Ribes* host leaves, motivating an understanding of spatio-temporal patterns of the rust on *Ribes*. We studied factors influencing rust severity on *R. missouriense* by dividing these multi-stemmed plants into separate ramets, or clones, leaving one set intact in different woodland sites and transplanting the other set into a common garden. Woodland ramets were exposed only to ambient inoculum while garden ramets were inoculated with common inoculum. An interesting result was found at the scale of woodland sites. Rust severity (AUDPC) on woodland ramets showed no difference in means between two sites in the first year (p-value 0.6). In contrast, during the second year mean AUDPC differed among woodland sites (p-values 0.01 and 0.05 with Bonferroni corrections). Within a common garden transplant cohort, AUDPC on garden ramets grouped by woodland site-of-origin showed no differences in means (p-values from 0.1 to 0.8), eliminating the possibility that plant genetic differences determined the differences in rust severity observed between woodland sites. At least three hypotheses could explain these results and will be discussed in the context of future research needs related to understanding spatio-temporal patterns of rust on both *Ribes* and pine hosts.

Effect of strobilurin fungicides and host resistance for control of gray leaf spot of corn

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Gray leaf spot (GLS) of corn, caused by Cercospora zeae-maydis is a common foliar disease that reduces corn yields in Tennessee and many other states. Two strobilurin fungicides (Headline® and Quadris®) have shown a high degree of control of GLS in tests conducted over the last three years (2006–2008) at the Research and Education Center at Milan, TN. Each fungicide was sprayed at 6 fl. oz/A with Penetrator Plus @ 0.125% v/v as an adjuvant. Four-row plots 30' long were randomized and replicated four times. Rows were on 30" centers and planted no-till in a field infested with GLS. The following three Pioneer corn hybrids with different levels of resistance to GLS were used: susceptible P 32T22, moderately susceptible P 33R76 and tolerant P 33V14. Each fungicide was sprayed once over the top at the VT growth stage (tassel) in 20 gallons of water per acre. Yield increases over the untreated control for the three-year period were significantly greatest for the susceptible hybrid for both Quadris and Headline. For this hybrid, the average three-year yield increase with Quadris was 24 bu/A and 23 bu/A respectively with Headline. The average three-year yield increase using the moderately susceptible hybrid was 18 bu/A with Quadris and 7 bu/A with Headline. For the tolerant hybrid, the average three-year increase in yield was 8 bu/A with Quadris and 6 bu/A with Headline respectively.

Effect of three fungicides and sodium bicarbonate for the control of *Penicillium digitatum* in vitro

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The green mold of citrus caused by *Penicillium digitatum* is one of the most important postharvest diseases in orange. The objective was to determine the effectiveness of pyrimethanil, thiabendazole, trifloxystrobin and sodium bicarbonate in controlling this pathogen. The fungus was isolated from 'Valencia' oranges with symptoms of the disease, coming from Alamo Veracruz, Mexico. Were evaluated three concentrations of each product. The experiment was carried out in a completely randomized design with five replications. Each treatment consisted of a Petri dish with PDA, in which the fungus was inoculated with a disc of mycelial growth of 5 mm in diameter. In the medium were diluted different doses of each fungicide treatment. The percentage of inhibition variant was evaluated. The variance analysis showed that the two studied factors (product and doses) were a statistically significant (Pr> F = 0.0001 and 0.00016, respectively) on the growth of the pathogen. The best treatment was thiabendazole 420 mg kg^{-1} and 840 mg kg^{-1} showed a 100% effectiveness. Although thiabendazole showed better efficacy than pyrimethanil and trifloxystrobin, both showed more than 80% efficiency. In contrast, the sodium bicarbonate in the different doses showed an effectiveness of 2.61%, it was very low relative to other products.

Detection and quantification of virulent strains of *Rhodococcus fascians* in plant material via a real-time PCR protocol

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Rhodococcus fascians is a gram-positive bacterium that causes bacterial fasciation on a wide range of ornamental plants. Effective control and eradication of the disease requires a reliable and sensitive detection method for *R. fascians* in infected plant materials. To address this need, a real-time PCR assay was developed. The oligonucleotide primers Rf-229F/Rf-408R and TaqMan probe Rf-366P were designed to detect part of the *R. fascians fas-1* gene, a plasmid-borne gene essential for virulence. DNAs from all virulent strains of *R. fascians* consistently tested positive, with detection limit of 30 fg. Quantification using Rf-366P probe was shown to be linear by regression analysis with R^2 = 0.994. No signal was detected with *R. fascians* DNA extracted from avirulent strains or other species of plant-associated bacteria. In repeated experiments with *R. fascians* pure culture, as few as 2.5 CFU per PCR reaction were reliably detected. However, due to strong inhibition of real-time PCR reaction by plant-derived compounds, sensitivity of *R. fascians* detection in plant tissues was much lower and required bacterial enrichment on nutrient media. As few as 10^2 CFU/100 mg plant tissue were successfully detected in our real-time PCR assays after 72 h enrichment on modified D2 (sodium azide 0.5 mg/L). This real-time PCR assay combined with enrichment on YDC media was successful in detecting *R. fascians* in several symptomatic clinical samples of *Chrysanthemum*, *Pelargonium*, *Phlox*, and *Veronica.*

Detection of potential reservoirs of *Tomato spotted wilt virus* by PCR analysis of crushed western flower thrips (*Frankliniella occidentalis*)

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Tomato spotted wilt virus (TSWV) is a major problem in tobacco and peanut production in Georgia. In tobacco, current management strategies include SAR inducers and control of thrips vectors. Thrips have to acquire the virus before they can infect tobacco plants in the spring. If the weed host reservoirs in the vicinity of tobacco fields can be identified, they could be targeted for elimination to reduce inoculum. There are over 2,000 hosts of TSWV, but only a few are likely to serve as a source of inoculum for tobacco. Individual *F. occidentalis* were collected from the surface of a white vehicle positioned at the edge of tobacco fields using a modified vacuum filtration system. Immediately after the return to the lab (~15 min.) collection containers were placed in a –80C freezer to immobilize thrips and halt metabolic activity until DNA extraction. Universal primers for the ITS region of nrDNA were used for PCR and the resulting products were sequenced. Sequences subjected to a BLAST search in GenBank matched ragweed and tomato among others. A time-course study, in which thrips remained active for varying time periods prior to freezing, indicated their food sources could be identified up to15 hours after feeding but not after 24 hours. Although adult thrips can't acquire the virus, this method has the potential of identifying plants that *F. occidentalis* not only prefers to feed upon but also reproduce.

***Xylella fastidiosa* strains causing bacterial leaf scorch of blueberry in Georgia are genetically distinct from those causing Pierce's disease of grape**

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Xylella fastidiosa is a genetically diverse species that has a wide host range. Recently it was confirmed to cause bacterial leaf scorch of blueberry (BLSB), a new disease of Georgia's most economically important fruit crop. The first step in characterizing the BLSB pathogen was to determine whether it is similar to strains of X. fastidiosa endemic to Georgia that cause Pierce's disease (PD) of grape. Polymerase chain reaction (PCR) assays using a published PD-specific primer pair did not produce an amplicon from any of 22 BLSB strains tested. Also, sequences of the 16S-23S rDNA intragenic spacer region from two BLSB strains were almost identical to that from strain M12, which causes almond leaf scorch disease (ALSD), and differed from that in Temecula1, a PD strain. Additional PCR assays using published and novel primer pairs compared BLSB strains with X. fastidiosa isolated from plum, oak, sycamore and oleander in Georgia, oak in Florida, and grape, almond and alfalfa in California. The results consistently indicated that BLSB strains are distinctly different from PD (G-type) strains of X. fastidiosa and are most like ALSD (A-type) strains. However, since almond is not grown in the southeastern United States, the BLSB pathogen likely is related to endemic X. fastidiosa strains in the phony peach group.

Effect of temperature and leaf wetness duration on the infection of wheat leaves by *Puccinia striiformis* f. sp. *tritici* in a controlled environment

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Stripe rust of wheat is afoliar disease caused by *Puccinia striiformis* f. sp. *tritici.* In recent years, this disease was more commonly observed in the Pacific Northwest and California, but had not been a significant problem in the Great Plains until 2001, 2003, and 2005. Recent studies on the pathogenecity and population genetics indicate that recent changes in the pathogen population contributed to these outbreaks. The effects of temperature and leaf wetness duration on infection was studied prior to the population shift, but remained undetermined for isolates representing the new population. The objective of this research was to determine infection conditions for isolates from the new population in a controlled environment. Two-week-old wheat seedlings were inoculated with spore suspensions of *P. striiformis* (5×10^6 spores/ml) and exposed to predetermined combinations of temperature between 4 to 20°C, and leaf wetness durations 5 to10 hours. Disease severity was visually assessed using a 0–3 scale after two weeks of incubation at 12°C. Results indicate that the isolate tested was ableto infect wheat when temperatures were between 4 to 20°C with 5 hours of leaf wetness duration. This isolate was able to cause severe infections at temperatures between 6 and 17°C with 10 hours of leaf wetness duration. Experiments comparing the infection requirements for isolates from the old and new populations are ongoing.

Prediction of deoxynivanenol accumulation for Fusarium head blight of wheat using empirical and mechanistic modeling approaches

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Fusarium head blight of wheat causes significant yield loss in many wheat-producing regions of the world. Grain damaged by this disease is often contaminated with the mycotoxin deoxynivalenol (DON). Models predicting the risk of mycotoxin contamination could help farmers evaluate the need for fungicides applications and identify strategies for dealing with DON contaminated grain. The objective of this research was to develop mechanistic and empirical models using weather information from time periods 10 days before and 7 days after 50% anthesis. Candidate models were developed using 2003–2006 season datasets from seven states, and preliminary results indicated that the accuracy of models forpredicting the risk of DON accumulation greater than 2 ppm ranged from 65–91%. In general, the accuracy of empirical models was greater than that of mechanistic models. Candidate models using weather information from only the pre-anthesis period resulted in up to 88% accuracy with the current dataset. Including weather information from during anthesis and early stages of kernel development further improved the accuracy of the models. However, with the timing of these predictions relative to crop development, the primary use of these post-anthesis models would not be for disease prevention. Model performance will be further tested using the observations collected during the 2007 and 2008 growing seasons.

The influence of nutrients and yeast on disease severity and lesion development by *Rhizoctonia solani* on *Festuca arundinaceae* leaves

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In tall fescue (*Festuca arundinacea*), brown patch caused by *Rhizoctonia solani* is characterized by irregular-shaped lesions that often coalesce resulting in extensive leaf necrosis. Presumably, nutrients released from wounding and death of foliar cells influence the microbial carrying capacity of these leaf surfaces. In a previous study we observed significant increases in the yeast carrying capacity of tall fescue infected with *R. solani* compared to non-infected leaves. In the present study we evaluated the impact that these yeasts have on lesion size and disease severity caused by *R. solani*. To determine if nutrients would affect yeast biocontrol efficacy, *R. solani* was grown on 2% Yeast Extract Agar and Tap Water Agar for 72 h. Five mm plugs from each treatment were then excised and applied to leaves of 6-week old tall fescue. Directly after inoculation with *R. solani*, four yeasts previously isolated from tall fescue were applied separately at the site of infection. Additionally, a control for each *R. solani* treatment where no yeast was applied was also utilized. Five replicate pots were used for both of the *R. solani* treatments for each of the four yeast applications plus the no yeast control. The experiment was repeated twice under growth chamber conditions. The results of this study offer valuable insight into the role that nutrients and yeast play in determining disease development on the phylloplane where nutrient competition may act as a mechanism of inhibition of foliar pathogens.

Genetic diversity of *Sclerotinia trifoliorum* infecting chickpea based on mycelial compatibility grouping, rDNA introns and multi-locus haplotypes

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Sclerotinia trifoliorum is recently reported as a new pathogen of chickpea in North America. The diversity and genetic structure of this heterothallic fungus is poorly understood. This study was designed to investigate the genetic structure and diversity of the pathogen. A collection of 133 isolates of *S. trifoliorum* was obtained from 9 locations from chickpea fields in Central and Sacramento valleys of California, plus three isolates from alfalfa. Three techniques were used to measure genetic diversity: mycelial compatibility groups (MCG), ribosomal DNA (rDNA) introns, and multi-locus haplotypes of microsatellite loci and sequence related amplified polymorphism (SRAP) markers. Very diverse MCGs were found among the populations. The 136 isolates were assigned to 80 MCGs. Variation in the rDNA introns divided the isolates into four rDNA haplotypes. Combination of 51 SRAP loci and one microsatellite locus divided the isolates into 69 multi-locus haplotypes. A strong correlation between MCGs and rDNA haplotypes was found. However no discernible patterns were observed between MCGs and multi-locus haplotypes. This study provides evidence for both clonality and recombination in *S. trifoliorum*. Disease management strategies should not only identify suitable rotation crops, but also consider the high levels of genetic heterogeneity in order to effectively control the disease.

Performance assessments of methyl bromide alternative fumigants in Sting nematode infested strawberry fields in Florida

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The objectives of the study were to evaluate the performance of methyl bromide alternative chemicals in over 60 commercial field locations. Fumigants evaluated include individual and or combined use of methyl bromide, chloropicrin, 1, 3-dichloropropene, metam sodium, and methyl iodide. A diversity of drip fumigants were also evaluated for relative strawberry yield determinations based on yield potentials extrapolated from end of season plant size distributions within each field. Plant size categories included small (<15 cm), medium (>15 cm and < 30 cm) and large (>30 cm) plants. In each surveyed field, plant sizes were enumerated in at least 42 randomly selected 15 m sections of row in each commercial field location. Each surveyed field was renown for recurring histories of problems with the sting nematode *Belonolaimus longicaudatus*. Meaningful differences in plant size distribution and of relative yield were observed between various alternative to methyl bromide chemical treatments. In general, fields receiving no fumigant treatment were the most variable and produced relative strawberry yields which were over 50% lower than that observed with methyl bromide chloropicrin. Drip applied Telone Inline produced the highest relative yields compared to methyl bromide chloropicrin treated fields. Due in general to higher incidence and severity of Sting nematode stunting of plants, relative strawberry yield was observed to decrease with field application rate of chloropicrin.

Viability, quality, and protein content associated with sorghum caryopses infected with grain mold fungi

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Grain mold (GM) of sorghum is a yield-limiting disease that impacts caryopsis viability and quality. Several fungi, including Fusarium thapsinum (FT) and Curvularia lunata (CL), colonize the caryopsis during development. The viability of caryopses (including Sureno, Tx2911, SC170, BTx623, BTx623, and Tx430) harvested after FT and CL inoculation treatments at anthesis was measured using a tetrazolium violet assay. Germination, emergence and vigor were also measured and re-isolation ratios (RIS) were calculated from harvested grain for FT and CL. Using the single kernel characterization system (SKCS), caryopses were measured for hardness, weight, moisture and diameter. Total protein and kafirins (gamma and non-gamma) were also measured. GM resistant (GMR) genotypes produced caryopses that showed improved viability staining, germination, vigor and decreased RIS compared to GM susceptible (GMS) types. SKCS data indicated that inoculations with FT and CL reduced weight and diameter of harvested caryopses. Inoculations had a significant effect upon total protein accumulation in grains ($P < 0.05$); the response varied by genotype and treatment. Levels of gamma-kafirin also varied by genotype and treatment, whereas non-gamma kafirins varied by genotype only. Gamma-kafirin content and hardness increased in FT- and CL-inoculated 'Sureno' (GMR). Further studies are needed to elucidate the underlying structural characteristics associated with high quality grain and resistance to GM fungi.

***In vitro* evaluation of *western white pine* partial resistance against rust pathogen *Cronartium ribicola* in Canada**

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Cronartium ribicola J.C. Fisch. ex Rabenh. is one of the most destructive forest pathogens of North American white pines. The pathogen infects pine trees through their stomata. Then it colonizes in the stem and produces canker on the stem in the next growing season. Different methods of screening have been used to characterize resistance against the disease. Partial resistant plants have been categorized in 4 major groups difficult-to-infect (DI), bark reaction (BR), slow canker growth (SCG) and needle shed (NS). We developed a disease assessment index, based on both *in vitro* and *ex vitro* techniques, to evaluate specific reactions to the pathogen of the DI plants. The preliminary

results from our DI screening experiments indicated a significant difference in the number of successful infections between DI and control populations. Further morphological investigation into the mechanism(s) responsible for these variations with electron microscopy revealed a considerable difference in the morphology of stomata. Also, the amount of epicuticular wax on the stomata of the resistant populations was significantly higher than the control plants. These adaptations could provide a greater structural defense system against white pine blister rust.

Influence of aphid vector on the quasispecies of CMV associated with disease epidemics in the Midwest

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RNA viruses are often genetically diverse and are composed of populations defined as quasispecies. High mutation rates create significant variability at the population level which can afford the virus a greater probability to evolve and adapt to new environments. *Cucumber mosaic virus* (CMV) is such an RNA plant virus with a very wide host range and transmitted by several aphid species. Recently, snap bean crops in Wisconsin have experienced significant increases in disease linked to the introduction of the soybean aphid (*Aphis glycines*). Presumably, the unique population biology and dispersal of this vector has influenced the population structure of CMV. Our understanding of how CMV has responded to the introduction and establishment of a new insect vector is not well defined. One of hypotheses is that the population structure, or CMV quasispecies, has been influenced by the soybean aphid which is known to have high transmission efficiency. To investigate this hypothesis, we inoculated snap bean plants with CMV strains collected from discrete regions of the US; specifically regions with and without established populations of the soybean aphid. Acquisition and inoculation studies have been performed using the soybean aphid from infected plants containing regionally specific CMV strains and the CMV quasispecies are compared before and after transmission using sequence analysis.

Evaluation of alternative nematicides for the control of root-knot nematodes in a commercial carrot field

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Phytopathology 99:S95

Nematodes are likely the number one pest of carrots grown in California, particularly root knot nematodes. Currently the preferred method of nematode control for carrots is with the use of pre-plant soil fumigants, which negatively impact the environment. Alternative methods of nematode control need to be studied to quickly identify other possible control strategies. The aim of this study is to evaluate the nematicidal properties of several botanical and biological products. Several biological and botanical products which have been reported to have nematicidal properties were applied either pre-plant or post post-plant or in pre and post plant combination in a commercial carrot field that had been identified as having a high nematode population. The roots of the carrots were harvested and evaluated for nematode damage at the end of the season. Pre-treatment and at harvest soil samples were also taken to evaluate the effect of these materials on nematode populations. Two of the products reduced nematode damage of the carrot roots. Another product which is not a biological product but a synthetic insecticide also reduced nematode damage. The significance of this research is that the use of fumigants is becoming under closer scrutiny by regulators due to human safety and environmental concerns. Identifying new materials that can be quickly phased into agricultural use is urgently needed.

Central American origin of the bacterial pathogen causing Pierce's disease of grape

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Pierce's disease of grape (PD) has long posed a serious threat to the wine industry in the United States. It is caused by infection of xylem vessels by a subspecific form of Xylella fastidiosa (subsp. fastidiosa). Although generally assumed to be native to the US, we present genomic data suggesting that all *X. fastidiosa* subsp. *fastidiosa* in the US are derived from a single introduction from Central America, probably just prior to the first recorded outbreak of the disease in the 1880s. This hypothesis is supported by the presence of extensive genetic variation among Costa Rican isolates, whereas the level of variation found in the US is consistent with radiation from a single common ancestor within the last 150 years.

Host susceptibility of tall fescue grass to *Meloidogyne* spp. and *Mesocriconema xenoplax*

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Phytopathology 99:S95

Preplant fumigant nematicides have traditionally been used to control *Meloidogyne* spp. and *Mesocriconema xenoplax* in peach in the Southeast. In recent years growers have faced economic hardships, making it difficult to afford costs associated with these chemicals. Finding an alternative to control these nematodes is warranted. Greenhouse trials were conducted to evaluate the susceptibility of E+ and E- tall fescue grass to *M. incognita* and *M. xenoplax*. Fescue lines evaluated included, i) Jesup EI (E+, wild-type endophyte present), ii) Jesup EF (E-, no endophyte present), iii) Max-Q (E+, but non-ergot producing endophyte), and iv) GA-5 (E+). Peach was included as the control. Nematode reproduction criteria were used in evaluating fescue susceptibility. Peach supported greater ($P < 0.05$) reproduction of *M. incognita* and *M. xenoplax* than all fescue lines. Differences in reproduction were not detected among the fescue lines for either nematode. All fescue lines were either poor or nonhosts for *M. incognita* and the endophyte does not appear to effect nematode reproduction. In contrast, *M. xenoplax* reproduction was detected in the fescue lines. An initial test evaluating Max-Q for susceptibility to *M. hapla* indicated that Max-Q did not support *M. hapla* reproduction compared to a tomato control. These results provide useful insights into the potential use of tall fescue grass as a preplant alternative to chemical control of *Meloidogyne* spp.

Effect of fall cover crops on tomato and pepper diseases and fruit yield under organic production in North Alabama

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Plant disease management in small organic vegetable production systems remains an important challenge for growers in the south. There is a need for improved small farm adaptable disease management strategies that enhance cash crop productivity, and long-term farm sustainability. Cover crops have several beneficial effects such as breaking disease cycles, suppressing weeds and improving soil nutrients. The objective of this study was to evaluate disease incidence, severity and fruit yield of tomato and pepper in field plots previously planted with 4 fall cover crops, crimson clover (*Trifolium incarnatum* L.), rye (*Secale cereale* L.), hairy vetch (*Vicia villosa* Roth), and Austrian winter peas (*Pisum sativum*). The experiment was part of a 5-year study to assess the effects of cover crops on soil health, weeds and crop yield. Tomato and pepper were planted in an RCB design with three replications in summer 2008 after fall covers were mowed. Plant diseases were rated using a 0–5 scale. Both foliar and fruit diseases (incidence and severity), plant height, fruit yield and number of fruits per plant were assessed. Incidence of bacterial leaf spot and early blight were observed in tomato, however, there was no significant difference between cover crop treatments. Fruit rot was significantly lower in hairy vetch and Austrian winter pea plots compared to the fallow plots. Pepper plants remained healthy in all treatments. The significance of these results for plant disease management in organic farms is discussed.

Attempts to naturally regenerate red pine can be threatened by Diplodia shoot blight damage to understory seedlings

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Changes in red pine (*Pinus resinosa*) management, due to aesthetic and biodiversity concerns, include creation of harvest units with irregular edges, long borders of mature trees, and retention of some overstory trees within a harvested area. Also, in contrast to traditional even-aged management in which trees of one age class are grown, clearcut at final harvest, and replaced by planted seedlings, there is increasing interest in natural regeneration and developing multi-aged red pine stands. However, crowns of red pines can be sources of abundant inoculum of the shoot blight pathogen *Diplodia pinea*. To determine if Diplodia shoot blight threatens young, naturally regenerated red pine in the understory, six replicate plots were established in each of four mature plantations in central Wisconsin. The frequency of standing, dead seedlings bearing shoot blight symptoms or signs of the pathogen, and the incidence and severity of shoot blight damage to live seedlings were recorded. Mean seedling mortality ranged from 13–30% and mean incidence of blighted living seedlings ranged from 94–100% at all sites. The mean frequency of live seedlings with their terminal leaders killed in the past was from 55–94%. Mean severity of damage to live seedlings, on a 0–3 scale, was ≥2.16 at all sites. Results of a PCR assay confirmed pathogen identity. These results support previous research and concern that shoot blight pathogens threaten young red pines in the understory.

Transport and retention of *P. citricola* zoospores vs. similarly-sized artificial propagules in an ideal soil

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The genus *Phytophthora* includes numerous pathogenic species of importance to agriculture and forestry. A unique feature in the life-cycle of these oomycetes is a water-borne stage in which motile and encysted zoospores are dispersed and transported by water to potential hosts. Our research focuses on how the interaction of zoospore motility and physical processes (fluid mechanics) affects retention and transport of root-infecting propagules as occurs when water moves through pores during rain and irrigation events. Using columns packed with uniform sand as an ideal 'soil,' we compared breakthrough of *P. citricola* zoospore suspensions with similarly-sized artificial propagules (latex microspheres) and a chemically inert tracer. Columns were dissected at the conclusion of transport experiments to reveal retention patterns, from which the influence of motility on retention mechanisms can be inferred. At low flow rates, where interstitial velocities were at or below the swimming speed of zoospores, there was significant retention and mean zoospore transport velocity was similar to that of the tracer. In contrast, at higher flow rates, there was less retention and mean zoospore velocity was nearer that of the artificial propagules, which was approximately 10% higher than the conservative tracer. These results provide insight into zoospore transport in soils and also have implications for filtration and micro-particle transport theory.

An RT-PCR procedure for detection and surveillance of Citrus leprosis virus C (CiLV-C) in post-entry quarantine stocks of citrus

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Phytopathology 99:S96

Citrus leprosis virus C (CiLV-C), which is transmitted both mechanically and by the mite *Brevipalpus phoenicis* (Geijskes) (Acari: *Tenuipalpidae*), causes significant disease damage in South and Central America. Citrus leprosis disease was first recorded in Florida in 1925, but is believed eradicated through improved mite control procedures. CiLV-C is a threat to citrus producing nations where it is not present, such as New Zealand and the USA, and a sensitive detection method is required for screening and biosecurity of suspect quarantine material. CiLV-C is mechanically transmitted, posses a bipartite RNA genome and was believed to be a rhabdovirus. After being sequenced, CiLV-C was proposed as the type member of a new genus, *Cilevirus*, related to several (+) ssRNA viruses. Of two known morphological types of CiLV particles, the cytoplasmic type (CiLV-C) is prevalent than the nuclear type (CiLV-N) in Brazil and elsewhere. A pair of diagnostic primers, amplifying a segment of 278 bp located at the RNA-2 p15 gene of CiLV-C was designed using the Web software pathway Primer3-mFOLD-BLASTn. A thermodynamically robust RT-PCR that performs well in a range of melting temperatures and specifically optimized for CiLV-C was developed, and is a feasible tool to be used in quarantine.

Association between post-anthesis infection and deoxynivalenol accumulation in grain from spikes without visual symptoms of Fusarium head blight

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Deoxynivalenol (DON) is a mycotoxin that accumulates in wheat spikes infected by *Fusarium graminearum*, the causal agent of Fusarium head blight (FHB). In general, DON levels are positively correlated with symptoms of FHB, but DON can also exceed the critical threshold of 2 ppm when no symptoms of FHB are visible. Wheat is most susceptible to infection at anthesis; however, late-season infections do occur and can result in DON contamination. This study examined the association between post-anthesis infection and DON in grain from asymptomatic wheat spikes. Three soft red winter wheat cultivars (Cooper, susceptible; Hopewell, moderately susceptible; and Truman, moderately resistant) were inoculated 1, 2 and 3 wk after anthesis with different spore concentrations. Milled grain from asymptomatic spikes was tested for DON. *F. graminearum* biomass was quantified by amplifying *Tri5* DNA from grain samples using quantitative RT-PCR. DON values ranged from not detectable (<0.05 ppm) to 3.1 ppm. The amount of *Tri5* DNA ranged from 0.02 to 2.53 ng/g of grain. There was a significant positive correlation between fungal biomass and DON across cultivar and infection timing ($r = 0.69$). The infection time with the strongest correlation was 1 wk post-anthesis, with $r = 0.86$. The strongest correlation for cultivar was for Hopewell ($r = 0.62$), the cultivar that accumulated the most DON and contained the greatest amount of fungal biomass.

Fungicide effects on different spore types of *Phytophthora infestans*

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Phytopathology 99:S96

Fungicide application is a widely used strategy for management of late blight of potato, caused by *Phytophthora infestans*. Assessment of pathogen sensitivity to fungicidal compounds is important for ensuring effective late blight control, long-term efficacy and low risk of resistance development. We assessed in-vitro growth of *P. infestans* isolates from divergent genotypes based on allozyme analysis on Rye-B media amended with chlorothalonil, mancozeb and cymoxanil at concentrations of 0, 10, 100 and 1000 ppm. Sporangia and zoospore germination, and oospore production potential of pathogen isolates were also determined. Significant differences ($P < 0.05$) in hyphal growth were detected among pathogen isolates, and varied with fungicide treatments and concentrations. Mean colony growth of isolates on media ranged from 0.7–6.9 cm after 16 days of incubation at 18°C. Sporangia germination varied among pathogen isolates and fungicides, ranging from 0 to 30%. Zoospore germination and oospore production potential were greatly inhibited on fungicide amended media at concentrations exceeding 100 ppm. Laboratory studies indicate over 85% inhibition of *P. infestans* spore germination and growth with chlorothalonil regardless of fungicide concentration. These studies indicate that fungicidal compounds with multi-site modes of action are relatively effective on different spore types of *P. infestans.*

Effects of weather parameters on southern stem rot incidence and peanut yield

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Sclerotium rolfsi is an important peanut pathogen that causes southern stem rot (locally known as white mold). Crop damage associated with the disease in Georgia increased from approximately $6.9 million in 2002 to $32.3 million in 2007, the highest since 1998 when approximately $30.7 million worth of damage was recorded. Differences among the annual level of damage might be partly due to variability in weather patterns that favor disease development during the growing season. The goal of this study was to examine how weather parameters affect southern stem rot incidence. The specific objectives were to determine the relationship between weather parameters and incidence of southern stem rot, and to determine the impact of southern stem rot on peanut yield. The incidence of southern stem rot (%) in 'Georgia Green' cultivar was recorded in field trials conducted between 1995 and 2007; the corresponding weather data were obtained for the nearest weather station of the Georgia Automated Environmental Monitoring Network (AEMN: www.georgiaweather.net). Results indicate that a combination of favorable weather parameters during the month of July and August resulted in a significantly high incidence of southern stem rot. There was a significant negative correlation between incidence of southern stem rot and peanut yield. Information from this study could potentially assist growers in fungicide application scheduling for managing southern stem rot disease in peanut.

Genetic variability of RNA1 and RNA2 within *Grapevine fanleaf virus* isolates in three naturally infected California vineyards

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Phytopathology 99:S96

Grapevine fanleaf virus (GFLV) causes fanleaf degeneration, one of the most important viral diseases of grapevines worldwide. GFLV belongs to the genus *Nepovirus* in the family *Comoviridae*. It is specifically transmitted from grapevine to grapevine by the ectoparasitic nematode *Xiphinema index*. GFLV has a bipartite (+)ssRNA genome, which is expressed into two polyproteins that are cleaved into at least eight individual proteins. Due to its error-prone replication and quasi-species nature, GFLV possesses great potential for genetic variation. The variability within the coat protein gene is well characterized for numerous GFLV isolates but other viral genetic information is scarce. Similarly, little is known on the diversity of GFLV isolates from the U.S. Our objectives were to (1) examine the genetic variability within RNA1 and RNA2 of GFLV isolates in three naturally infected vineyards in California and (2) compare the genetic structure of these California isolates with other isolates from various geographic origins. GFLV-infected grapevines were identified in vineyards by monitoring fanleaf disease symptoms and testing leaf tissue by ELISA. Immunocapture-reverse transcription-PCR, cloning, and sequencing were used to determine nucleotide variability among GFLV isolates. Results will be presented and discussed

with regard to the development of improved GFLV diagnostic tools and the design of constructs for GFLV control through genetic engineering.

Resistance to wheat stem rust in spelt wheat, wild emmer and triticale
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Phytopathology 99:S97

A group of races of *Puccinia graminis* f. sp. *tritici* in the TTKS (or Ug99) lineage possess broad virulence to wheat cultivars worldwide, and only a few genes in the adapted cultivars have resistance to these races. In attempts to identify novel stem rust resistance genes effective against race TTKSK, we evaluated triticale (X *Triticosecale*), spelt wheat (*Triticum aestivum* ssp. *spelta*), and wild emmer (*T. turgidum* ssp. *dicoccoides*) for resistance to TTKSK and other stem rust races with broad virulence at the seedling stage. A high frequency of TTKSK resistance was observed in triticale, as 440 (78% of 567 accessions) exhibited low infection types. Resistance in triticale was highly diverse, with infection types ranging from 0; to 2+. Resistance was less frequent in wild emmer and spelt wheat, as only 16.6% (26 of 157) and 3.3% (16 of 495) accessions were resistant to race TTKSK, respectively. Low infection types 2 and 2+ to race TTKSK were predominant in wild emmer and spelt wheat. Accessions resistant to TTKSK have been further characterized for their reaction to other races in the TTKS lineage and additional races. Resistant accessions from diverse geographic origins and exhibiting different infection types were selected to develop crosses in an attempt to determine the genetic control of TTKSK resistance, to develop mapping populations, and to introgress genes into cultivated wheat.

What role could macroarrays play in plant pathology?
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We have explored the use of a macroarray for detection and identification of bacterial plant pathogens in multiple situations. For this macroarray, oligonucleotide probes were designed to anneal to the 23S rRNA and *rpoC* genes, with 23S oligos designed to identify genera, focusing on *Pseudomonas*, *Xanthomonas*, *Pectobacterium*, and *Clavibacter*, and *rpoC* oligos to distinguish among clades within genera. The oligos were fixed to nylon membranes and hybridized with PCR amplified, alkaline phosphatase-labeled bacterial DNA. After addition of a chemiluminescent reagent, membranes were exposed to light-sensitive films. We found that the array was specific and reproducible, but not sensitive. A test with field samples from a *Clavibacter michiganensis* outbreak in potato showed that the array could detect the pathogen in samples that, with ELISA, had an OD_{405} of over 1 and, with qRT-PCR, had a Ct value of under 28. Thus, macroarrays are not useful when high sensitivity is required. This array was able to distinguish among the *Pseudomonas syringae* pathovars and *Pectobacterium* species tested, thus could play a role in pathovar or species identification. The array was able to detect multiple *Pectobacterium* clades in diseased field samples, and therefore may be useful for pathogens commonly present in high levels in mixed infections. Testing the macroarray with field samples infected with *Pseudomonas* is underway.

Phenotypic characterization of *Phytophthora* isolates from North Carolina greenhouse ornamentals
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A collection of *Phytophthora* isolates from floriculture crops in North Carolina were characterized phenotypically for fungicide sensitivity and mating type. Isolates that grew at 1 ppm mefenoxam were evaluated further for the effective concentration of fungicide providing 50% growth inhibition (EC_{50}). Isolates within species were divided into groups based on location, host of origin, and initial fungicide sensitivity. Representative isolates from each group were grown on CMA amended with three mefenoxam concentrations appropriate for the initial resistance level. A regression analysis was conducted, and the resulting slope was used to estimate the EC_{50}. *Phytophthora drechsleri* isolates exhibited the greatest mefenoxam resistance with three of four groups having EC_{50} estimates over 700 ppm mefenoxam. All groups of *P. tropicalis* had EC_{50} values of less than 1 ppm. EC_{50} estimates for *P. nicotianae* groups were 246, 319, 339, 349, 386, and 435 ppm. Heterothallic *Phytophthora* species were evaluated for mating type. Collected isolates were paired with testers of known mating type on amended V8 agar. Both mating types of *P. nicotianae* and *P. tropicalis* were found. At two locations, the A1 and A2 mating types of *P. nicotianae* were found. Only the A1 mating type of *P. drechsleri* was found. Characterization of *Phytophthora* species attacking floriculture crops will provide information for disease management strategies.

Chemical control of green mold (*Penicillium digitatum*) with the fungicide Pyrimethanil in Persian lime
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Phytopathology 99:S97

Persian lime (*Citrus latifolia*) is the principal citrus fruit exported from Mexico to USA. Green mold is the most important postharvest disease that affects this fruit. Chemical control is used in the packing houses. We evaluated three concentrations of the fungicide Pyrimethanil (Shield-Brite, Penbotec®: 500, 1,000 and 2,000 ppm + wax) for the control of this disease. Imazalil was included as check. Fruits were inoculated (25 fruits per treatment and four replicates) with a stainless steel rod tip immersed in spore suspension (1.0×10^6 spores/ml) and causing a 2 mm deep wound on each fruit. After 16 hours of inoculation, the fruits were submerged for 20 seconds in the fungicide solution, whereas for the wax treatment, the fungicide was mixed in the wax and applied by hand. After treatment, the fruits were stored in a humid chamber at 20°C during 10 days, and then the disease was evaluated. All the treatments with Pyrimethanil reduced the incidence and severity of the disease; however, the best treatments were Pyrimethanil at 1,000 ppm and Pyrimethanil + wax at 2,000 ppm, registering 5.5 and 3.3% of affected fruits, respectively. Pyrimethanil and Imazalil, both at 500 ppm had 8.5 and 11.0% of infected fruits, respectively. The control fruit (only inoculated and without fungicide) showed 100% of disease incidence.

***Penicillium digitatum*, causal agent of green mold in Persian and Mexican lime fruits**
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Green mold is the most important disease affecting Mexican (*Citrus aurantifolia*) and Persian (*Citrus latifolia*) lime fruits in postharvest. *Penicillium digitatum* was isolated and identified as the causal agent of the disease. Koch's postulates were successfully completed to relate the fungus with the disease. *P. digitatum* produced a colony with mycelium grayish green to olive and white margins. Conidia are elliptical to cylindrical, greenish white to pale green, measuring 5–9 × 3–6 µm. To know the optimal concentration of inoculum of *P. digitatum* to causes symptoms, we evaluated four concentrations (1.0×10^5, 4.0×10^5, 7.0×10^5 and 1.0×10^6 spores/ml) and a control without inoculation. The fruits were inoculated (25 fruits per treatment and four replicates) with a stainless steel rod tip immersed in spore suspension and causing a 2 mm deep wound on each fruit. The treatments were incubated at 20°C ± 2. At 10 days after inoculation, all concentrations recorded a 100% fruit affected in both citrus species. The severity of green mold in Mexican lime was 98 to 100% of diseased fruit area and there was no significant difference between concentrations. In Persian lime, the greatest severity was achieved with 1.0×10^6 and 7.0×10^5 spores/ml, recording 93.7 to 100% of damage, respectively.

Colonization of nonwounded and wounded creeping bentgrass (*Agrostis stolonifera*) by virulent and hypovirulent isolates of *Sclerotinia homoeocarpa*
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Sclerotinia homoeocarpa causes dollar spot disease of turf grass. Hypovirulent isolates of *S. homoeocarpa* contain the mitochondrial virus *Ophiostoma novo-ulmi mitovirus 3a* (*OMV3a*). In this study, colonization and lesion production by *S. homoeocarpa* on nonwounded (NW) and wounded (W) leaves of creeping bentgrass were characterized using light microscopy. On NW leaves, hyphae initially infected the leaf by forming appressoria along cell walls and over stomates by 48 h. Inter- and intracellular hyphae were not common until the NW leaves were heavily colonized at 96 h. Mycelia grew ahead of the lesion for 50 h on both NW and W leaves. On W leaves, mycelia grew directly into and infected wound sites as soon as 8 h, and inter- and intracellular hyphae rapidly developed into a primary infection front. At 16 h, secondary infections developed from superficial mycelia that formed appressoria along cell walls and over stomates. Appressoria gave rise to infection hyphae, spherical infection vesicles, and primary intracellular hyphae. Hypovirulent isolates seldom colonized NW or W leaves past 25 mm after 240 or 168 h, respectively. These results indicate that *S. homoeocarpa* is a hemibiotroph which colonizes and causes disease more rapidly on W than

NW grass; an important factor for turf grass management. The etiology of virulent and hypovirulent isolates were similar, except for slower progression of hypovirulent colonization and disease.

Maize land races from Mexico harbor resistance to diverse aflatoxin-producing fungi

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Maize is the critical staple of billions in several regions across the globe. Domestication occurred in Mexico about 7000 years ago. Maize is frequently contaminated with aflatoxins, highly toxic carcinogenic secondary metabolites produced by members of *Aspergillus* section Flavi. Contamination occurs both pre- and post-harvest. Ethnic groups in Mexico maintain many genetically distinct maize land races that have been adapted to diverse climates and soil types. Previously, in laboratory tests we found great variation in resistance to aflatoxin contamination among 34 maize land races collected in 2006, 2007 and 2008 from two regions of Mexico. However, the previous evaluations were performed with one L strain isolate representing only a small portion of the diversity of aflatoxin-producing fungi infecting maize in Mexico. The current work compared maize land race resistance to contamination by 12 phylogenetically diverse aflatoxin producers isolated from Mexico. The resistant land race accumulated lower concentrations of aflatoxins than the susceptible land race when challenged with any of the examined fungi. This suggests that maize land races possess resistance mechanisms effective against the diverse groups of aflatoxigenic fungi associated with maize in Mexico. Integration of resistance genes from the maize land races into hybrid breeding programs may contribute to development of commercially acceptable maize with improved resistance to aflatoxin contamination.

***Phyllachora* "tar spots" on *Bauhinia* species from the Brazilian Cerrado**

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Native *Bauhinia glabra* and *B. rubiginosa* are fabaceous shrubs frequently found as part of the Cerrado vegetation. Both species showed symptoms of "tar spot" due to infection by *Phyllachora* species. Each host was associated with a different *Phyllachora* species. The species on *B. glabra* showed: *Anamorph* present; and *Teleomorph* under tar spots measuring *ca*. 1–9 mm diam, epiphyllous, irregularly shaped, sometimes with reddish-brown areas at margin; *ascomata* 100–250 × 188–438 µm, multiloculate, conic to pyriform, occupying the leaves all across, with *wall ca*. 10–22 µm thick; *clypeus* 45–67.5 µm thick; *paraphyses* 2–3 µm diam. hyaline, simple; *asci* 70–96 × 10–18 µm, cylindric to clavate, short-stalked, with a clear truncate apical structures, 8-spored; *ascospores* 13–18 × 5–8 µm, uniseriate or biseriate, guttulate, hyaline, cylindric-ellipsoidal, with gelatinous sheath. The species on *B. rubiginosa* did not show anamorph. The *teleomorph* was under epiphyllous "tar spots" with 2–17 mm diam, irregularly shaped; *ascomata* 180–300 × 264–600 µm, stromatic, subepidermal, ellipsoidal to pyriform, *wall ca*. 10.5–24 µm thick; *clypeus* 55–80 µm thick; *paraphyses* 2–3 µm diam., hyaline, branched, septate; *asci* 53–121 × 8–12 µm, cylindric to clavate, short-stalked, with a wide pex, 8-spored; *ascospores* 10–14 × 6–11 µm, uniseriate to biseriate, guttulate, hyaline, ellipsoidal to cylindric-ellipsoidal, thick walled, with gelatinous sheath. The two pathogens probably belong in new *Phyllachora* species.

Nursery stock is a potential source of *Blueberry scorch virus* in new plantings

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Phytopathology 99:S98

Blueberry scorch virus (BlScV) is one of the most pervasive pathogens of highbush blueberry. BlScV exhibits a latent period between infection and symptom expression that may extend to years. The virus is vectored by aphids and typically spreads in clustered patterns. However, we have observed BlScV symptom expression that is randomly distributed in young fields and this is inconsistent with an insect vectored introduction. It was therefore speculated that the virus was introduced on infected nursery stock. To examine this possibility, the following study was conducted. Initially, commercial nurseries were surveyed to test for infected mother plants. Mother plants are pruned so that they do not flower and therefore do not express symptoms. Cuttings of the cultivar Duke were collected from infected and non-infected mother plants and rooted in propagation beds. The survival and infection of cohorts of cuttings from different mother plants was determined one year after planting. A greater proportion of cuttings survived from uninfected mother plants (71%) than from infected mother plants (47%). The resulting plants were tested for BlScV and only cuttings originating from infected mother plants tested positive. Of the cohort propagated from infected mother plants approximately 42% tested positive. This study identifies infected nursery stock as an important source of BlScV spread and underscores the importance of having mother plants routinely virus tested.

Russian isolates of *Potato spindle tuber viroid* exhibit low sequence diversity

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Phytopathology 99:S98

The introduction of a series of improved diagnostic methods has led to the virtual eradication of *Potato spindle tuber viroid* (PSTVd) from potatoes grown in North America and Western Europe, but this pathogen remains wide-spread in seed potatoes grown in Russia. There are no regional seed potato certification laboratories in Russia, and farmers lack access to reliable methods of viroid identification. Characterization of 39 PSTVd isolates collected over a 15-year period from widely separated areas in Russia revealed the presence of 17 different sequence variants, all but one of which were previously unknown. Most variants were recovered only once, but two were more widely distributed; one of these was a mild variant previously isolated in Germany, the second was a novel variant that induces symptoms similar to those of the type strain in tomato. Despite this apparent lack of population diversity, several informative PSTVd variants were recovered. Sequence changes in the pathogenicity and variable domains were particularly common, but previously unknown changes were also detected within the loop E motif in the central domain, a structural motif known to play a key role in PSTVd replication and host range determination.

The impact of strobilurin fungicides on disease development and yield in corn and cotton

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Phytopathology 99:S98

Strobilurin fungicides are the products of choice for managing endemic foliar diseases of wheat and soybean in the Mid-South. Recently, strobilurin fungicides have been promoted for use in corn and cotton based on crop growth stage instead of disease thresholds. To determine if these fungicides are necessary, university tests were conducted across the Mid-South. The fungicides Headline and Quadris were applied at various rates and timings and evaluated for their efficacy on naturally-occurring diseases, plant development, yield, and quality. Fungicides were evaluated in small plots and producer fields. A single application to corn was made at tasseling (VT). Single and multiple applications were made to cotton at flowering and 2 weeks post-flowering. Plants were monitored for disease development and diseases were quantified if present. In the majority of tests, disease occurred late-season. Foliar fungal diseases and boll rots occurred in cotton, and rusts and leaf blights were observed in some corn tests but differed between locations. Plant development was recorded during the growing season. In some tests, stalk densities were calculated to determine if fungicides preserved stalk strength. Results varied considerably across locations and within tests. Cotton treated with a fungicide did not consistently increase yield or fiber quality than non-treated cotton. Similar results for yield were observed in corn; and, stalk densities did not differ among treatments.

Management of zoosporic root-infecting pathogens by amending the nutrient solution in re-circulating hydroponic systems with sodium salicylate and Neem

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Phytopathology 99:S98

We previously reported that N-Serve, a nitrification inhibitor, when added to the re-circulating nutrient solution in hydroponic systems, caused a surge in the population of indigenous fluorescent pseudomonads and suppression of diseases caused by root-infecting zoosporic pathogens. In our ongoing search for an effective strategy to manage these diseases in re-circulating systems, we have been using *Phytophthora capsici* – pepper combination to evaluate the efficacy of some non-fungicidal alternatives such as sodium salicylate and Neem. Sodium salicylate was chosen for its reported ability to increase the

population of indigenous fluorescent pseudomonads in the soil. The selection of Neem was based on the report that it, like N-Serve, has nitrogen stabilizing ability and contains ingredients such as fatty acids and glycerides which could act as carbon sources for bacterial growth. In repeated experiments, sodium salicylate, compared to inoculated controls, caused a significant delay in the onset of *Phytophthora capsici* root rot of peppers grown in re-circulating hydroponic system. However, Neem's ability to suppress the disease was inconsistent. Furthermore, both sodium salicylate and Neem caused a surge in the population of indigenous bacteria. The population size and the diversity of fluorescent pseudomonads within the total bacterial population in samples taken before and after the addition of either sodium salicylate or Neem were extremely variable.

Colonization of maize seedlings under drought conditions by two ochratoxin A producers species within the *A*. section *Nigri*
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Phytopathology 99:S99

Some species of black-spored aspergilli (*Aspergillus* section *Nigri*) are able to cause disease in several plant hosts, including peanut and maize seedlings. Besides the economical impact of black-spored aspergilli infections, several species within this section are well known mycotoxin producers, specifically the teratogenic, nephrotoxic, and potential carcinogenic ochratoxin A. The interactions between maize and *A*. section *Nigri* species are poorly understood, especially since endophytic colonizations are symptomless. In this study, *A. carbonarius* and *A. foetidus* were transformed with green, yellow, and red fluorescent proteins, and used to measure colonization of maize seedlings under drought stress conditions. Maize kernels were infected with 5 × 10^4 spore/ml suspension. After 3 weeks of inoculation, seedlings were drought stressed, and the colonization of lateral roots, meristem, and stems was monitored at different time points with the aid of laser scanning confocal microscopy. The use of a fluorescent tagging approach determined the different colonization patterns employed by black spored aspergilli species during maize seedling colonization.

Effects of antagonistic *Pseudomonas* strains on soil and airborne populations of *Aspergillus flavus* and *Fusarium verticillioides*
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Phytopathology 99:S99

Pseudomonas chlororaphis strain JP1015 and *Pseudomonas fluorescens* strain JP2175 were previously isolated from Mississippi corn field soil samples and selected for their growth inhibition of *Aspergillus flavus* and *Fusarium verticillioides* in laboratory culture. In this study, we determined the antifungal activity of these bacterial strains against *A. flavus* and *F. verticillioides* in soil coculture. Growth of *A. flavus* was inhibited up to 100-fold by *P. chlororaphis* and up to 58-fold by *P. fluorescens* within 3 days following soil coinoculation. *A. flavus* propagule populations remained 7-fold to 20-fold lower in soil treated with either bacterial strain. *F. verticillioides* growth was inhibited up to 40-fold by *P. chlororaphis* and up to 30-fold by *P. fluorescens* after 3 days of soil coculture, and remained up to 6-fold lower after 16 days. One mechanism by which corn may become infected by these fungi is by spore transmission via wind. Using a bench-scale wind chamber, we demonstrated that bacterial treatments of soil led to significant reduction in the number of airborne spores dispersed across a 1-meter distance. These results suggest that corn field soil amendment using these bacterial strains may be effective in reducing the populations of mycotoxigenic fungi, thereby limiting fungal spore formation, and ultimately decreasing the potential for corn infection via airborne transmission.

Biochemical characterization of effects of plant essential oils on *Ralstonia solanacearum* by laser Raman spectroscopy
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Phytopathology 99:S99

Essential oils of palmarosa, lemongrass and eucalyptus show promise for biological control of bacterial wilt, caused by *Ralstonia solanacearum* (Rs). Palmarosa and lemongrass oils (0.04, 0.07, 0.14% v/v) had bactericidal effects on Rs whereas eucalyptus oil was bacteriostatic in culture amendment studies. The effects of treatments at different concentrations were evaluated using Raman spectroscopy using a 785 nm near infrared laser. All bactericidal treatments except palmarosa at 0.04% v/v caused reduction in levels of phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), nucleic acid bases, carbohydrates, amides and lipids as indicated by reduction in peak height of Phe at 621, 1003 and 1031 cm^{-1}; Tyr at 643, 827, 852, 1158 and 1172 cm^{-1}, Trp at 758 cm^{-1}; nucleic acid bases at 725, 782, 1337 and 1578 cm^{-1}; carbohydrates at 1097 cm^{-1}, amides at 1663 cm^{-1}; and lipids at 1450 and 2932 cm^{-1} compared to controls. Eucalyptus oil treatments showed less reduction in these peak heights. The bactericidal and bacteriostatic properties of the oils were confirmed by visual analysis with scanning- and transmission- electron microscopy and cell viability studies using epifluorescence microscopy supporting the data obtained by Raman spectroscopy. The Raman studies provide evidence that essential oils degrade cell components, thus confirming their potential use in biological control.

Fungi isolated from cankers and galls on hickories exhibiting crown decline or dieback
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Phytopathology 99:S99

Diffuse cankers without surrounding callus, callused-over annual cankers and globose galls were commonly found on stems of hickories with crown decline or dieback during 2007 and 2008 field surveys in Indiana, Iowa, Minnesota, New York, Ohio and Wisconsin. Crown decline exhibiting sparse crown with small, chlorotic leaves was distinguished from crown dieback with dead tops but normal green leaves below. Samples (1.5 cm^3) from discolored sapwood associated with cankers or galls were kept in moist chambers to stimulate formation of fungal fruiting structures while smaller wood chips were plated on lactic acid-amended potato dextrose agar. Spore masses were transferred to streptomycin sulfate-amended malt yeast extract agar. Of 160 diffuse cankers on 32 trees exhibiting crown decline, 66 cankers representing 26 trees yielded *Ceratocystis* spp. while 28 cankers from 14 trees yielded *Fusarium* spp. For 112 annual cankers on 22 trees with crown dieback, *Fusarium* spp. were isolated from 32 cankers from 16 trees. Only three of seven trees with galls yielded *Ceratocystis* spp., *Fusarium* spp., or *Phomopsis* spp. The majority of the *Ceratocystis* isolates were tentatively identified as *C. smalleyi*; the remaining isolates were *C. caryae*. If confirmed, these results will constitute the first report of *Ceratocystis* spp. on hickory in IN, MN, NY and OH. The *Ceratocysis* spp. and *Phomopsis* spp. have been reported in the other states as pathogens on hickory, but *Fusarium* spp. have not.

Proteomic analysis of soybean accessions resistant and susceptible to *Phakopsora pachyrhizi* urediniospores from Louisiana
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Phytopathology 99:S99

Asian soybean rust, caused by *Phakopsora pachyrhizi*, is an emerging disease in the continental U.S. since its discovery in late 2004. This disease has the potential to cause severe yield reduction and billions of dollars in economic losses due to that all U.S. commercial soybean varieties are susceptible to this disease. In an effort to understand host-pathogen interaction at the molecular level, sixteen accessions were evaluated with rust spores collected in Louisiana. Two accessions showed consistent immune response in both detached leaf assay and greenhouse inoculation. Protein profiles of two resistant and two susceptible lines were compared to identify proteins differentially expressed between resistant and susceptible lines with or without rust infection. Differentially expressed proteins were observed in both resistant and susceptible lines. Some of them matched with previously identified proteins, such as pathogenesis related protein 10, chalcone flavonone isomerase, and β-1,3-endoglucanase. The identities of other differentially expressed proteins are being determined through peptide sequencing. The transcript levels of differentially expressed proteins also will be determined using qRT-PCR. The potential importance of these differentially expressed proteins in soybean-*P. pachyrhizi* interaction will be discussed.

Phytophthora and Pythium Databases: A growing cyberinfrastructure supporting the identification and monitoring of major pathogen groups
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Phytopathology 99:S99

The movement of non-indigenous pathogen species and exotic variants of indigenous ones will likely increase due to the rapid expansion of global commerce and human travel. However, to date efforts to study and manage this threat have been fragmented, mostly regional, and limited to coping with immediate crises. Here we will present the scope and progress of global efforts to document and catalog the genetic and phenotypic diversity of economically important pathogens. The Phytophthora Database (http://www.phytophthoradb.org), an online, forensic database, was established to support rapid detection and diagnosis of Phytophthora species. To support the identification of new Phytophthora isolates via comparison of

their sequences at one or more loci (up to nine loci) with the corresponding sequences derived from the isolates archived in the database, sequence data from more than 1,700 isolates representing >90 species in the genus were generated and deposited. Data search and analysis tools in the database include BLAST, Phyloviewer, and Virtual Gel. The database also provides a customized means of storing and sharing data via the web. A parallel international project to construct a database for the genus Pythium, termed the Pythium Database (http://www.pythiumdb.org), is also in progress. An overview of database functions and how to use these databases to determine the species identity of new isolates will be discussed.

Online outreach: *Phytophthora* training for nursery growers
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Phytopathology 99:S100

Phytophthora spp. cause some of the most important diseases of nursery crops nationwide. The quarantine pathogen *P. ramorum,* the cause of ramorum blight and sudden oak death, is of special concern. In partnership with the Oregon Department of Agriculture, we created an online course to teach growers how to reduce the incidence of *Phytophthora* diseases in their nurseries. This free, non-credit course includes three modules that focus on 1) biology, symptoms and diagnosis, 2) disease management, and 3) *Phytophthora ramorum.* Both English and Spanish language versions of the course are available. After completion of the course, nursery growers can take an optional online exam for a fee. If they pass the exam, they earn a certificate of mastery from Oregon State University Extended Campus and qualify for pesticide recertification credits. The course may be accessed at http://ecampus.oregonstate.edu/phytophthora. The development and efficacy of online training courses for grower education will be discussed.

Recovery of *Phytophthora* species from critical control points in horticultural nurseries
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Phytopathology 99:S100

We previously reported results of a systems approach study that elucidated critical control points (CCPs) for *Phytophthora* contamination in Oregon nursery production systems. A CCP is the best point at which significant hazards of contamination can be prevented. CCPs included contaminated gravel substrates, re-used containers, potting media, and irrigation ponds. We now report the identity of *Phytophthora* isolates associated with each of these CCPs. *Phytophthora* isolates were identified to species by direct sequencing of the internal transcribed spacer (ITS) rDNA and blast searches at www.phytophthora-id.org. Of 449 total *Phytophthora* isolates, 364 isolates (81%) belonged to 15 *Phytophthora* species, 13% matched *Phytophthora* taxa without species designations, and 6% did not match any sequence in the database. The most frequently isolated species from symptomatic plants were *P. citricola, P. cinnamomi*, and *P. syringae*. From gravel substrates, pots, and soil, the predominant species were *P. citricola, P. cinnamomi*, and *P. cryptogea*. From irrigation ponds, most isolates were *P. gonapodyides* or other *Phytophthora* taxa belonging to ITS Clade 6. *P. parsiana*, not previously reported from nurseries, was also detected. *P. cinnamomi*, the species most frequently isolated from plants, was never recovered from water. These results provide insights on *Phytophthora* pathology and ecology in nurseries.

Effect of rootstock genotype on functional and taxonomic diversity of rhizosphere communities and endophyte communities of grapevine in California
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Phytopathology 99:S100

As in many other perennial production systems, viticulturist use rootstocks to manage poor soil conditions and pathogens. Developing an understanding of the bacterial community inside the grapevine and in the grapevine rhizosphere is needed to provide a baseline of information for further research concerning disease control and growth promotion. Five popular grapevine rootstocks were selected to examine the influence of host genotype on the functional and taxonomic diversity of the microbial community of grapevines. Vines growing in Yolo fine sandy loam were sampled at three points throughout the growing season. The functional diversity of the rootstock rhizosphere communities was separated by carbon utilization profiles (CUP). Utilization rates of some carbon sources, like D-mannitol, were unaffected over the season. Others, like 2-hydroxy benzoic acid, were significantly affected as a function of host genotype and time. Teleki 5C and Ramsey consistently had similar CUPs; alternatively, 110R was typically different from other rootstocks and had comparatively depressed utilization rates. In terms of taxonomic diversity, rootstock rhizosphere communities and endophytic communities had unique and common taxa with varying degrees of taxa isolation frequency. As an example, greater isolation frequency occurred with Variovorax paradoxus in St. George and Teleki 5C in the rhizosphere, but only Ramsey had greater isolation of V. paradoxus from inside roots.

Isolation of *Phytophthora inundata* from a flue-cured tobacco field in Virginia
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Phytopathology 99:S100

Tobacco black shank, caused by *Phytophthora nicotianae*, is widespread within the tobacco growing regions of the US. Increased disease losses have been reported due to altered population structure. Our objective in this research was to characterize the *Phytophthora* populations in tobacco fields in VA. One hundred and seventy five isolates were collected from diseased burley and flue-cured tobacco plant samples from 30 different fields located in 12 counties in VA. Single strand conformation polymorphism (SSCP) analyses of PCR-amplified ribosomal DNA indicated that two of these isolates, 01A3 and 01A4, belong to *P. inundata* instead of *P. nicotianae*. Both 01A3 and 01A4 were collected from the same flue-cured tobacco (cv. K326) field in Nottoway County in 2007. Both isolates produced non-papillate, ovoid - to - obpyriform sporangia proliferating internally, but neither produced oospores in single culture. These morphological characteristics and sequences of their ITS regions confirmed that 01A3 and 01A4 are *P. inundata*. *Phytophthora inundata* has been reported as a pathogen of trees and shrubs in flooded soil. This is the second report of *P. inundata* in the US, after a 2006 report on alfalfa in California. Tests on the pathogenicity of the two isolates to tobacco are currently underway.

Combining rust resistance genes in snap beans for Eastern Africa
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Phytopathology 99:S100

Snap bean (Phaseolus vulgaris) production is a significant source of income for smallholder farmers in Eastern and Southern African countries such as Kenya, Tanzania, Uganda, Zambia, Zimbabwe, and other countries in North and Central Africa. Common bean rust, caused by Uromyces appendiculatus, is the most important constraint to snap bean production in this region. Rust severity is exacerbated by the intensity of snap bean production in this region and the use of mostly rust-susceptible varieties in different stages of growth. The intensity of bean rust has led to excessive use of fungicides. To reverse this situation we, ARS-USDA and Cornell University, are developing snap beans with genetic resistance to bean rust. We are initially developing snap bean lines that combine the Ur-4 and Ur-11 rust resistance genes. Ur-4 is from a snap bean of the Andean gene pool. Conversely, Ur-11 is from a dry bean from the Middle American gene pool and it is known to be very effective in Eastern and Southern Africa and other parts of the world. These two genes complement each other in the snap bean lines that we have developed. These lines are resistant to about 100 different races of the rust pathogen maintained at Beltsville and are expected to be resistant to the races of the rust pathogen infecting snap beans in Eastern and Southern Africa. In addition to rust resistance, we are selecting snap bean lines that also have tolerance to high temperatures.

Evidence for the role of Type VI secretion during *Lysobacter enzymogenes* pathogenesis of fungal hosts
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Phytopathology 99:S100

The bacterial biocontrol agent *Lysobacter enzymogenes* is a pathogen of a wide range of lower eukaryotes including the fungi, nematodes and lower plants. Pathogenesis proceeds through a series of stages initiated by polar attachment, followed by intracellular infection, replication, and eventual lysis of the host cell resulting in release of bacteria into the surrounding environment. The mechanisms *L. enzymogenes* uses to infect its hosts remain unclear; however, analysis of the *L. enzymogenes* genome sequence has revealed the presence of several genes thought to be involved in pathogenesis. Among these are two type VI secretion systems (T6SS), which are known to function as intricate translocation machinery of effector molecules associated with bacterial pathogenesis of higher plants and animals. Analysis of the *L. enzymogenes* T6SS indicates both systems contain *hcp* (hemolysin-coregulated protein) genes, which are thought to serve a structural purpose and function as a secreted component of the T6SS apparatus. Strains containing mutations within the *hcp* gene of either T6SS displayed a reduction in the rate of intracellular colonization of fungal hosts compared with the wild

type strain. Host cells treated with either *hcp* mutant strain were also reduced in the rate at which non acidic vesicle-like structures appeared. Taken together, these observations support a role for VI secretion in *Lysobacter* pathogenesis of fungal hosts.

Pyrenophora tritici-repentis isolates cause necrosis in a wheat cultivar Glenlea without the _ToxA_ gene

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Phytopathology 99:S101

Pyrenophora tritici-repentis causes tan spot disease in wheat and produces multiple host specific toxins (HSTs) such as Ptr ToxA, Ptr ToxB, and Ptr ToxC in sensitive wheat. Ptr ToxA induces necrosis while Ptr ToxB and Ptr ToxC cause chlorosis. Pathogenic races of *P. tritici-repentis* have been identified using a set of standard differential cultivars. Among these differential cultivars, Glenlea is sensitive only to Ptr ToxA and not to Ptr ToxB and ToxC. *P. tritici-repentis* isolates have been collected from the Arkansas and those isolates were checked for the presence of the ToxA gene by polymerase chain reaction. Three isolates were found not to contain the ToxA gene. These three isolates were placed in race 1 category (1) based on the Lamari's classification (2) which suggested that all three isolates produced necrosis on susceptible cultivar Glenlea, indicating the presence of possible additional toxin in *P. tritici-repentis.* (1) Friesen et al. 2005. Phytopathology 95:1144-1150. (2) Lamari et al. 1995. Can. J. Plant. Pathol. 17:312-318.

Changes in expression patterns of pathogenesis-related genes in wheat after treatment with chemical inducers against _Pyrenophora tritici-repentis_

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Phytopathology 99:S101

Tan spot, caused by *Pyrenophora tritici-repentis* (Ptr), is economically an important disease in wheat worldwide. To determine the effects of chemical inducers such as brassinolid (BL), benzo (1,2,3) thiadiazole-7-carbothionic acid S-methyl ester (BTH), jasmonic acid (JA), and salicylic acid (SA) on tan spot development in wheat, spring wheat cultivar ND 495, which is susceptible to Ptr, was pre-treated with four chemical inducers and challenged with *P. tritici-repentis* race 1. Disease progression was monitored and scored from 0 to 7 days after pathogen inoculation (DAI). Total RNA was isolated and converted to cDNA from the leaf tissues to examine the expression profiles of four pathogenesis-related (PR) genes PR-1, PR-2, PR-4 and PR-5. The expression of each PR gene was estimated using quantitative real-time PCR assay. The results of this study indicated that plants pretreated with BTH, JA and SA induced higher disease severity compared to BL. In contrast, plants applied with BL reduced disease severity significantly from 0 to 4 DAI and induced PR genes (except PR-4) from 2 to 6 DAI. We conclude that BL could play an important role in plant defense by activating PR genes against Ptr.

Inducing sporulation by the fungus _Cladosporium caryigenum in vitro_

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Phytopathology 99:S101

Cladosporium caryigenum, the causal agent of pecan scab, is characterized as a slow growing fungus with minimal spore production when mycelial plugs are inverted onto traditional media, such as potato dextrose agar (PDA) and malt extract agar (MEA). Vegetative growth and spore production were evaluated on five media. Mycelial plugs from six fungal isolates were macerated in 1 ml of sterile water with 3, 3-mm glass beads at 4600 rpm on a bead beating apparatus for 10 seconds. Media types tested were Sabouraud dextrose agar (SDA), PDA, MEA, potato carrot agar amended with 50% lactic acid (PCAL), and water agar (WA) as a negative control. Twenty µl of suspended hyphal material from each isolate and a water drop control were placed on each media type. Media were observed for growth and sporulation for 10 days at 12 hour intervals. While media were not significantly different (P = 0.07), sporulation on PCAL, PDA, and MALT were comparable while SDA was lower. No sporulation occurred on WA. A significant interaction (P < 0.01) of media and sporulation of isolates was found due to the non-sporulation of two isolates on all media types. Increased sporulation observed in this trial may be due using a maceration technique on hyphal tissue prior to plating.

Immuno-capture of _Ralstonia solanacearum_ by an EPS-specific monoclonal antibody enhances sensitivity of PCR

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Phytopathology 99:S101

Detection of *Ralstonia solanacearum* (*Rs*) in field soil and irrigation water is complicated by significant heterogeneity within the species complex and insufficient bacterial populations for most detection assays. DNA-based methods, though sensitive and specific, are not very practical since it only accommodates a small sample volume, usually 1–5 microliters, which may not contain enough bacterial DNA for detection. Therefore, an anti-*Rs* antibody was developed with the aim of concentrating dilute populations of bacteria from large volumes of irrigation or drainage water. The antibody was developed using traditional monoclonal hybridoma technology with a pool of 10 Rs strains as the antigen. The strong reacting antibody 3.H7 (IgG_3 with kappa light chain) is specific for all Rs EPS (109 strains from diverse hosts and geographical origins, representing 3 phylotypes) with only one exception (one BDB strain from banana). Detection of dilute concentrations of *Rs* was not possible when aliquots of large water samples were directly added to PCR reactions (using fliC or 759/760 primers); however, aliquots of 3.H7-immuno-captured cells were successfully amplified by PCR from these same water samples.

Generation of monoclonal antibodies based on phylogenetic relationships of _Dickeya_ sp. associated with pineapple heart rot disease

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Phytopathology 99:S101

Bacterial heart rot of pineapple caused by uncharacterized *Dickeya* sp. (formally *Erwinia chrysanthemi*) was first observed in Hawaii in 2003. Monoclonal antibodies (MAb) were generated to facilitate rapid detection and identification of the pathogen in diseased plant material. The previously reported MAb 19C2G4 had appropriate specificity but lacked adequate affinity. Therefore, in this study we used phylogenetic data from a *gyrB* DNA marker to select three strains, grouped exclusively in a clade containing only virulent pineapple pathogens (clade A), for making a MAb antigenic cocktail. Upon screening hybridoma cell lines, only two antibody specificities were detected. The first (i.e. Clone #1D1H6H7, IgG_3) reacted strongly with all strains in clade A and the Hawaiian, but not the Malaysian, strains that grouped into another clade (clade B) sister to clade A. This antibody did not react with any other *Dickeya* sp. except for one strain isolated from Hawaiian irrigation water, which clusters with *D. dadantii*. The second specificity (Clone #2D11B8G10, IgG_1) reacted with all strains in clade A and B in addition to a sister clade of weakly virulent pineapple strains and several other *Dickeya* species. Antigen selection based on phylogenetic data facilitated generation and screening of hybridoma cell lines to obtain MAbs of desired specificities that should prove useful in epidemiological studies.

Genetic diversity of _Enterobacter cloacae_

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Phytopathology 99:S101

Enterobacter bulb rot, caused by the bacterium *Enterobacter cloacae*, results in an storage onion bulb decay. This disease is characterized by watersoaking and a discoloration of the inner scales. Numerous bacterial isolates were obtained from onion bulbs in Washington during the 2006–2008 storage seasons that exhibited rot. They were identified as facultative anaerobes that were arginine dihydrolase positive, indole negative, and unable to degrade pectin, all characteristics typical of the genus *Enterobacter.* Currently, no species-specific probes or primers are available for these taxa and it is important to confirm the identity of the causal isolates. A multilocus phylogenetic analysis of these bacterial strains is being used to genetically characterize the putative *E. cloacae* isolates from onions and to clarify their evolutionary relationships with environmental and medical isolates of *E. cloacae*. Genomic DNA was isolated from representative isolates and portions of the housekeeping genes *acnA*, *gapA*, *icdA*, *mdh*, *mtlD*, *pgi*, and *proA* were PCR amplified and sequenced. Representative GenBank sequences from other plant-pathogenic facultative anaerobes such as *Enterobacter sakazakii*, *Enterobacter agglomerans*, *Dickeya dadantii*, *Pectobacterium atrospecticum, P. carotovorum* subsp. *carotovorum*, *P. wasabiae*, *P. betavasculorum*, and *Erwinia rhapontici* were also included in the analysis.

Baseline sensitivities of isolates of _Colletotrichum acutatum_ to strobilurin (QoI) fungicides

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Phytopathology 99:S101

Anthracnose fruit rot, caused by *Colletotrichum acutatum*, is one of the most important diseases of strawberry in Florida and worldwide. Baseline sensitivities of *C. acutatum* populations to azoxystrobin and pyraclostrobin were developed using 21 isolates collected from 1994 to 1999, before the

registration of these fungicides. Fifty two isolates collected afterwards, from 2001 to 2008, were evaluated to determine if *C. acutatum* populations have lost sensitivity to those fungicides. All isolates were tested using the spiral gradient dilution (SGD) method. The average EC_{50} value for azoxystrobin for isolates collected from 1994 to 1999 was 0.30 µg/ml and for isolates collected since registration was 0.13 µg/ml. The EC_{50} values for pyraclostrobin were 0.014 µg/ml for the years before registration and 0.011 µg/ml for the years since the product was registered. No isolate was recovered with a sensitivity level differing widely from the range determined before these products were utilized. Thus, there is no indication that highly resistant isolates have developed nor that the sensitivity of isolates of these pathogens to the strobilurin fungicides has been increasing over time. Due to the potential for development of resistance pathogens, sensitivity levels need to be monitored periodically.

Micro and macrospatial distribution of the genetic diversity of *Teratosphaeria* (*Mycosphaerella*) *nubilosa* on *Eucalyptus nitens* in South Africa

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Phytopathology 99:S102

The genetic diversity of the important *Eucalyptus nitens* leaf pathogen *Teratosphaeria nubilosa* was studied at different spatial levels. These included diversity within individual and coalescing lesions, within and among leaves on a single tree and within and among plantations located in the main *Eucalyptus* growing areas of South Africa. A total of 823 *T. nubilosa* isolates were analyzed using five microsatellite loci. Discrete lesions contained only a single genotype, confirming the homothallic nature of this fungus since pseudothecia are formed on these lesions. As many as seven genotypes were observed in coalesced lesions and despite meticulous sampling of coalesced areas, there was no evidence for outcrossing amongst genotypes. The gene and genotypic diversity was as high for isolates from a single tree as between trees in plantations. There was no evidence of population differentiation among populations located in the main *Eucalyptus* growing areas in South Africa, suggesting that both the continuity in the geographical distribution of the host as well as wind dispersal of ascospores are important in structuring the genetic diversity of *T. nubilosa* at macrospatial scales.

Native Myrtaceae and introduced *Eucalyptus* sharing Botryosphaeriaceae species in Uruguay

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The expanding areas planted to exotic *Eucalyptus* in Uruguay provide new opportunities for biological exchanges among pathogens of exotic and introduced trees. In this regard, Uruguay has a wide variety of native tree species residing in the Myrtaceae and thus related to *Eucalyptus*, which could facilitate such host shifts. The aim of this study was, therefore, to identify Botryosphaeriaceae infecting native and introduced Myrtaceae and to test the pathogenicity of isolates on *Eucalyptus grandis*. Symptomatic and asymptomatic material was collected countrywide from *Eucalyptus* spp. and native Myrtaceae. Single spore cultures were identified based on conidial morphology and comparisons of DNA sequences. Results revealed a strong relationship between Botryosphaeriaceae infecting *Eucalyptus* and native trees. *Botryosphaeria dothidea*, *Neofusicoccum eucalyptorum* and isolates identified in the *N. parvum-N. ribis* complex were found on both native and introduced Myrtaceae. *Lasiodiplodia pseudotheobromae* was found only on *Myrcianthes pungens* and two novel species, *Diplodia* sp.1 and *Spencermartinsia* sp.1, were found only on native trees. Pathogenicity tests showed that isolates of *L. pseudotheobromae*, *N. eucalyptorum* and *N. parvum-N. ribis* complex obtained from native trees are pathogenic to *E. grandis*. These findings illustrate the relevance of surveying native forest trees for early detection of potential threats to *Eucalyptus* plantations.

Avocado, banana, carambola and mango are hosts of members of the sooty blotch and flyspeck complex

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The sooty blotch and flyspeck (SBFS) complex is comprised of ascomycetes that blemish pome fruits in temperate regions of the world. SBFS fungi colonize the epiculticular waxes on host surfaces and produce generally similar smoky grey to minutely stippled signs. At least 60 species have been reported as SBFS agents worldwide. We examined symptomatic fruit of four tropical fruit crops that are produced commercially in South Florida: avocado (A), *Persea americana*; banana (B), *Musa* spp.; carambola (C), *Averrhoa carambola*; and mango (M), *Mangifera indica*. Phenotypically diverse fungi were recovered on artificial media and their morphological characters and rDNA regions (ITS and LSU) were compared with previously described members of the SBFS complex. Isolates distantly or closely related to previously identified SBFS species included (ITS bp homologies): *Peltaster* sp. (291/376bp) (C), *Schizothyrium pomi* (B and C) (510/520bp), and *Stomiopeltis* sp. (A, B and M) (380/450bp). In contrast, several taxa had not been associated previously with the SBFS complex: *Melanopsamma* sp. (481/590bp) (C), *Acremonium implicatum* (489/550bp) (M), *Guignardia mangiferae* (607/607bp) (A, C and M), and *Cyphellophora* sp. (576/598bp) (C). Koch's postulates are currently being conducted with these fungi. Other than *Peltaster* sp. on carambola, these are new national or global records for these host plants.

Suppression of Cylindrocladium black rot of peanut with Proline in-furrow and foliar sprays of Provost

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Two field trials were conducted each year to measure suppression of Cylindrocladium black rot (CBR) with foliar sprays of Provost 433SC (prothioconazole 0.085 kg a.i./ha + tebuconazole 0.169 kg a.i./ha) and Proline 480SC (prothioconazole 0.2 kg/ha) in-furrow in 2006, 2007 and 2008. Treatments were: 1) Echo 720 1.26 kg a.i./ha, 2) Provost sprays, 3) Proline in-furrow + Provost sprays, and 4) metam 32 kg a.i./ha prior to planting + Proline in-furrow + Provost sprays. Proline was applied in a volume of 47 liter/ha. Echo and Provost were applied 3 or 4 times in 140 liter/ha. Metam was applied 25 cm under rows with a chisel shank. Plots were four, 10.6 m rows spaced 0.9 m apart. Treatments were randomized in four complete blocks. Stand and leaf spot control were excellent in all trials. CBR was not suppressed significantly by Provost, but Proline + Provost reduced CBR incidence significantly (44% in low and 31% in high CBR pressure). Metam + Proline in-furrow + Provost reduced CBR incidence more than any treatment (76% in low and 69% in high CBR pressure). Yields for Echo, Provost, Proline + Provost, and metam + Provost + Proline averaged 5649, 6029, 6565, and 6831 kg/ha in low and 1657, 2172, 2529, and 3794 kg/ha in high CBR pressure, respectively. Yields for Provost were increased significantly by Proline in low but not high CBR pressure. Metam + Proline + Provost increased yields significantly above that of Proline + Provost in high CBR but not low CBR pressure.

A National Plant Disease Recovery Plan for laurel wilt of avocado

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The National Plant Disease Recovery System (NPDRS), initiated after 9-11, ensures that the tools, infrastructure, communication networks and capacity that are needed to mitigate high-impact diseases are understood and available. Its ultimate goal is to enable reasonable, ongoing levels of production of susceptible crops. We describe a NPDRS recovery plan for laurel wilt of avocado. It provides backgrounds on the disease, the newly described fungal agent, *Raffaelea lauricola*, the exotic ambrosia beetle vector, *Xyleborus glabratus*, and similar scolytid-vectored diseases. Standard Operating Procedures (SOPs) that were compiled by the National Plant Diagnostic Network (NPDN) to facilitate diagnosis of the disease, vector and pathogen, are referenced in the plan, and are available for use by interested/responsible laboratories and agencies (www.npdn.org). Critical recovery components that are recognized in the plan include: i) the identification of disease-resistant genotypes; ii) the development of safe, effective and economical control

measures; iii) a more complete understanding of vector and pathogen biology and behavior; and iv) additional information on the insect and fungus host ranges and the disease's epidemiology. State and federal extension and education efforts are outlined to increase stakeholder and policymaker awareness.

Efficacy of plant oils on Cytospora canker in stone fruits in Colorado
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Preliminary studies evaluated topical application of six plant oils for efficacy against Cytospora canker of stone fruit in western Colorado. Neem, camphor, thyme, clove, and cinnamon oil were used at 1% v/v; mustard oil was used at 25% v/v. Treatment solutions were prepared in water and in alcohol separately w/5% white latex paint as a carrier liquid and applied to drip via a hand-pump sprayer to existing Cytospora cankers on June Pride and Cresthaven peach and Bing sweet cherry. Water only and alcohol only were applied as controls. Initial disease incidence and severity (as reflected in the gum exudation and canker extent), assessed just before treatment application in Feb 2008, were greater in sweet cherry than in peach. Disease symptom expression (gum exudation) and canker growth were evaluated and measured again in Dec 2008. Mustard, cinnamon, and clove oil consistently reduced or eliminated gum exudation and halted canker expansion in size in peach but not in sweet cherry. Possible explanations include a difference in the causal fungi between peach and sweet cherry and a reduction in treatment efficacy due to the severity of initial canker infection (higher in cherry than peach). Further studies will repeat these studies and also look at the causal fungi for differences in susceptibility to these plant oils. Identification of effective management options would benefit both organic and conventional stone fruit growers.

Fairy ring disease of cranberry: New developments and characterization of the causal agent
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Fairy ring is a disease affecting cultivated cranberries in New Jersey and Massachusetts. The disease causes vine dieback resulting in yield loss and shortens the productive lifespan of cranberry beds, which normally endure over 50 years. The disease spreads across cranberry beds as advancing 'rings' that grow for several years. Although the causal agent is reported as *Psilocybe agraiella*, this is likely incorrect. In late 2008, vines with dark lesions collected from a ring perimeter were found to be covered with 'infection pads'. This observation was consistent for 22 active fairy rings. Further examination revealed fungal strands growing from the infection pads on the root and stolon tissues. A fungus was isolated and tentatively identified as a *Helicobasdium* sp., based on DNA sequence analysis. Signs of the pathogen were found on stolons up to 15 cm below the soil surface. This helps explain why control with fungicides is problematic and likely due to limited soil penetration of the active ingredient. Fungicide trials over the past two years have shown increased efficacy in applications with higher water volume. An alternative method of control may be through the use of debilitating (i.e. hypovirulent) fungal viruses. Some species of *Helicobasidium* are known to contain such viruses. In a preliminary screen of cranberry isolates, we identified a double-stranded RNA virus. If successful, this method of biocontrol could provide effective long-term management.

Evaluation of inoculation methods to assay wheat for resistance to Fusarium crown rot
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Crown rot is a major biotic constraint on rainfed wheat production systems throughout the world and in the Pacific Northwest (PNW) of the U.S. Caused by a complex of *Fusarium* species, of which *F. pseudograminearum* and *F. culmorum* are the most important, crown rot reduces wheat yields by an average of 9% in the PNW. Many groups have attempted to develop a genetic map and identify QTLs for crown rot resistance. However, adequate *Fusarium* screening systems must be established to appropriately phenotype the population for accurate QTL identification. The objective of this research was to find the inoculation method with the greatest consistency and least variation. Methods of inoculation were to 1.) grow *Fusarium* on millet seed which was placed near the germinated seedling; 2.) soak germinated seedlings in a liquid conidial suspension (10^6 conidia per ml), reported as the 'Nicol method'; 3.) place a 10 µl droplet of a liquid conidial suspension (10^6 conidia per ml) in water or methylcellulose on the stem base (10 days post-germination), reported as the 'Mitter method'; or 4.) place an agar-based suspension of conidia (10^6 conidia per ml) in short 4- cm drinking straws at the base of the stem (10 days post-germination). The millet seed placement and the conidial-agar straw inoculation methods resulted in the most consistent virulence, differentiation between resistant ('2-49') and susceptible ('Seri') varieties, and the least amount of variation.

Optimization of Real Time Quantitative PCR (Q-PCR) for *Fusarium pseudograminearum* and *F. culmorum* on wheat
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Fusarium crown rot of wheat is caused by a complex of *Fusarium* species, of which *F. pseudograminearum* and *F. culmorum* are the most important. Crown rot reduces wheat yields by an average of 9% in the Pacific Northwest. Traditional methods of species identification have included morphological characteristics of macroconidia. With the advent of Q-PCR techniques and the development of primers for *F. pseudograminearum* and *F. culmorum,* the potential exists for more accurate species identification and fungal DNA quantification from infected wheat stems. Primers developed in previous studies were evaluated for use in Q-PCR and DNA extraction kits were tested and optimized to accurately assess *Fusarium* species and DNA concentrations in wheat stem tissue. The 'OPT' primers (Shilling et al. 1996) for the amplification of *F. culmorum* and the 'FPG' primers (Williams et al. 2002) for the amplification of *F. pseudograminearum* yielded the most consistent results. The MO-BIO© Ultra Clean Soil Kit for DNA extraction produced the most consistent Q-PCR amplification from infected wheat tissue. The most optimal results were obtained by grinding with liquid nitrogen and soaking prior to bead beating (using a ceramic bead (MP Biomedicals© - FastDNA extraction kit)) and a Fast Prep speed of 5 for 45s. Addition of polyvinylpolypyrrolidone (PVPP) was necessary for adequate DNA extraction.

Systemic spread of *Beet yellows virus* following aphid inoculation
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Beet yellows virus (BYV) is an aphid-transmitted, phloem-associated closterovirus. We used *Myzus persicae* aphids to study early events following aphid inoculation and preceding BYV systemic spread in *Nicotiana benthamiana* plants. A genetically modified, GFP-tagged virus was transmitted by green peach aphids into *N. benthamiana* leaves which were allowed to initiate BYV replication and then mechanically detached at various time-points, from 15 hours to 7 days. Systemic spread of the GFP-tagged BYV was then observed in upper, non-inoculated *N. benthamiana* leaves, as well as in the detached inoculated leaves kept on a wet filter paper in a Petri dish. Surprisingly, initiation of systemic spread of BYV following aphid inoculation occurred quite fast – between 30 and 45 hours post-inoculation (PI). Systemic spread could be easily observed in upper, non-inoculated leaves of *N. benthamiana*, and also in the detached, inoculated leaves by 7 days PI, but only if inoculated leaves were detached 45 or more hours PI. In contrast to aphid inoculation, mechanically inoculated plants rarely became systemically infected, and this could only be observed around 40–50 days PI. In mechanically inoculated *N. benthamiana* plants, BYV infection was usually confined to a small number of mesophyll cells and was not found associated with vascular tissues. These data suggest that aphids deliver BYV to specialized leaf cells where it can replicate, and quickly, within 30–45 hours, initiate systemic spread through the vascular system.

Crop hosts of soybean cyst nematode in the northern Great Plains
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Sixty two cultivars of twelve crops grown in the northern Great Plains were evaluated for suitability as hosts of the soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) (HG type 0) using soybean Lee-74 as the susceptible host. 'Conetainers' with autoclaved sand were infested with 2,000 eggs placed into a 2 cm × 1 cm hole and then a 3 day-old germinated seed was placed in the hole. 'Conetainers' were placed in sand in plastic pots immersed in a water bath at 27 degrees C in the greenhouse. Plants were harvested at 30

to 40 days, and females were extracted and counted. Canola, clover, lentil, and sunflower were non-hosts, while field pea, nyjer, camelina, cuphea, and safflower, were poor hosts for SCN with only a few females per plant. Borage, crambe and lupines were moderately good hosts of SCN. Purple borage averaged 5% of the number of females on Lee-74 while white borage had only a few females per plant. The five cultivars of crambe and lupines averaged 4 to 8%, and 42 to 57%, respectively, of the number of females on Lee-74. This is the first report of reproduction of SCN on crambe, cuphea and nyjer.

Root-expressed *Carica papaya* genes regulated by *Phytophthora palmivora*: A promising new system for comparative genomics of *Phytophthora*-plant interaction

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Phytophthora species cause devastating diseases to important crops world-wide. The fruit tree, *Carica papaya*, has relatively fewer disease resistance gene homologs vs. other sequenced angiosperms, is highly susceptible to the broad-host-range pathogen *P. palmivora*, and can serve as a new model system for comparative genomics of compatible *Phytophthora*-plant interactions. In this study, the expression of genes isolated from *C. papaya* seedling roots (cultivar SunUp) inoculated with *P. palmivora* were evaluated for pathogen regulation. An open reading frame (ORF) encoding a predicted ascorbate peroxidase was found to be upregulated in leaves but not in roots while another peroxidase ORF was downregulated in roots. Genes predicted to encode a β-1,3-glucanase and ferulate 5-hydroxylase (F5H) were upregulated in roots, and an ORF encoding a hypersensitive-induced response protein was induced by *P. palmivora* in both roots and leaves. Finally, an ORF predicted to encode an aquaporin with normally high root expression was down regulated following inoculation. Although many host genes regulated during *Phytophthora* infection are associated with a generalized response to microbe associated molecular patterns (MAMP), others are required for pathogenicity. Expression patterns revealed in this study can be used to identify host genes regulated by *Phytophthora* for pathogenicity. In addition, the pathogen up-regulated genes provide a source of promoters for engineering novel transgenic resistance.

Efficacy of phosphorous acid in managing Aphanomyces root rot on processing peas

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Phytopathology 99:S104

Aphanomyces root rot (ARR) caused by *Aphanomyces euteiches* f. sp. *pisi* (*Aep*) is a major disease of fresh and dry pea throughout the world. The absence of genetic resistance and effective chemical control has made managing this disease challenging. Strategies to reduce or prevent ARR, and improve yield were investigated. The efficacy of phosphorous (PA) and phosphoric (PC) acids to manage ARR on green pea was tested in five field and four greenhouse studies at two locations in Washington from 2003 to 2008. Plants receiving in-furrow + foliar or two foliar applications of PA in field trials had significantly lower ($P \leq 0.05$) root disease severity than the non-treated controls (NTC) in 2 of 3 trials from 2003 to 2005 and, 3 of 5 trials from 2003 to 2006, respectively. Green pea yield for plants receiving two foliar applications of PA was either numerically or significantly higher ($P \leq 0.05$) than the NTC in all of the five field trials. When field applications of PA (phosphite) or PC (phosphate) were adjusted to deliver equivalent amounts of elemental phosphorus, results indicated PA was the only active material that reduced ARR. A single foliar application of PA at 0.90 kg/ha a.i. at plant emergence was as effective as two foliar applications at the same rate applied at emergence and one week later, in reducing ARR in two greenhouse tests. Based on this research PA was determined to be an effective chemical management tool to reduce root rot severity due to ARR.

Relative susceptibility of quince, pear, and apple cultivars to fire blight following greenhouse inoculation

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Fire blight caused by *Erwinia amylovora* (*EA*) is one of the most serious diseases of plants in the family *Rosaceae*, and Quince (*Cydonia oblonga* Mill.) is considered one of the most susceptible host genera. Apple (*Malus* sp.) and pear (*Pyrus* sp.) cultivars ranging from most susceptible to most resistant have been identified and are used as international standards. The response of six quince cultivars to a virulent isolate of *EA* was compared to that of four standard apple and seven pear cultivars. Young leaves of actively growing, recently grafted trees (6 trees/cultivar) were inoculated by cutting with scissors dipped in a suspension of *EA* strain Ea153 at 1×10^9 CFU/ml and maintained in a greenhouse. Assays were conducted in May 2008, and repeated in September. Length of fire blight lesions after 3 weeks ranged from 0 to 24 cm. 'Jonathan' apple and 'Forelle' pear developed the longest lesions. Apples 'G-41' and 'Robusta 5' and pear 'Old Home' were the most resistant with either no disease symptoms or lesions < 1.0 cm. No significant difference in lesion length was found between quince cultivars 'Aromatnaya', 'Limon', 'Quince A', 'Smyrna' and 'Van Deman'. Disease severity in quince was comparable to standard apple and pear cultivars considered intermediate in susceptibility. Apple and pear standards responded as expected to inoculation with *EA* and each of the quince cultivars was more resistant to fire blight than the most susceptible apple or pear clones.

Taxonomy of *Rathayibacter* species on cereals

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Rathayibacter tritici (Rt) and *R. iranicus* (Ri) cause a gumming disease of wheat. Since only a single strain of Ri has been described, no taxonomic study of Ri has been conducted. Recently a large number of *Rathayibacter* strains were identified from wheat seeds in Turkey (Postnikova et al., Plant Pathology, In press). The genotypic relatedness among strains of Rt, Ri, and other *Rathayibacter* species on grasses and cereals was determined. The 16S rDNA sequencing not only confirmed the identification as Ri, but also revealed a possible new *Rathayibacter* sp. (R.sp.). DNA/DNA reassociation analysis showed 86% similarity among Ri strains and 49.3% between Ri and R.sp strains. AFLP analysis showed the Ri taxon had a nearly identical similarity (88%) to ICPB 70005^T; (FH-6^T; CI 148^T), type strain of Ri with 60% similarity to R.sp. taxon. Thus, Ri and R.sp. were very different from Rt by DNA/DNA reassociation (less than 20% related) and AFLP (less than 45% related).

An improved method of DNA extraction from *Diaphorina citri* for HLB detection

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Phytopathology 99:S104

Huanglongbing (HLB) is a devastating disease of citrus that is transmitted by two citrus psyllids. *Diaphorina citri* transmits *Candidatus* Liberibacter asiaticus (Las) and *Ca.* L. americanus (Lam), and *Trioza erytreae* transmits *Ca.* L. africanus (Laf). *Ca*. Liberibacter species can be detected in DNA extracted from infected plants and psyllids by polymerase chain reaction (PCR). However, an efficient method for DNA extraction and PCR detection of HLB from individual psyllids is not available. We describe a high throughput DNA extraction for use with individual psyllids. The method utilizes single steel bead, 2–3 mm in diameter (Biospec Products) in each well of a ninety-six well racked collection microtube plate (QIAGEN) along with a single psyllid and 200 µl of DNazol direct (MRC). Plates are shaken at 1,800 rpm on a TissueLyser (QIAGEN) two times for 90 s, followed by a short centrifugation at 3,000 rpm. The supernatant can be used immediately as a template for PCR. DNA can also be extracted from batched psyllids by varying the number of beads and volume of DNAzol Direct. Time to extract DNA from 200 psyllids is less than two hours.

Induced expression of pathogenesis-related protein genes in soybean is associated with *avrXg1* in *Xanthomonas axonopodis* pv. *glycines* Race 3

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Xanthomonas axonopodis pv. *glycines* Race 3 elicits a hypersensitive response (HR) on specific pustule-resistant cultivar. In this study, we demonstrate that strain KU-P-SW005 Race 3, carrying *avrXg1,* induces expression of defense-associated genes in soybean responses in soybean. Expression of genes involved with the production of defense related-enzymes and pathogenesis-related (PR) proteins during HR were identified. The proteome of 15-day old resistant soybean cv. Williams82 inoculated with KU-P-SW005 expressed defense related proteins including catalase, lipoxygenase-4 (LOX-4), and phenylalanine ammonia-lyase. Williams82 also showed enhanced expression of PR-2, PR-4, PR-6, PR-10, and LOX genes for HR induction following inoculation with KU-P-SW005. These protein genes have been categorized

into two groups, PR-10 is predominantly limited to cells proximal to the point of treatment, whereas genes for the elicitor-releasing endoglucanase (PR-2), a WIN-like protein (PR-4), and a Kunitz trypsin inhibitor (PR-6) are seen in proximal, near-proximal, and distal cells. Furthermore, KU-P-SW005 also enhanced the speed and magnitude of expression of phenolics and β-1,3-glucanase within 24 h and reached maximum at 48 h after inoculation. Methyl jasmonate induced strong gene expression for PR-6 and the ethylene precursor, and jasmonic acid (JA) enhanced β-1,3-glucanase and PR-4 were connected. The results are discussed in the context of the recently observed Race 3, AvrXg1 elicitor and JA-induced systemic defense potentiating in soybean.

The effects of water on virus titer growth of *Wheat streak mosaic virus* in hard red winter wheat

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Phytopathology 99:S105

Wheat streak mosaic virus is one of the most common wheat viruses found in North Texas. The majority of wheat grown in North Texas is irrigated and it has been noted that the incidence and severity of wheat streak mosaic appear to be more severe in years of extreme drought. Greenhouse studies were conducted to determine if water had an effect on virus and disease development. Plants were grown in three different water regimes of .32, .84, and 2.77 bar. After inoculation with WSMV, plants were grown for approximately 4 weeks. During this period, clippings were taken from the second to bottom leaf every 5–6 days for virus titer analysis, using relative quantification Real-time PCR. Disease severity evaluations were also made during collections, and root weights were evaluated after harvest. Significant increases were found in virus titer at consecutive collection dates ($P < .0001$), and a positive correlation was found with increasing virus titer and severity of disease ($P < .0001$) However, no significant difference in disease severity or virus titer was found among the different water treatments at any collection date. These results indicate that the amount of water does not have a direct effect on WSMV titer in infected plants or disease severity, and that the high incidence and severity of wheat streak mosaic often observed during drought years likely is due to environmental conditions that favor vector reproduction or spread.

Great Plains wheat virus survey 2008

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Wheat viral diseases are an important factor affecting wheat production throughout the western Great Plains. A nine-state regional wheat virus survey was conducted by diagnosticians within the Great Plains Diagnostic Network (GPDN) to determine incidence and distribution of a number of wheat viruses, in addition to time of infection and disease symptoms. Wheat samples were submitted to diagnostic labs in CO, KS, MT, ND, NE, OK, SD, TX, and WY throughout the 2008 season, and samples were tested by ELISA, using the appropriate antibody. Viruses identified included *Wheat streak mosaic virus* (WSMV), *Wheat mosaic virus* (WMoV), *Triticum mosaic virus* (TriMV), *Barley yellow dwarf virus*-PAV (BYDV-PAV), and *Cereal yellow dwarf virus*-RPV (CYDV-RPV). Additional data collected for each sample included location and date of collection, and symptom type and severity. A total of 754 samples were processed for disease identification. WSMV was detected in all states at high percentages (27–83%). WMoV was also detected in all states, with first reports in MT and WY. TriMV was identified in CO, KS, NE, OK, SD, TX, and WY. Incidence of BYDV-PAV and CYDV-RPV was low. A high incidence of double infection by WSMV/TriMV and WSMV/WMoV were found, particularly in KS, NE, OK, and TX.

Epiphytic bacteria and yeasts on apple blossoms and their potential as antagonists of *Erwinia amylovora*

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Apple blossoms were sampled for indigenous epiphytic populations of culturable microorganisms during different stages of bloom at two locations in central Washington State and one site in Corvallis, Oregon. Frequencies and population sizes of bacteria on stigmas of apple were lower in Washington than at Corvallis, where average relative humidity was higher and possibly favored greater colonization; however, bacteria at Corvallis were mainly pseudomonads, whereas those in Washington were diverse, composed of several genera. In Washington, yeast as well as bacteria were isolated from both stigmatic and hypanthial surfaces. Sampled blossoms were processed immediately to assess microbial populations, or after a 24-h incubation at 28°C and high relative humidity, which broadened the range of detectable taxa evaluated as potential antagonists. Identifications were based on FAME and rDNA-sequence analyses. Yeasts or yeast-like organisms were detected at frequencies similar to or greater than bacteria, particularly in hypanthia. When microbial isolates were tested for their capacity to suppress *E. amylovora* on stigmas of detached crab apple flowers, many were ineffective. The best antagonists were bacteria *Pantoea agglomerans* and *Pseudomonas* spp. and a few yeasts identified as *Cryptococcus* spp. Further evaluation of these taxa on flowers could lead to the discovery of additional biocontrol agents for fire blight.

Taxonomy of powdery mildews on *Rhododendron* spp. in the Pacific Northwest

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Powdery mildew is increasingly problematic on cultivated evergreen and deciduous *Rhododendron* spp. and cultivars in the Pacific Northwest. The causal agent has never been fully characterized. This study was initiated to determine the powdery mildew species occurring on *Rhododendron* species and cultivars in the region by characterizing morphological features and ITS sequences. Collections of powdery mildew-infected foliage from urban botanical gardens and residential gardens in Oregon, Washington, and British Columbia were studied. Symptoms were variable, ranging from almost absent to purple or yellow discoloration. Mycelial mats occurred on adaxial and/or abaxial leaf surfaces. Anamorphic states in all collections were morphologically similar, forming conidia singly on conidiophores with kinked foot cells. Differences in teleomorph morphology and ITS sequences suggested that two distinct species occur. Chasmothecial appendages in some collections were numerous, relatively short and straight, and dichotomously branched at the apices. A second species, with longer, flexuous, and unbranched chasmothecial appendages also was found. Morphological analysis of the pathogens suggests that the fungus with short, straight chasmothecial appendages is *Erysiphe azaleae* and the one forming flexuous chasmothecial appendages is *Erysiphe vaccinii*.

Effect of production media on *Sclerotinia sclerotiorum* inoculum fitness

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A laboratory study was conducted to evaluate the fitness of *Sclerotinia sclerotiorum* sclerotia produced in different substrates. Fitness was measured as the ability of sclerotia to produce apothecia (carpogenic germination, CG), and the ability of ascospores to germinate and produce oxalic acid (OA) in artificial medium. Substrates used to produce sclerotia were sunflower, canola, and dry bean plants, and sand-corn-meal medium, potato dextrose agar, and bean juice agar. CG was estimated after three weeks of incubation at 18°C and 16 hr light/8 hr dark. Ascospore germination was assessed after 24 hours of incubation at 21°C. OA production, expressed as percentage of colonies that changed the color of the medium they grew on, was measured after 48 hours of incubation at 21°C in dark. In general, plant-produced sclerotia had higher ($P = 0.002$) CG and more vital ascospores than media-produced sclerotia. Sunflower was the best substrate with 45% CG, 97% ascospores germination, and a similar percentage of ascospores changing the color of the medium. Dry bean and canola-produced sclerotia had <10% CG, and <20% ascospore germination, although their ascospores cleared the medium at rates similar to that of sunflower produced sclerotia. No differences in CG were detected among media, but in general, their ascospores were six times less effective in changing color of the medium than plant-produced sclerotia.

An antiviral metabolite from a potential biocontrol actinomyces strain V-15

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Actinomyces strain V-15 was isolated from soil of great wall in the suburb of Beijing, China. According to the morphological, cultural, physiological and

biochemical characteristics, strain V-15 was identified as a *Streptomyces* sp. Use the method of half-leaf and inoculated with both virus and the fermentation broth of V-15 to check the ability of antivirus, it presented a stable and strong inhibitory activity against the plant viruses such as Cucumber Mosaic Virus (CMV) and Tobacco Mosaic Virus (TMV) and the local lesion on tobacco leaves reduced average 23% to 76%; the potted biological test in greenhouse showed the symptom on tomato was markedly suppressed by the fermentation broth of V-15, and the control efficacy was similar compare with those of antiviral products. The phenylalanine aminolyase (PAL), peroxidase (POD), superoxide dismutase (SOD) and polyphenoloxidas (PPO) were tested their activity by using the tomato leaves and treated with fermented broth of V-15 after inoculated by virus. Results showed that enzyme activation of tomato leaves were all improved obviously after being treated with fermented broth of V-15 and all of fore enzymes increased their activities. Also the peak of enzyme increasing appears in different treated time from 20 h to 144 h and PAL, PPO found more important to reduce the viral symptom. The present study revealed a new producing strain to antivirus and potential application as a biological control agent for plant viral diseases.

Roles of rhizoxin and 2,4-diacetylphloroglucinol in suppression of *Fusarium* spp. by the rhizobacterium *Pseudomonas fluorescens* Pf-5

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Pseudomonas fluorescens strain Pf-5 is a rhizosphere bacterium that acts as a biocontrol agent of soilborne plant diseases and produces at least 10 different secondary metabolites including several with antifungal properties. We derived site-directed mutants of Pf-5 with single and multiple mutations in the biosynthetic gene clusters for the antifungal metabolites 2,4-diacetylphoroglucinol (2,4-DAPG), pyrrolnitrin, pyoluteorin, hydrogen cyanide and rhizoxin. These mutants were tested for suppression of the pathogens *Fusarium verticillioides* and *Fusarium oxysporum* f. sp. *pisi* on several culture media. Rhizoxin and 2,4-DAPG were found to be primarily responsible for fungal antagonism by Pf-5 Previously, other workers showed that the mycotoxin fusaric acid (FA), which is produced by many *Fusarium* species including *F. verticillioides*, inhibited the production of 2,4-DAPG by *Pseudomonas* spp. In this study, amendment of the culture medium with FA decreased 2,4-DAPG production of Pf-5 and reduced transcript levels of 2,4-DAPG biosynthetic genes, assessed using RT-qPCR. Therefore, the mycotoxin influenced antibiotic production by Pf-5 at the transcriptional level. Our results demonstrated the importance of two compounds, rhizoxin and 2,4-DAPG, in suppression of *Fusarium* spp. by Pf-5, and confirmed that an inter-species signaling system mediated by FA influenced 2,4-DAPG production by the bacterial biological control organism.

Comparison of molecular and mycelium assay for determining benzimidazole resistance in field populations of *Venturia inaequalis* in Indiana

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Phytopathology 99:S106

Apple scab, caused by the fungus *Venturia inaequalis*, is the most destructive disease on apples in the Midwest and is controlled primarily by fungicide application. Fungicide resistance has become a problem in orchards. Knowing if resistance is present is important for disease management, as it allows for prompt modifications to spray programs to exclude or tank-mix the reduced efficacy fungicide. *V. inaequalis* isolates were collected from orchards throughout Indiana and screened through mycelium assays, a process that takes 4 weeks, for resistance to Topsin M® (thiophanate-methyl). Isolates were found to be either resistant (91%) or susceptible (9%). DNA was extracted from pure cultures and the target β-tubulin gene was amplified and digested. The restriction enzyme *Bst*UI was used to verify a restriction fragment length polymorphism (RFLP) in codon 198 that corresponded to high fungicide resistance in other studies. We found that 71% of resistant isolates were positive for the polymorphism. Despite this shortcoming, we plan to use this approach to directly test for fungicide resistance with DNA extracted from leaf lesions; therefore, demonstrating that direct assay of the lesion can be used to screen for fungicide resistance in 1 to 2 days. However, when determining fungicide resistance, this approach should be used with caution as it only selects for the known resistant mechanisms and as we found, may under-report resistant types.

Resistance in tomato and wild relatives to *Phytophthora capsici*

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Phytophthora capsici causes root, crown, and fruit rot of tomato, a major vegetable crop grown in every country of the world. The objective of this study was to determine whether different varieties and wild relatives of tomato show resistance to *P. capsici*. Each of four *P. capsici* isolates were used to inoculate 6-week-old seedlings (1 g *P. capsici*-infested millet seed/10 g soilless medium) of 42 varieties and wild relatives of tomato in a greenhouse. Experiments were conducted three times and included a control group. Plants were evaluated for wilting and plant death. All *P. capsici* isolates tested incited disease in seedlings with significant differences observed. Interactions between isolate and variety occurred. A wild relative of cultivated tomato, *Lycopersicon hirsutum* accession LA407, showed complete resistance to all *P. capsici* isolates used. Partial resistance to all isolates was identified in the varieties Ha7998, Fla7600, Jolly Elf, and Talladega. *P. capsici* was most frequently recovered from root and stem tissue of the inoculated seedling; the phenotype of the recovered isolate matched the phenotype of the inoculum. This study is a step forward towards the development of tomato varieties for use in fields infested with *P. capsici*.

A new member of the family *Reoviridae* isolated from crumbly fruited 'Meeker' red raspberry

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Phytopathology 99:S106

A virus induced crumbly fruit disease of red raspberry has been observed in northern Washington, USA and British Columbia, Canada. DsRNA extracted from symptomatic plants in northern Washington yielded more than 12 bands on agarose gels. DsRNA bands, except those corresponding to RBDV were gel-purified and cloned for sequencing. Thus far, sequencing results showed the presence of at least two viruses in addition to RBDV. One has significant amino acid sequence identity to *Rice ragged stunt virus* (RRSV), a ten-dsRNA-segmented oryzavirus that belongs to the family *Reoviridae*. The complete sequence for the segments that correspond to RNA S1, S3, S4 and S7 of RRSV has been determined. Partial sequences of segments S2, S5, S9, and S10 are also known and are being used to generate the complete genomes using poly A tailing of the 3' ends. In addition, *Raspberry mottle virus* (RMoV), a recently characterized member of the genus *Closterovirus* in the family *Closteroviridae,* was also identified from raspberries with severe crumbly fruit. These findings along with the lack of severe crumbly fruit symptoms in 'Meeker' red raspberry singly infected with RBDV in Oregon, suggest the existence of a novel virus complex associated with severe crumbly fruit in red raspberries. The complex may involve RBDV, RMoV and/or this new reovirus. Transmission studies are underway to determine the affect of each of these viruses singly and in all combinations on crumbly fruit symptom development in 'Meeker' red raspberry.

Soybean root defense responses to *Fusarium virguliforme* infection reveals a role of defense related genes during resistance

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Phytopathology 99:S106

Sudden death syndrome of soybean is an important disease, caused by the semi-biotrophic fungus *Fusarium virguliforme*. This fungus colonizes soybean roots causing rot, and releases a phytotoxin that is translocated to leaves causing interveinal chlorosis and possible defoliation. In this study, we report on an Affymetrix analysis measuring transcript abundances in resistant and susceptible roots upon infection by *F. virguliforme*. Real time RT-PCR was used to measure fungus infection progress and to determine that the ideal time points for analysis were 5 and 7 days post infection. Analysis of root response to *F. virguliforme* infection versus mock inoculated plants, identified 1279 transcripts as being differentially expressed at an fdr adjusted p-value of <0.01. Many of up regulated genes were common between resistant and susceptible plants, including genes related to the phenylpropanoid pathway, defense, signal transduction, and transcription factors. Gene expression comparisons between this experiment and another that was deigned to study the effect of translocated phytotoxin on soybean leaves, indicated that most of the genes showed similar expression patterns in both leaves and roots, suggesting that many defense responses are shared between these two very different tissues upon *F. virguliforme* infection. The transcript levels of many genes were induced to a greater degree in the susceptible plants probably as a result of the more rapid colonization of the susceptible plants.

Real-time PCR systems aid in quantitative detection of *Colletotrichum* spp. in spatial dispersal studies of strawberry anthracnose
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Anthracnose of strawberry is an economically important disease in the Southeast US. Detection and quantification of quiescent infections aid in deciding preventive management strategies for this disease. Primers and a hybridization probe were designed on the basis of the internal transcribed spacer (ITS) region of the nuclear rRNA genes for a highly sensitive real-time PCR. R-T PCR with DNA extracts of petioles and leaves from controlled environment-grown plants that were artificially inoculated with different levels of *C. acutatum* inoculum showed a significant correlation with levels of quantification expressed by Ct values. Spatial dispersal of *C. gloeosporioides* in a strawberry nursery was reliably quantified with the newly developed protocol that showed higher precision and accuracy compared to a traditional paraquat protocol. Inoculum dispersal in the nursery showed a good fit in the inverse power law model as quantified by real time PCR. Quiescent infection of leaves at young, middle and older stages originating from inoculation with the same number of spores indicated middle aged leaves were best for assessing quiescent infection. These leaves are best used to predict the need for adopting preventative control measures. Leaves proved to be a significantly bigger reservoir of inoculum, as quiescent infections, compared to petiole or crown tissue. Overall, the real-time PCR protocol showed >100 times sensitivity compared to regular PCR or the bioassay.

Aurantioideae: Phylogeny and susceptibility to *Citrus* huanglongbing
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Phytopathology 99:S107

Citrus belongs to the sub-family Aurantioideae and family Rutaceae. Citrus is grown as a grafted plant and is sexually and graft-compatible with several related genera. The recent introduction of the Asian citrus psyllid, *Diaphorina citri*, and the bacterial pathogen, *Candidatus* Liberibacter asiaticus, the causal organism of citrus greening (huanglongbing or, HLB) into the USA have renewed interest in understanding the relationships of citrus relatives with objectives of identifying alternative hosts of HLB, understanding the sources of disease resistance and possibilities of identifying new tolerant rootstocks. In this study, we have selected 35 genera and 61 species belonging to Rutaceae and sequenced a 1 Kb fragment of a nuclear gene, malate dehydrogenase. The region of this house-keeping gene included in the analysis has both introns and exons. Introns were rich in parsimony informative characters. Sequences were aligned, partitioned and used for phylogenetic studies using PAUP and Mr. Bayes analyses. The phylogenetic information generated provides a source of SNP markers and aids in a better understanding of the relationships among the different accessions studied. Rutaceous plants collected from HLB infested areas of Florida were tested for the presence of *Candidatus* Liberibacter asiaticus by quantitative PCR of the plant samples. The information generated will be useful in formulating guidelines for preventing the spread of HLB.

Genetic analysis of a novel *Xylella fastidiosa* subspecies found in the Southwestern United States
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Phytopathology 99:S107

Xylella fastidiosa, the causal agent of Pierce's disease, is associated with leaf scorch symptoms in *Chitalpa tashkentensis*, a common ornamental landscape plant used throughout the Southwestern United States. Phylogenetic analysis of multiple loci was used to examine the *Xylella fastidiosa* infecting chitalpa strains from New Mexico, Arizona and trees imported into New Mexico nurseries. These loci were compared with previously reported *X. fastidiosa* strains. Loci analyzed included the 16S ribosome,16S-23S ribosomal intergenic spacer region, gyrase-B, simple sequence repeat sequences, *X. fastidiosa* specific sequences, and the virulence associated protein (VapD). This analysis indicates that the *X. fastidiosa* isolates associated with infected chiltalpa trees in the Southwest are a highly related group that is distinct from the four previously defined taxons *X. fastidiosa* subsp. *fastidiosa (piercei), X. fastidiosa* subsp. *multiplex*, *X. fastidiosa* subsp. *sandyi* and *X. fastidiosa* subsp. *pauca*. We propose classifying this chitalpa infecting group as a new subspecies *X. fastidiosa* supsp. *tashke.*

QTL mapping of resistance to powdery mildew in lettuce
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Erysiphe cichoracearum causes powdery mildew on most compositae including lettuce and chicory. Variation in susceptibility has been documented both in cultivated lettuce and wild relatives. Little is known about the genetic architecture of resistance to the pathogen, but monogenic resistance has been reported. We have used a set of recombinant inbred lines between *L. sativa* var. Salinas and *L. serriola* to map quantitative resistance to powdery mildew through the application of newly developed genomic and EST-SSR markers distributed along the lettuce genome. Quantitative assessment of disease development took place in the greenhouse in Salinas, CA during the winter of 2008–2009 using a 0–5 scoring scale. Disease development was faster in *L. serriola* than in *L. sativa*. Phenotypic data were normally distributed, indicative of a quantitative trait. A framework map was obtained from the Compositae genome project database and our SSR markers were assigned to known linkage groups. Resistance data were added both as disease assessment at scoring dates and as an approximation of AUDPC. A QTL for resistance was located to linkage group 2 associated with the EST-SSR marker SML22, it explains about 15% of the variation. QTLs for susceptibility were found in linkage groups 1, 4 and 8 making them interesting areas for further research.

Detection of mycotoxigenic fungi and indirect competitive ELISA for fumonisin B_1 in sorghum
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Mycotoxins are hazardous secondary metabolites produced by filamentous fungi and pose a threat to human and animal health. Fumonisin B_1 (FB1) contamination is a major concern in grain sorghum in India. One hundred and seventy sorghum samples were collected from various market yards to evaluate the incidence of mycotoxigenic fungi and fumonisin contamination across Andhra Pradesh, India. An indirect competitive enzyme-linked immunosorbent assay (ELISA) was performed to access the levels of FB1 in these samples. Among these 170 samples, 85% of the sorghum samples contaminated with FB1 0.37 µg/kg to 7892.67 µg/kg. India is one of the largest producers of sorghum grain in the world and consumed as a staple food. Thus, this study aimed to explore the menacing FB1 levels in sorghum to safeguard the public health.

Secretome analysis of dollar spot fungus *Sclerotinia homoeocarpa*
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Dollar spot, caused by *Sclerotinia homoeocarpa*, is one of the most devastating diseases of golf course turfgrass worldwide. This pathogen produces oxalic acid *in vitro* as confirmed through Liquid Chromatography Mass Spectrometry (LC-MS). Whole secretome analyses of *S. homoeocarpa* were performed using transcriptomic [Sequencing By Synthesis (SBS)], proteomic (Multi-dimensional Protein Identification Technology) and metabolomic (LC-MS) approaches to identify the differentially expressed genes, secretory proteins and metabolites, respectively. Four libraries were constructed using SBS technology. A total of 4 to 7 million mRNA signatures were sequenced from each of these four libraries. Bioinformatic analyses revealed the expression of many putative pathogen and host-specific defense related genes. Proteomic and metabolomic approaches revealed the secretion of various proteins, organic acids and other metabolites by *S. homoeocarpa*. Comprehensive analysis of the *S. homoeocarpa* secretome may lead to an enhanced understanding of the molecular mechanisms involved in host pathogenesis.

A root rot of soybean (*Glycine max*) caused by *Phytophthora sansomeana* sp nov.
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In 1990 a serious root disease of soybean in Indiana was associated with an unknown *Phytophthora* species, now named *P. sansomeana*. Plants in affected fields suffered severe yellowing and stunting after a period of unusually cool, wet weather in July. Symptoms were severe on varieties with the Rps1-k gene for race-specific resistance to *P. sojae*. Some isolates from diseased plants could not be assigned to race because of non-specific response in a set of 8 differential cultivars used for *P. sojae* race-classification, and showed certain morphological differences from *P. sojae*, but shared similarities with an unknown *Phytophthora* spp. isolated from Douglas-fir seedling roots in the

Pacific Northwest and from herbaceous weeds in New England states. Soybeans grown at 15 and 20°C in sand infested with soybean isolates of *P. sansomeana*, and subjected to periodic flooding, showed yellowing, stunting, and root disease compared with un-infested controls. Soybeans grown in naturally infested field plots developed yellowing, stunting and root disease, compared with Ridomil treated plots. In all cases *P. sansomeana* was recovered from diseased roots. Stem cankers usually characteristic of infection with *P. sojae* were not common. Soybean root disease caused by *P. sansomeana* appears to be favored by cool soil temperatures and flooding, and is not affected by race-specific resistance to *P. sojae*.

Population structure and diversity of *Eutypa lata* from Mediterranean grape-growing regions

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Eutypa lata is an ascomycete fungus causing dieback of grape (*Vitis vinifera*). We examined the genetic structure of eight vineyard collections using nine polymorphic microsatellite loci. In California, isolates were collected from four vineyards (CS1, CS2, M1 and M2) separated by distances of 50 m to 21 km. In Australia, isolates were collected from four vineyards (CS3, Sh1, Sh2 and Sh3) separated by distances of 80 to 410 km. Among the 145 isolates analyzed 134 unique multilocus haplotypes were identified based on unique combinations of alleles among the microsatellite loci. Among all vineyards, gene diversity (H = 0.56 to 0.62) and genotypic diversity (G = 0.85 to 1) were high, and there was no linkage disequilibrium among loci ($P < 0.01$). We found that >93% of genetic variance was found within vineyards ($P < 0.01$) and 3.24% of variance could be attributed to differences between continents ($P < 0.05$). Three pairwise comparisons revealed significant genetic differentiation between continents: Sh3 vs. both CS2 and M2 ($F_{ST} = 0.03$ and 0.10, respectively; $P < 0.05$), and Sh2 vs. M1 ($F_{ST} = 0.05$; $P < 0.05$). These findings, coupled with our finding of moderately low genetic differentiation between continents ($F_{ST} = 0.03$; $P < 0.05$), suggest that *E. lata* collections are part of a single panmictic population at the regional scale within continents, and gene flow is restricted between the two continents.

Perception of CLE peptides in Arabidopsis during Cyst Nematode pathogenesis

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Sedentary endoparasitic cyst nematodes induce enlarged, multinucleate feeding cells in host roots that serve as the sole nutrient source for the nematode to complete it's life cycle. The developmental changes in the host root that occur to form feeding cells require secretory molecules encoded by parasitism genes expressed within the esophageal gland cells of the nematode. *Heterodera species* produce small secreted peptide ligands sharing functional similarity with plant CLAVATA3(CLV3)/ESR (CLE) signaling peptides involved with several aspects of plant development including maintenance of stem cell pools in the root meristem. Although the role of CLE signaling pathways in the root remain to be elucidated, the receptor-like proteins (RLPs), CRN and CLV2, have been shown to mediate CLE signaling in roots. It is not yet known however how the nematode CLEs are being perceived in the roots. A screen of Arabidopsis RLP mutants for resistance to synthetic *Heterodera* CLE peptides, infection assays of peptide resistant mutants, and localization of candidate receptors in the infection site has identified several RLPs that appear to be involved in nematode CLE perception. The identification of host plant receptors of nematode CLE peptides will help to unveil the role these peptides play in feeding cell formation.

A new tea tree oil-based organic fungicide for the control of grape powdery and downy mildews

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Phytopathology 99:S108

The use of copper and sulfur for disease control is under growing pressure and increasing limitations due to their environmental concern and in grapevines due to their negative impact on wine fermentation. The new organic fungicide Timorex Gold (TG) containing 23.8% Tea Tree Oil (TTO) was found to be effective against broad spectrum of plant pathogenic fungi and was evaluated in table and winegrapes against powdery (PM) and downy (DM) mildews. Spraying TG at 0.35–0.5% as prophylactic treatment effectively controlled powdery mildew and suppressed the existed colonies of *Erysiphe necator*. Spraying at 0.5% provided an excellent prophylactic protection of downy mildew and reduced sporulation in *Plasmopara viticola*-infected tissue. Field trials conducted during 2006–09 in Israel, Chile and other countries revealed that spraying TG at application rate of 0.5% controlled powdery and downy mildews on berries and leaves, respectively, and was as effective as sulfur and systemic fungicides against powdery mildew when applied at 14-d intervals. TG was also as effective as sulfur and better than DMI fungicide in controlling PM on leaves developed at late season. TG does not harm beneficial insects and bees, has no residues and may substitute sulfur and copper products in both organic and conventional as well as an attractive tool for anti-resistance programs.

Hyperspectral remote sensing for detection of Rhizoctonia crown and root rot in sugar beet

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Rhizoctonia crown and root rot (RCRR), caused by *Rhizoctonia solani* AG-2-2, is an important disease of sugar beet in Minnesota and North Dakota. Disease severity ratings are based on subjective, visual estimates of root rot severity (0–7 scale; 0 = healthy; 7 = 100% rotted, foliage dead). Remote sensing of hyperspectral reflectance was evaluated as an objective method to assess RCRR. Field plots of sugar beet were inoculated with *R. solani* AG 2-2 IIIB at the 10-leaf stage. At 2 wk after inoculation, data were collected for 1) reflectance from the sugar beet canopy with a handheld spectroradiometer (1.2-m above canopy) and 2) visual ratings of RCRR. Additional data were collected biweekly for 6 weeks. Seven narrowband and five wideband vegetation indices (VIs) and reflectance in the green, red, and near infrared regions were calculated and correlated with visual ratings of RCRR. There was strong non-linear correlation between VIs and RCRR. VI values were constant until disease ratings reached 4 (when foliage was slightly chlorotic and 25–50% of root surface rotted) and then decreased significantly as disease severity increased. Three VIs associated with chlorophyll content had the strongest relationship with RCRR values: the wideband Optimized Soil Adjusted Vegetation Index, narrowband Pigment Specific Simple Ratio for chlorophyll *a*, and modified Spectral Ratio. Hyperspectral remote sensing can detect RCRR but not before initial appearance of foliar symptoms.

Evaluation of pattern recognition receptors for durable disease control in crops

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Plants detect conserved molecules referred to as PAMPs (pathogen-associated molecular patterns) by pattern recognition receptors (PRRs). PAMPs are essential conserved molecules that cannot be mutated or lost, and so PRRs could potentially offer durable resistance to pathogens. Arabidopsis thaliana provides an excellent model system to study PAMP-triggered immunity (PTI), and detects a variety of PAMPs from bacteria and fungi. The related LRR receptor kinases (LRR-RLKs) FLS2 and EFR are the PRRs for bacterial flagellin and EF-Tu, respectively whereas CERK1 is required for response to chitin, the main component of fungal cell walls. However, PRR responses in agricultural crops have not been characterised. Based on our proof-of-concept findings that PRRs can be transferred across plant families and confers broad spectrum resistance, we have also transformed EFR and CERK1 into wheat to test whether their function is preserved. In addition, we have initiated an investigation into PAMP-perception and PRR function in wheat, barley and Brassica. We are evaluating the range of PAMP-mediated responses in the diverse accessions of these key crop species, and testing how the environment influences their activity. Our research will enable us to evaluate whether PRRs can be developed in agriculture to develop broad spectrum disease resistance.

Potential for a bromoageliferin analogue biofilm inhibitor-dispersant to enhance control of phytopathogenic bacterial diseases

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The roles of quorum sensing (QS) and biofilm formation in survival, colonization and pathogenesis are increasingly demonstrated to be important for many phytopathogenic bacteria. Disruption of these processes could lead

to novel methods for bacterial disease management. It is known that marine natural product extracts particularly from Caribbean sponges of the family Agelasidae and the macroagla *Delisea puchra* possess antibacterial activity including anti-biofilm activity. Analogue derivatives of the marine natural product bromoageliferin were shown to possess anti-biofim activity against medically important strains of *Pseudomonas aeruginosa* (Mol. BioSyst. 2008, 4, 614). Using the microplate, crystal violet straining, spectrophotometry method, an analogue, U01 (now Agilyte™), was tested against a strain of the pepper and tomato bacterial spot pathogen, *Xanthomonas euvesicatoria*, and found to provide 86% biofim inhibition at 20 uM concentration. During the 2008 growing season, this analogue at 100 uM (35 mg/L) was evaluated in the field as a foliar spray against a copper-resistant strain of *X. euvesicatoria*. Disease control with U01 and Kocide 3000 1.4 kg/935 L/ha (1.25 lb/100 gal/acre) used individually did not differ significantly ($\alpha = 0.05$) from the non-treated check. The tank-mixture of U01 and Kocide 3000, however, provided significantly ($\alpha = 0.05$) better disease control than the non-treated check and U01 and Kocide 3000 applied alone.

Grafting tomato with inter-specific rootstock provides effective management for southern blight and root-knot nematodes

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Southern blight (*Sclerotium rolfsii*) and root-knot nematodes (*Meloidogyne* spp.) cause severe losses on tomato in the SE US. Cultivated tomato hybrids do not confer resistance to *S. rolfsii* and although root-knot nematodes (RKN) are managed with the *Mi* gene, resistance can break down. Field trials were initiated in 2007 and 2008 to determine the efficacy of inter-specific rootstocks to manage SB and RKN. In Alamance Co., 'Maxifort' and 'Beaufort' were completely resistant to SB while non- and self-grafted plants had 18–46% terminal SB incidence. Similarly, 'Beaufort', 'Maxifort' and 'Big Power' rootstocks had excellent resistance to SB in Sampson Co. Terminal SB incidence of non- and self-grafted plants was 79% and 72%, respectively, and ranged from 1–5% among the three rootstocks. 'Beaufort' and 'Maxifort' reduced the severity of RKN galling and reduced AUDPC values as compared to non- and self-grafted plants ($P < 0.01$). At first harvest, RKN soil populations among 'Beaufort' and 'Maxifort' treatments were lower than non- and self-grafted plants ($P < 0.01$). Plants grafted onto 'Big Power' maintained high resistance to RKN, and little galling was seen throughout the season. At first harvest, RKN populations in plots with 'Big Power' were <1% of those in non- and self-grafted plots ($P < 0.01$), and this was the only treatment that reduced RKN populations at terminal harvest ($P < 0.01$). Grafting with inter-specific rootstock provides an excellent conduit for rapid deployment of host resistance.

Immunodiagnosis of *Groundnut bud necrosis virus* (GBNV) using polyclonal antiserum to recombinant nucleocapsid protein of GBNV-mungbean isolate

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Thrips-transmitted tospoviruses cause significant losses to vegetable, pulse and ornamental crops worldwide. Of 19 tospoviruses reported, five are present in India. *Groundnut bud necrosis virus* (GBNV) is the most destructive of them all and is endemic in several states. Availability of good quality antiserum is crucial to accurate detection of the virus and to apply timely control measures. The production of polyclonal antiserum to nucleocapsid (N) gene of Tospoviruses is difficult since it is not easy to purify them. So an in-vitro expression strategy was used to produce polyclonal antiserum against the N gene of GBNV. The N gene of GBNV isolate on mungbean from Delhi (GenBank Accession No: AY871098), was sub-cloned in 6X Histidine tagged expression vector. Optimal expression of the protein was achieved with 1 mm IPTG. The fusion protein was found to be in the insoluble fraction of cell lysate and had a molecular mass of ~32kDa. Ni-NTA agarose resin was used to purify the recombinant protein. 500 µg protein was injected into rabbit intra-muscularly at weekly intervals for the production of polyclonal antiserum. The antiserum was able to detect presence of GBNV in various crops and locations in India in different immunodiagnostic tests. It could detect GBNV in crude sap of infected plants at dilutions of 1: 4000 and 1:8000 as well. The antisera performed well even without cross absorption.

A diversity of species of *Phytophthora* found on floriculture crops

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Phytophthora species are among the most important pathogens attacking floriculture crops in South Carolina and elsewhere. Previous research in our lab demonstrated that *P. nicotianae* is the most common species attacking these crops in South Carolina but that other species of *Phytophthora* also attack these plants. Therefore, we examined 87 isolates of *Phytophthora* spp. that had been recovered from floriculture plants and not studied previously; 83 isolates came from samples submitted to the Clemson University Plant Problem Clinic between 1996 and 2008 and four isolates came from other states. Isolates were identified based on morphological (chlamydospores, sporangia, antheridia, oogonia, oospores) and molecular (RFLP fingerprints and DNA sequences for ITS and *cox* regions) characters. We found 12 different species among the 87 isolates—including eight known species and three previously undescribed species. Of the known species, we found 31 isolates of *P. nicotianae*, 13 *P. cinnamomi*, 13 *P. palmivora*, 9 *P. drechsleri*, 6 *P. cryptogea*, 4 *P. citrophthora*, 2 *P. capsici*, 2 *P. citricola*, 1 *P. cinnamomi* var. *parvispora*, and 1 isolate of a potential *P. nicotianae* × *P. cactorum* hybrid. The undescribed species had ITS sequences that were the same or similar to those from isolates previously referred to as *P*. sp. "*niederhauserii*" (3 isolates), *P*. sp. "*kelmania*" (1 isolate), and *P*. sp. "*lagoariana*" (1 isolate). In summary, there is an interesting diversity of species of *Phytophthora* that attack floriculture crops.

Effects of foot traffic and sand topdressing on anthracnose severity of annual bluegrass putting green turf

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Phytopathology 99:S109

Sand topdressing, a common practice on putting greens, can improve surface characteristics, which enhances aesthetics and playability of the turf. Anthracnose, caused by *Colletotrichum cereale* Manns, is a disease of annual bluegrass [*Poa annua* L. f. reptans (Hausskn) T. Koyama] that is more severe on stressed turf and is thought to be enhanced by sand topdressing particularly in combination with foot traffic. A two year field study was conducted to evaluate the effect of foot traffic and sand topdressing on anthracnose severity in North Brunswick, NJ. A split-plot design with foot traffic [none and 327 footsteps m^{-2} d^{-1} (200 rounds d^{-1})] as the main factor and sand topdressing (none and 0.3 L m^{-2} wk^{-1}) as the subplot factor was used on annual bluegrass turf maintained at 3.2 mm in 2007 and 2008. Anthracnose was rated using a line-intersect grid counting method. Surprisingly, foot traffic reduced anthracnose severity as much as 27% during both years. Moreover, traffic did not enhance anthracnose severity of sand topdressed turf. Sand topdressing initially increased disease slightly in 2007, although continued applications decreased disease by August 2007 and throughout 2008. The combination of foot traffic and sand topdressing resulted in the best turf quality by the end of both seasons. Results indicate that the practice of topdressing should not be terminated when anthracnose develops on annual bluegrass putting greens.

Detection and characterization of a plant virus in wild raspberry, *Rubus idaeus* L., in Alaska

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In 2008, mosaic leaf symptoms were detected on wild raspberry plants, *Rubus idaeus* L., in north central Alaska. They were growing on remnant patches within developing agricultural sites. Partially purified virus samples were obtained by differential centrifugation of homogenized leaves according to established protocols. Protein extractions from the samples revealed a putative coat protein (CP) ~30 kDa on 10% SDS-PAGE. Virion RNA was extracted from the sample preparations and visualized on 1% non-denaturing agarose gels. Two prominent single-stranded-RNA species of ~5.9 kb and~1.9 kb were depicted. Virus samples that were mechanically inoculated to a plant host range resulted in mosaic leaf development on *Nicotiana benthamiana* L. and definite local lesions on *Chenopodium quinoa* Willd. Virus samples assayed by ELISA using Agdia kits (Elkhart, IN) for detection of *Raspberry bushy dwarf virus,* (RBDV), *Raspberry ringspot virus*, *Tobacco streak virus*, and *Tobacco ringspot virus* were all negative. Based on the sizes of the bipartite genome and CP, and susceptibility to *C. quinoa* Willd., we concluded that the virus was most similar to RBDV in the genus *Idaeovirus*. This is the first report of a virus occurring in *Rubus* sp. in Alaska. Its presence is significant since it may be a threat to domestic raspberry crops in Alaska.

Isolation and antibiotic characterization of *Erwinia amylovora* from flower samples of apples from Chihuahua, Mexico

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Streptomycin, oxytetracycline and gentamycin are the antibiotics currently used in Mexico for the control of fire blight of apple caused by *Erwinia*

amylovora. Resistance of *E. amylovora* to streptomycin was first reported in California in 1971, in Michigan in 1999, and in New York in 2002. Antibiotic resistance has not been reported in isolates of *E. amylovora* in Mexico, but farmers are concerned about the possibility of *E. amylovora* resistance to streptomycin and oxytetracycline. The purpose of this research was to determine the minimum inhibitory concentration (MIC) to confirm the possible resistance of *E. amylovora* isolates to these antibiotics. During blossom, asymptomatic flowers were monitored for *E. amylovora* using the stigma printing method in six orchards from the apple region of Chihuahua. Sixty five isolates were identified and screened for antibiotic resistance using the Kirby bauer method. All the isolates were found to be highly resistant to both oxytetracycline and streptomycin according to the recommended dose of the antibiotic products. All the isolates were resistant to oxytetracycline and 87.6% of them to streptomycin. The MIC of oxytetracycline ranged from 0.25 to 10 kg/ha of active ingredient (10–400 µg/ml) and from 0.5 to 5 kg/ha (20–300 µg/ml) of streptomycin in most of the isolates studied. This is the first report of *E. amylovora* resistance to streptomycin and oxytetracyclin in the main apple region of Chihuahua, Mexico.

Molecular and morphological characterization of *Monilinia fructicola* isolates from Mexico

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Brown rot causes important losses on peach fruit production in many countries around the world. *Monilinia fructicola*, *M. fructigena* and *M. laxa* have been reported as causal agents of brown rot in America and Europe, respectively. Wide range of symtoms and atypical cultures were observed on Monilinia isolates from peach. It is unknown if several species are involved inducing brown rot of peach in Mexico or pathogen variability is responsable of our observations. The objective of this research was to identify and characterize Monilinia isolates in terms of molecular and morphological characteristics. Fifty monoconidial isolates were obtained from symtomatic peaches collected in Michoacán, Morelos, Aguascalientes, Chihuahua and Zacatecas states. Pathogenicity was tested by inoculation of monoconidial cultures on three fruits of var. 'Diamante criollo'. There were differences of pathogenicity between strains, but not between states. Based on molecular and morphological characteristics the isolates correspond to M. fructicola. Colony morphology and rate of growth on PDA and peach-agar (PA) were evaluated at 24, 72 and 144 hours at 22°C. In average, Monilia isolates grew faster on PA (8.4 cm diameter) compared to PDA (5.01 cm) after 144 h incubation. The strains isolated in the state of Morelos, had higher growth compared to other states. Molecular characterization was based on multiplex PCR and sequenced by ITS.

Wild cucurbit species as reservoirs for *Potyviridae* in Puerto Rico

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Phytopathology 99:S110

Potyviridae is the most frequent family of viruses currently reported in cucurbits in PuertoRico. Yield losses have been substantial in watermelon, pumpkin, zucchini, melon and squash and incessant virus outbreaks have reduced the profitability for those crops. Identification of the virus reservoirs is an important step for development of an integrated pest management program. Three common species of wild cucurbits, balsampear (*Momordica charantia* L.), West Indian gherkin (*Cucumis anguria* L.) and hedgehog gourd (*Cucumis dipsaseus* Ehrenb. ex Spach) were collected in different areas of Puerto Rico. These species were usually found naturally growing near cucurbit crops and along fences, secondary roads, and irrigation channels. ELISA, immunostrip tests, RT-PCR and sequencing of a coat protein gene fragment were conducted to identify viruses infecting the wild species. Mechanical transmission to *Cucurbita moschata* 'Waltham' was conducted and expression of symptoms was observed 2 weeks after inoculation. Serologic tests and the sequence of the amplicons revealed that the three wild species were infected by at least one strain of *Zucchini yellow mosaic virus* and/or *Papaya ringspot virus*.

Validation of a single nucleotide polymorphism genotyping method for Wheat streak mosaic virus

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Phytopathology 99:S110

Wheat, one of the three most valuable crops in the United States, ranks first in crop exports. However, wheat pathogens threaten both national and international commerce, and could be deployed intentionally to impact trade streams. Attribution of such agricultural crimes will require new forensic tools that are more stringent and targeted. A method using primer elongation with fluorescent dideoxynucleotides at potential single nucleotide polymorphic (SNP) sites was developed and tested for its ability to discriminate reliably among plant pathogen strains using *Wheat streak mosaic virus* (WSMV) as a model. Consistent, distinguishable SNP fingerprints, consisting of patterns of chromatographic peaks, were obtained using three test strains of WSMV. Similar fingerprints were obtained by a second operator, using the same protocol at a different laboratory, suggesting that the assay is reproducible. The assay also showed that one field sample contained a mixture of WSMV strains, based on the numbers and positions of peaks within the fingerprint. Further validation of the protocol, including its application to additional WSMV strains, is in progress. The SNP genotyping method shows promise as a method for forensic discrimination among WSMV strains.

An agricultural biosecurity decision tool: Is it natural or intentional?

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Phytopathology 99:S110

Distinguishing between natural and intentional outbreaks of plant disease can be challenging because the time between inoculation of a plant pathogen and detection of a plant disease can be days, weeks, months, or even years. To assist law enforcement personnel in determining if an agricultural crime has been committed, a decision tool was developed using wheat streak mosaic (WSM) caused by *Wheat streak mosaic virus* as a model system. Criteria relevant to the decision, such as weather, geographic location, surrounding elements, and indicators of vector presence were identified and formulated into a table. Each criterion was given a weighted value, which is a number representing the importance of the criterion. Upon investigation of an infected field or site, the user assigns an assessment value (a number representing presence or absence) to each criterion. Together, the weighted and assessment values are used to estimate the probability that an incident was the result of an intentional inoculation. An accompanying worksheet and fact sheet also were created to facilitate application of the tool in a real-world setting. The tool is being evaluated for its usefulness in Oklahoma wheat fields in which outbreaks of WSM were either natural or resulted from intentional inoculation. The decision tool will be valuable in field investigations of potential agricultural crimes and their subsequent attribution.

Genomics tools for *Frankliniella occidentalis*, an arthropod vector for *Tomato spotted wilt virus* (TSWV)

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Phytopathology 99:S110

Thrips are members of the insect order *Thysanoptera,* and *Frankliniella occidentalis* (the western flower thrips) is the most economically-important pest within this order. *F. occidentalis* is both a direct pest of crops and an efficient vector of plant viruses, including *Tomato spotted wilt virus* (TSWV). Despite the world-wide importance of thrips in agriculture, there is little knowledge of the *F. occidentalis* genome or gene functions at this time. Our long-range goal is to use thrips genomics tools to develop biologically-based strategies that specifically control insect populations and prevent TSWV spread. To generate genomics tools to study the molecular interaction between *F. occidentalis* and TSWV, we have constructed a normalized cDNA library from larval thrips and sequenced 17,664 clones. Bioinformatics analysis of our high quality sequence data revealed 887 contigs and 11,025 singletons, a total of 11,912 expressed sequence tags (ESTs). We found that 37% of these ESTs shared significant sequence similarity with genes in the NCBI non-redundant (nr) protein database, and 26% were functionally-annotated using Blast2GO. We identified 75 and 6 ESTs with homology to proteins associated with insect innate immunity and the siRNA pathway, respectively. With these tools, we will perform gene expression studies and functional assays to identify a suite of candidate insect genes that may play important roles in thrips recognition of and response to TSWV.

An OmpA family outer membrane protein is required for both disease symptom development and sugarcane stalk colonization by *Xanthomonas albilineans*

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Phytopathology 99:S110

Xanthomonas albilineans (Xa) is a systemic, xylem-invading pathogen that causes sugarcane leaf scald. Leaf symptoms vary from a single, white, narrow,

sharply defined stripe to complete wilting and necrosis of infected leaves, leading to plant death. Xa produces the toxin albicidin that blocks chloroplast differentiation, resulting in disease symptoms. Albicidin is the only previously known pathogenicity factor in Xa, yet albicidin-deficient mutant strains are still able to efficiently colonize sugarcane. We used Tn*5* (transposome) mutagenesis in an attempt to identify additional Xa pathogenicity factors. Sugarcane cultivar CP80-1743, moderately susceptible to leaf scald, was inoculated by the decapitation method with 780 independently derived Tn*5* insertions in Florida strain XaFL07-1. Leaf scald symptoms were recorded on emerging leaves one month after inoculation, and stalk colonization by the pathogen was determined two months after inoculation. In addition to the previously identified albicidin biosynthetic gene cluster mutations, four new Tn*5* mutants were identified that produced no or very few leaf symptoms. These mutants produced albicidin *in vitro* but did not efficiently colonize sugarcane stalks. The transposon insertion site of all four mutants was found to be located in Orf XALc_0557 of the Xa genome. This gene is predicted to encode an OmpA family outer membrane protein, a previously unrecognized and apparently essential pathogenicity factor in Xa.

Diverse stem rust races found in a single field in Washington, USA
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Phytopathology 99:S111

In 2007, a spring barley field in northeastern Washington State was severely infected by stem rust and a bulk sample was collected. Preliminary testing on wheat stem rust differentials suggested that the sample consisted of many different virulence types. To further characterize the virulence diversity, we derived 83 single-pustule isolates. The isolates were race-typed in two replicates on the 20 North American stem rust differential lines and eight supplemental wheat lines, viz. Line E, Chinese Spring, LMPG-6, Little Club, Rusty, Morocco, Federation, and Gabo, most of which are considered to be widely susceptible to *Puccinia graminis* f. sp. *tritici*. Twenty-three races were identified from the 83 isolates. The supplemental lines further differentiated the isolates because isolates within each of the races frequently displayed different reactions on the supplemental lines. The isolates were genotyped with 20 SSR markers. The SSR data indicated the presence of three clonal lineages (JCBBB, QHMJC, and QCMBB) within this population. Besides these lineages, the SSR data for the remaining isolates is consistent with a sexual population. The SSR data demonstrate that this population of *P. graminis* is more diverse than virulence alone would indicate. Overall, the results demonstrate the vast diversity of stem rust races present at this location. It is likely that this population consisted of *formae speciales* in addition to *P. graminis* f. sp. *tritici*.

Stem rust resistance in *Aegilops tauschii* germplasm
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Phytopathology 99:S111

Aegilops tauschii, the D genome donor of hexaploid wheat, has been used extensively for the transfer of agronomically important traits to wheat, including stem rust resistance genes *Sr33* and *Sr45*. In order to identify potentially new stem rust resistance genes in *Ae. tauschii* germplasm, we evaluated 456 non-duplicated accessions of *Ae. tauschii* deposited in the USDA National Small Grains Collection and Wheat Genetic and Genomic Resources Center collection, with races TTKSK (Ug99), TRTTF, TTTTF, TPMKC, QFCSC, and RKQQC of *Puccinia graminis* f. sp. *tritici*. Results indicated that resistance in *Ae. tauschii* germplasm is largely race-specific. Sixty-eight accessions (15%) were identified as resistant to race TTKSK. However, only 8 of these accessions were also resistant to TRTTF, TTTTF, and RKQQ. A broad range of infection types were found in accessions resistant to TTKSK (; to 2+). Infection type data indicate the presence of novel gene(s) for resistance to TTKSK. Screening with additional races and crossing among *Ae. tauschii* accessions are needed in order to determine the allelic relationship of novel genes with *Sr45* and *Sr33*. We have begun introgressing resistance from selected accessions into wheat.

Simultaneous detection and differentiation of *Citrus tristeza virus* genotypes using a hexaplex reverse transcriptase polymerase chain reaction assay
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Phytopathology 99:S111

Simplex and hexaplex reverse transcriptase polymerase chain reaction (RT-PCR) assays were developed to detect various genotypes of *Citrus tristeza virus* (CTV). Based on symptoms, nucleotide homology and phylogenetic analysis CTV was grouped into five genotypes. The type members were designated as T36, T30, T3, VT and B165 genotypes. Degenerate and specific primers were designed based on the genotypic CTV nucleotide gene sequence data from GenBank. The degenerate primer pair for ORF8 [p18] was utilized for reliable detection of all CTV genotypes using simplex RT-PCR. The simultaneous detection of all the five genotypes including the p18 gene control was established using the hexaplex RT-PCR (Hex-RT-PCR). This method saves time and reduces the cost as compared to other conventional methods for genotype classification and detection of CTV. Six different fragments specific to CTV and its five different genotypes were simultaneously amplified using Hex-RT-PCR and were identified on the basis of molecular sizes. The consistency of Hex-RT-PCR was compared with simplex PCR for detection of all CTV genotypes. The Hex-RT-PCR results were confirmed by sequence analysis. Hex-RT-PCR provides a useful rapid method for detecting existing CTV genotypes from tristeza infected plants and will assist in the certification programs for production of CTV free citrus plants.

Resistance evaluation and detection methods of *Leifsonia xyli* subsp. *xyli* in sugarcane cultivars
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Phytopathology 99:S111

The sugarcane is sensitive to diverse pathogen attacks and among them is the *Leifsonia xyli* subsp. *xyli* what causes the Ratoon Stunting Disease, one of the most important of that culture, causing damages superior to 30% in productivity. One of the biggest problems to researchers is the evaluation of the cultivars in relation to resistance and endurance to the disease, that's because most of the time, it doesn't show any specific external symptoms. To evaluate the sensibility of 3 methods for the detection of the bacteria in the evaluation of the resistance of 20 sugarcane cultivars to Ratoon Stunting Disease, it was conducted an experiment in the experimental Station of Federal University of Paraná-Brazil. It was realized evaluations to Agronomy parameters: weight, number of stalks, general grading and height of stalks. The methods used for the diagnose were the Dot Blot EIA (dot blot enzyme immunoassay), EB-ELISA (and evaporative-binding enzyme-linked immunosorbent assay) and PCR (polymerase chain reaction). The method that presented better performance for the detection was the EB-ELISA that beyond pointing 7 clones with positive result, quantified the bacterium. The Dot Blot showed 2 cultivars with positive result and the PCR, 3 positive results to the bacteria. By comparing the results of the evaluations, it was found that 5 cultivars were classified as enduring, 3 as susceptible and 12 as intermediate.

Effect of fruit thinning on *Botryosphaeria obtusa* severity in New York
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Phytopathology 99:S111

Botryosphaeria obtusa is the causal agent of black rot of apple, which can cause losses in the form of fruit rots and cankers. Mummified fruitlets retained in the canopy following thinning of certain varieties are believed to serve as a source of primary inoculum. The objective of this study was to evaluate the effect chemical bloom thinners have on Botryosphaeria colonization of immature apple fruit. Chemical thinners naphthaleneacetic acid (NAA) and 6-benzyladenine (BA), alone and in combination with carbaryl (Sevin®), were applied to mature apple trees in summer 2007. Three application timings based on fungicide application timing for black rot management were also compared: petal fall (early), 10–12 mm fruit diameter (late), and both early and late timings. The experiment was conducted at two orchard locations with at least 4 treatment replications. Immature fruitlets were collected over the course of the growing season to follow the progression of fungal colonization. Water and air sampling were used to determine the timing of spore release in New York orchards. Thinning treatments adequately reduced fruit set although at one location treatment with fish oil + lime sulfur defoliated trees. Preliminary results indicate thinning treatment did not negatively affect severity of black rot. Mummy colonization was low at the initial collection date but increased over time, indicating that infection during bloom may be less important than previously believed.

Biological control of soilborne pathogens on cucumber in organic agriculture
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Phytopathology 99:S111

Soilborne pathogens cause severe losses on cucumber, a major crop in Lebanon. This study explored the identity of the soilborne pathogens on

cucumber in an organic agriculture farm and the comparative efficacy of different biocontrol agents for their management. Biocontrols used were Promot®, Fulzime®, *Trichoderma harzianum*, *Trichoderma viride* and a mixture of *T. harzianum and T. viride*. One biocontrol experiment was carried out on cucumber planted in plastic tunnels. Another was conducted in pots in a greenhouse. The pathogenicity of the isolated and identified soilborne fungi: *Fusarium oxysporum*, *Fusarium nivale*, *Pythium ultimum* and *Rhizoctonia solani* was assessed in a greenhouse pot experiment. The growth and yield of plants treated with *T. harzianum and T. viride* was significantly higher than that of any of the other treatments in the plastic tunnels. In the pot experiment, the mixture of *T. harzianum and T. viride*, *T. harzianum,* and Fulzime® each gave a significantly higher yield in comparison to that of any of the other treatments. In vitro studies showed that Promot® suppressed the growth of all the soilborne pathogens. Fulzime®, *T. viride* and *T. harzianum* suppressed the growth of the *Fusarium* spp, but showed a lower suppressive activity against *P. ultimum* and *R. solani.* The mixture *T. harzianum* and *T. viride* suppressed the growth of *Fusarium* spp., and grew over the mycelia of *P. ultimum* and *R. solani* thus suppressing their further growth and development.

Sequencing the Pokeweed mosaic virus genome, the final act of a century-long characterization

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Phytopathology 99:S112

Pokeweed mosaic is one of the first described viral diseases, originally reported in1902. It is widespread in the Southeastern United States and is caused by a Pokeweed mosaic virus (PkMV), a potyvirus that was characterized from biological, ultra-structural and serological stand points in the late 1960's. Curiously, its genomic sequences were never determined. Taking into consideration that PkMV is one of the most widespread viruses in Mississippi, it was decided to complete its characterization by genome sequencing. As in other potyviruses, the PkMV genome contained a long open reading frame encoding a polyprotein with hallmark motifs of the members of the genus Potyvirus (fam. Potyviridae). Its coat protein shared the highest (75%) identity with recently described Araujia mosaic virus and 70–73% with different isolates of Chilli veinal mottle virus, Pepper veinal mottle virus and Yam mild mosaic virus. The entire polyprotein shared the highest identities with Potato virus A (56%) and Turnip mosaic virus (53%). Virus specific primers confirmed the presence of Pokeweed mosaic virus in several locations of Southern, Central and Northeast Mississippi.

Unraveling the phytovirus world of the Great Smoky Mountains National Park

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Phytopathology 99:S112

Plant viruses described so far from non-agronomic environments, especially forest ecosystems, represent just a tiny portion of currently recognized plant viral species. Considering the extreme richness and diversity of plant species present in the Great Smoky Mountains National Park, we started an investigation on plant viruses within the framework of an on-going All Taxa Biodiversity Inventory (ATBI) activities in the Park and supported by Discover Life in America Inc (DLIA). To this end, symptomatic and apparently healthy specimens of different mono- and dicots were collected and analyzed via dsRNA/random-primed reverse transcription/cloning/ sequencing strategy for RNA viruses and applying DNA extraction/degenerate primer PCR for major taxa of DNA viruses (i.e. begomoviruses, badnaviruses, etc). We have detected and molecularly characterized dozens of undescribed viral species belonging to various current taxa (i.e. families Caulimoviridae, Tymoviridae, Luteoviridae, Partitiviridae; genus Umbravirus, etc), or to genera yet to be erected (i.e. Southern tomato virus-like viruses). In addition, a number of sequences resembling mycoviruses were also encountered during this work. The project is still continuing.

Grapevine virus Q: The first phytovirus with inverted RdRp motifs

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A new marafivirus, for which the name Grapevine virus Q (GVQ) is proposed, was identified in several samples and its genome was sequenced. The monocistronic genome of GVQ is ca 6.5 kb in size and encodes a polyprotein with an estimated molecular mass of 230 kDa which contains hallmark domains of viruses belonging to the genus Marafivirus, family Tymoviridae. Unlike other marafiviruses, motifs of viral RNA dependent RNA polymerase of this virus were inverted and organized in an unique arrangement, so far unreported in plant viruses. Several independent cloning and sequencing experiments, including RT-PCR using different sets of primers and primer walking strategy, confirmed this peculiarity in GVQ genome. Other analyses, including pairwise comparisons and phylogenetic studies, confirmed that GVQ is an undescribed and unusual marafivirus.

Association of a new marafivirus with ring spot symptoms in giant ragweed (*Ambrosia trifida* L.) in Mississippi

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Phytopathology 99:S112

Peculiar chlorotic ringspot symptoms were observed on a group of giant ragweed plants in Yazoo County, Mississippi. Partially purified preparations showed the presence of an isometric virus with a surface arrangement of protein subunits resembling members of the family Tymoviridae. "Universal" tymovirid primers generated a PCR product of expected size from reverse-transcribed total nucleic extracts from three diseased plants. No PCR product was generated from symptomless giant ragweed samples used as a control. Pairwise comparison showed that amplicons shared more than 95% common amino acids, thus proving that the same virus is present in all three tested plants. The genome of this virus is typical of marafiviruses and is characterized by the presence of a large, single open reading frame. Pair-wise comparisons showed that levels of amino acid identities with known marafiviruses is much lower than the species demarcation threshold proposed by the Tymoviridae Study Group of the International Committee on Taxonomy of Viruses, thus indicating that this virus is an undescribed species in the genus Marafivirus (fam. Tymoviridae). The name Giant ragweed ring spot virus (GRRSV) is proposed for this virus.

Identification and molecular characterization of a new member of the genus Petuvirus (family Caulimoviridae) from rhododendron

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Phytopathology 99:S112

Several clones containing sequences similar to plant pararetroviruses were obtained from a Great rhododendron specimen (Rhododendron maximum L.; syn. Great Laurel, Rose Bay, American Rhododendron) as part of a project on phytoviruses in the Great Smoky Mountains National Park (GSMNP). Based on the sequence of the initial clones, specific primers were designed and used to clone the rest of the viral genome. Complete sequencing revealed that the double-stranded DNA genome of this virus is 7.1 kbp in size and contains a single open reading frame coding for a large polyprotein with conserved motifs of movement proteins (MPs) and reverse-transcriptases (RTase). Phylogenetic analyses and pair-wise comparisons revealed a close evolutionary relationship of this virus, for which the name Rhododendron virus N (RhVN) is proposed, with Petunia vein clearing virus (PVCV), currently the sole member of the genus Petuvirus, family Caulimoviridae. RhVN shared 57% and 75% common amino acids with PVCV in MP and RTase regions, respectively. A PCR-based survey of rhododendrons showed that the virus is present in several different locations in the GSMNP and in the Pacific NorthWest.

Nucleotide sequences and detection of a new flexivirus from blackberry

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Phytopathology 99:S112

Native blackberry plants displaying symptoms resembling blackberry yellow vein disease were examined and resulted positive for the presence of high-molecular-weight dsRNAs. Extracted dsRNAs were used as a template for cloning and sequencing. Sequence data from two specimens revealed infections by a new virus for which the name Rubus virus R (RVR) is proposed. The amino acid sequences of the RVR RNA dependent RNA polymerase (RdRp) and coat protein (CP) share limited levels of identical residues with the corresponding regions of members of the genera Foveavirus, Potexvirus and Carlavirus. A virus-specific RT-PCR based method was developed and study on the importance of this virus in cultivated blackberries in Southeastern United States is ongoing.

Hypervariability of badnavirus-like sequences in *Canna indica* L.

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Phytopathology 99:S113

In order to study the population of badnaviruses infecting canna plants, specimens were collected from public landscapes, private gardens, ornamental nurseries and retail stores from various locations in Mississippi. Electron microscope observations of partially purified preparations revealed the presence of badnavirus-like particles in randomly examined tissue. For molecular purposes, DNA was extracted from 100 mg of leaf tissue applying a modified DNeasy Plant Mini kit (Qiagen Inc) and submitted to PCR with "universal" primers designed on conserved sequences of the open reading frame III (ORF III) of badnaviruses as reported in literature. PCR products were cloned and multiple selected clones were sequenced for each tested sample. Pair-wise comparisons of sequenced clones showed the presence of highly diversified sequence variants. Significant differences up to 40% were observed not only between samples, but also among clones generated from the same plant specimen indicating on-going multiple infections. Many of the sequences shared differences significantly exceeding species demarcation thresholds (20%) with current definitive or tentative members of the genus Badnavirus. Further characterization of these putative new badnaviruses is under progress.

A detection method for endornaviruses from various plant species

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Phytopathology 99:S113

Endornaviruses infect hosts in the Kingdoms Plantae, Fungi and Chromista. Conserved genome segments were used to design a set of degenerate primers to detect the presence of endornaviruses in different plant species. The primers were successfully used in preliminary tests, amplifying the predicted DNA segment of 375-384 bp (depending on endornavirus species) from reverse-transcribed dsRNA templates from rice, bean, barley, and avocado. Further work was performed on total RNAs extracted from plant tissue. Primers were also used to detect putative endornaviruses from additional plant hosts and to generate the first data on their genomes. The presence of endornaviruses was confirmed by electrophoretic analyses. Phylogenetic analyses performed on sequences generated in this investigation together with endornavirus data available in the NCBI/GenBank did not distinguished between myco- and plant endornaviruses suggesting their common origin.

RAPD Marker as a criterion to study differentiation of isolates of *Rhizoctonia solani* and *Rhizoctonia bataticola* (*Macrophomina phaseolina*)

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Phytopathology 99:S113

Rhizoctonia solani Kuhn and *R. bataticola* (*Macrophomina phaseolina*) are two of the most destructive soil born plant pathogens causing diseases in a wide range of hosts. In this study, sixty one isolates of *R. solani* and *R. bataticola* from different hosts such as sugar beet, potato, soy bean, and sesame were evaluated for their genetic diversity using RAPD markers. Five decanucleotide primers were selected for RAPD analysis. The results of RAPD revealed a wide DNA polymorphism among the isolates. The similarity value of RAPD profiles in all isolates ranged from 0.1 to 0.95 with an average of 0.52. The results indicated that isolates of both the *Rhizoctonia* species were distinguished into two separate groups. Analysis of RAPD profiles confirmed the separation of different AGs and revealed considerable genetic diversity among and within AGs. According to these results, it seems that RAPD technique, in spite of its simplicity, could be used to separate different isolates of these two important plant pathogens.

Pathogenic variability among the isolates of *Rhizoctonia solani* recovered from potato tubers and sugar beet

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In this study, aggressiveness of the *Rhizoctonia* isolates recovered from potato and sugar beet was examined on radish, tomato, potato and sugar beet. Results revealed a high degree of diversity in virulence of the isolates. Among plants tested, sugar beet and potato were the least and the most susceptible hosts for *R. solani*, respectively. Isolates of AG-4 and AG-3 caused the highest and lowest amount of disease on sugar beet respectively. Of the four hosts, tomato and radish were the most susceptible plants used in this study against the isolates of AG-4. These results indicated clearly that AG-3 isolates have a preference for potato whereas no host preference was observed among isolates of AG-4. In other words, AG-4 isolates have the highest pathogenicity on the all plants tested. Results of this study indicated that it would be possible to separate the isolates based on their virulence on radish and tomato better than potato and sugar beet respectively. These results suggest that it is preferable to replace sugar beet and the potato seeds with radish and tomato seeds when these types of experiments need to be done.

Characterization of *Rhizoctonia solani* isolates from potato and sugar beet

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Rhizoctonia solani is a world wide soil-borne pathogenic fungus showing the tremendous variation in characteristics such as morphology, host specificity and pathogenecity. In this study, hyphal anastomosis reaction, virulence, RAPD and ITS-RFLP of isolates representing *R. solani* recovered from sugar beet root rot and potato tuber with black scurf were compared. Entirely, three anastomosis groups AG-3, AG-4 and AG-5 recovered from both potato and sugar beet. Since, anastomosis reaction alone generally did not provide adequate evidence for genetic diversity among different AGs, pathogenic variability and host range were evaluated in *In vitro* condition on four different plants, radish, tomato, potato and sugar beet seeds. Results revealed a high degree of diversity in virulence of isolates. Genotypic variation among all isolates was evaluated with RAPD analysis. Fifteen decanucleotide primers were selected for RAPD analysis. The results of RAPD revealed a wide DNA polymorphism among all isolates. AG-3, AG-4 and AG-5 isolates of potato were separated from AG-3, AG-4 and AG-5 isolates of sugar beet respectively. Genetic similarity among all isolates was evaluated with ITS-RFLP. A DNA fragment of 700-710 bp in size was amplified from rDNA preparations of all isolates with the ITS5&4. The amplified products of DNA for all the isolates digested with the enzymes *Taq* 1, *Bsur* 1, *EcoR* 1, and *Tru*91. ITS-RFLP showed that isolates of different anastomosis group generated very distinct patterns and were separated based on AG.

Differential response of soybean cultivars to the toxin phaseolinone extracted from *Macrophomina phaseolina*

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Phytopathology 99:S113

Macrophomina phaseolina is one of the most damaging soil borne plant pathogens, infecting about 500 plant species worldwide and causing charcoal rot on soybean in warmer growing areas. In order to assess the tolerance of soybean cultivars to charcoal rot, a cell free culture filtrate of the fungus and phaseolinone extracted from a fungal culture were used to develop a bioassay for this purpose. The amount of phaseolinone present in a two-week-old culture of the fungus was determined with High Performance Liquid Chromatography (HPLC). HPLC was also used to purify the toxin. Ten-day-old seedlings of soybean were used in the assays. The seedlings were cut just above the soil and treated with culture filtrate and the extracted toxin. Alternatively, seedlings with complete root systems were treated with the toxin or the culture filtrate. With seedling cuttings, wilting of leaves was observed within two hours, tissue decay and dessication of leaves occured within eight hours of treatment. Similar symptoms were observed with 10-day-old seedlings with complete root systems within 72 hrs of treatment. The time required to show the first symptoms was indirectly proportional to the concentration of the phaseolinone used in the treatment. Both crude culture filtrate and purified phaseolinone are suitable to use for screening soybean germplasm for resistance to charcoal rot. Soybean seedlings were, however, more sensitive to the purified phaseolinone than the culture filtrate.

The complete nucleotide sequence and genome organization of Calibrachoa Mottle Virus (CbMV)

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Phytopathology 99:S114

Calibrachoa is becoming an important new horticultural plant in Europe and in the United States. Commercial reproduction of *Calibrachoa* plants and maintenance of genetic mother stock are done by means of vegetative propagation. A virus with spherical particles was isolated from *Calibrachoa* plants. The infected plant showed leaf mottling, chlorotic blotch and interveinal yellowing symptoms. The causal agent of this disease was named Calibrachoa Mottle Virus (CbMV). Based on the particle morphology and dsRNA profile, CbMV resembles a carmovirus. In this study, the complete genome of CbMV was sequenced, characterized and analyzed. CbMV has a single stranded, positive-sense RNA genome. The complete genome sequence contained 3,919 nucleotides and five open reading frames (ORFs) were identified. The 5'-proximal ORF encodes a 28-kDa protein that terminates with an amber codon. If the amber codon is read through, the ORF generates a read through protein of 87-kDa (ORF1 plus ORF2). Two small, centrally located ORFs encoded an 8-kDa (ORF3) and a 9-kDa (ORF4) protein, respectively. The 3'-proximal ORF5 encodes a 37-kDa capsid protein (CP). Comparison of the genome organization with other viruses supports the classification of CbMV in the genus *Carmovirus*; family *Tombusviridae*.

Spread of *Xylella fastidiosa* in a pecan orchard and presence of potential vectors in orchards

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Phytopathology 99:S114

Pecan bacterial leaf scorch (PBLS) disease caused by the bacterium *Xylella fastidiosa* is prevalent in commercial pecan orchards in the southeastern United States. In some cultivars, PBLS can cause defoliation in excess of 50% and nut yield reduction of 13–18% annually. To determine the rate and pattern of disease spread, PBLS was monitored over a 6-year period in an orchard with three cultivars beginning when the orchard was 14 years old. The first symptoms of PBLS on newly infected trees in this orchard were generally on current season vigorous growth near the tops of the trees. PBLS increased from 5% infection to 64% infection in one cultivar (Cape Fear) and from no infection to 10.5 and 2.6% in two less susceptible cultivars (Stuart and Candy, respectively) in the orchard. Analysis suggested that the pathogen was being transmitted from infected trees within the orchard with no clear indication of an outside pathogen source. The probability of a tree becoming infected was not strongly influenced by its distance from a previously infected tree. A year-long survey of five Louisiana pecan orchards revealed that several potential vectors of the pathogen were common in pecan orchards. Five species of leafhoppers were regularly detected in all of the orchards, including the glassy-winged sharpshooter, *Homalodisca vitripennis*, a major vector in other hosts along with the pecan spittlebug, *Clastoptera achatina*, recently shown to be a vector.

Evaluation of quinoxyfen and acibenzolar-s-methyl for suppressing bacterial spot of bell pepper

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Phytopathology 99:S114

Bacterial spot of pepper caused by the bacterium, *Xanthomonas campestris* pv *vesicatoria*, is a major disease of bell pepper grown in Georgia that occurs every year. Copper + maneb sprays can be effective at suppressing bacterial spot, however, copper resistance is common and these sprays do not provide adequate protection in periods of high disease pressure. The efficacy of Quintec (quinoxyfen) and Actigard (acibenzolar-s-methyl) were evaluated for the suppression of bacterial spot of pepper. Greenhouse screening trials were conducted in two consecutive years on bell pepper seedlings (*Capsicum anuum* 'Early Cal Wonder) grown in 200 cell trays arranged in a randomized complete block design with four replications. At the four leaf stage, four plants in the middle of each tray were inoculated with *X. campestris* pv. *vesicatoria* pepper race 1. Beginning one week prior to inoculation, Quintec sprays were applied biweekly and Actigard sprays were applied either biweekly, weekly, or once every three weeks. All applications were applied with a backpack sprayer with a single, hooded nozzle calibrated to deliver 80 gpa. Plots were evaluated by counting plants that expressed disease symptoms and by rating defoliation. Quintec and Actigard were effective at suppressing bacterial spot when compared to the untreated checks in both years. There was no difference in the amount of bacterial spot suppression between Quintec, Actigard and Kocide 3000 + Manex.

Incorporation of rice residues and swine manure in soil for control of Phytophthora fruit rot in vegetables in Nueva Ecija, Philippines

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Phytopathology 99:S114

A study was conducted in 2008 at the Central Luzon State University, Nueva Ecija, Philippines, to test the hypothesis that rice straw residues and swine manure would reduce Phytophthora fruit rot in vegetables. Seedlings of eggplant (*Solanum melongena*, cultivar Casino) and tomato (*Lycopersicon esculentus*, cultivar Marimar) were transplanted into soil in which rice straw and composted swine manure were incorporated and infested with *Phytophthora infestans*. Two factors were examined: (i) presence or absence of rice straw and (ii) composted swine manure applied at three levels: none (0 kg/m^2), low (0.5 kg/m^2, equivalent to 5 tons per hectare), and high (1 kg/m^2, equivalent to 10 tons per hectare). Data was collected on the following variables: (i) disease incidence, defined as the number of fruit with Phytophthora rot; (ii) disease severity, defined as the percentage of fruit surface with rot symptoms; and (iii) marketable fruit yield. In general, in plots treated with swine manure, disease incidence and disease severity were greatly reduced while marketable yields were greatly increased in contrast to plots not treated with swine manure. This trend was more pronounced in treatment with rice straw and high swine manure than in other treatments. Results suggest that incorporation of rice straw and swine manure into field soil may serve as an effective option for reducing Phytophthora fruit rot in vegetables in Nueva Ecija.

Prevalence of *Phymatotrichopsis omnivora* in alfalfa fields affected by root rot in southeastern New Mexico

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Phytopathology 99:S114

Field surveys were conducted in summer 2007 to identify microorganisms associated with alfalfa root rot in southeastern New Mexico. Surveys were completed before or after crop harvest. Symptomatic plants were found in semi-circular patterns with radius varying from 1 m to over 50 m. Five to 10 samples of plants with root rot were collected in each of 10 fields. Affected plants displayed browning of the entire root system with sloughing of cortical tissues or discoloration in the vascular tissues. Fungal structures such as acervuli and rhizomorphs were observed on crowns and roots, respectively, of some symptomatic plants. Further microscopic observations of rhizomorphs revealed the presence of acicular hyphae, a characteristic feature of *Phymatotrichopsis omnivora*, causal agent of Phymatotrichopsis root rot. Tap roots were washed free from adhering soil, surface-sterilized in 0.5% sodium hypochlorite, rinsed in sterile distilled water, and plated on acidified potato dextrose agar. Emerging mycelial colonies were transferred to potato dextrose agar medium for identification. On the basis of preliminary results, microorganisms recovered included *P. omnivora, Fusarium oxysporum, Rhizoctonia solani, Collectrotrichum* sp., and other unidentified mycelial microorganisms. *Phymatotrichopsis omnivora* was found in 9 out of 10 fields surveyed, indicating that this fungal pathogen is more prevalent than other mycelial microorganisms in alfalfa grown in southeastern New Mexico.

Plant pathogenic Phytophthora species found in Tennessee commercial nurseries

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Phytopathology 99:S114

Phytophthora has been associated with aerial blight, stem dieback and root rot in commercial nurseries. A survey of 17 nurseries in middle Tennessee revealed the presence of several species of Phytophthora including unclassified species isolated from plant tissues, rhizosphere soil in field and container-grown plants, and from various irrigation water sources used by commercial nurseries. Phytophthora ramorum was not found. Out of 171 different isolates identified as Phytophthora or Phytophtora-like, 10 isolates from irrigation water including six unclassified Phytophthora, two P. hydropathica, P. megasperma, and P. citricola were selected for pathogenicity tests on 26 woody nursery plant species. The flowering dogwood (*Cornus florida*) was most susceptible to all ten Phytophthora isolates, Pieris. was susceptible to nine isolates, Viburnum to eight, silky dogwood (*C. amomum*) and kousa dogwood (*C kousa*) were susceptible to five isolates. Red maple was least susceptible to four isolates. Out of the ten Phytophthora isolates, P. citricola was the most virulent on the hosts tested. The in-vitro bioassay

technique using detached leaves allows rapid evaluation of Phytophthora pathogenicity on a large number of hosts using limited space and inoculum resource. Other bioassay techniques such as inoculation by spray, drench on roots are in progress to provide a better understanding of the virulence of these Phytophthora isolates.

Variants of antagonist *Cryptococcus flavescens* OH 182.9 with improved efficacy in reducing Fusarium head blight in greenhouse and field environments

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Phytopathology 99:S115

The inclusion of biological control agent *Cryptococcus flavescens* OH 182.9 in the integrated management of Fusarium head blight (FHB) has potential for significantly contributing to the reduction of FHB and deoxynivalenol (DON) in wheat. Experiments were conducted to determine if liquid culture growth conditions could be modified to promote the production of stable variants of OH 182.9 with improved efficacy compared to the wild-type progenitor strain. A protracted exposure of OH 182.9 to conditions adverse to cell growth resulted in the isolation of variants OH 182.9 3C and OH 182.9 4C. In greenhouse tests, variant 4C reduced head blight severity by 83% compared to 36% for the wild type (WT) strain. In two field trials on susceptible winter wheat, variants 3C and 4C reduced severity by 34% and 42% compared to the control ($P < 0.05$, Bonferroni) in one trial and 21% and 24% in another (NSD) while the WT strain had no consistent effect. For both field trials, treating flowering wheat heads with the fungicide Proline and variant 3C after 24 hours was the most successful treatment in reducing FHB severity (control vs. treated averages of 10.0% vs. 1.4%, respectively) and DON (10.0 vs 2.3 ppm, respectively). Similar trends, though to a lesser degree, were observed in field trials on a moderately FHB-resistant winter wheat. Enhanced efficacy variants of antagonist OH 182.9 warrant further study regarding their inclusion in the integrated management of FHB.

Local adaptation and global biogeography of antagonistic Streptomyces

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Streptomyces are ubiquitous soil-borne saprophytes that often inhibit the growth of other microorganisms through antibiotic production. Though little data exists, selection for antibiotic phenotypes is expected to depend on available nutrients (plant litter, root exudates, etc.) and interactions with other microorganisms through resource competition and antibiotic inhibition. We sought to explore local (small-scale) and global patterns in inhibitory and resource use phenotypes among Streptomyces. Specifically, we determined carbon source utilization and inhibitory phenotypes for a global collection of Streptomyces isolates. Preliminary results indicate large variation in inhibitory and nutrient use phenotypes among Streptomyces from different locations. The data also show localized selection for resistance to antibiotics produced by coexisting isolates. Within locations, antibiotic inhibition is also more frequent among isolates that have high nutrient use overlap. Co-evolution among coexisting Streptomyces may be significant to soil microbial community function, including disease suppression. Understanding the factors that influence antagonistic Streptomyces communities will provide insight into how we may manage soil microbial communities to enhance disease suppression.

Development and use of fluorescent antibody and qPCR protocols for the electrostatic spore trap

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Fluorescent antibody (FA) and qPCR protocols were evaluated for the newly developed aerobiological sampler (Ionic Spore Trap™), which depends upon electrostatic deposition of particulates onto a 25 mm aluminum disk (stub). This device was originally designed for assessment of captured particulates by scanning electron microscopy (SEM), which could be augmented with automated scanning of stubs and machine vision. The trap was used to monitor airborne populations of *Phakopsora pachyrhizi*, causal agent of soybean rust, in Florida and Louisiana in 2008. Urediniospores were observed by SEM analyses several weeks before first symptoms were found at both locations. A standard compound microscope also may be used to examine clear adhesive tape affixed to the stub. We now report on the successful development of FA and qPCR protocols, which greatly enhance the versatility and utility of this device. FA detections were conducted with clear double-stick tape and with adhesive carbon disks affixed to the stubs. These protocols will be especially useful for fungi in which spore morphology may not be adequate for identification to species or biotype levels, and they allow for more precise quantification. In addition, access to a SEM and a trained microscopist are not needed.

Comparative analysis of whole bacterial genomes and derivation of RIF, a DNA identification marker for bacterial phytopathogens

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We have identified a region of the replication initiation factor gene (RIF) as a useful DNA marker for identification of phytopathogens below the species level. RIF, which is present as a single copy in all genomes examined (*Clavibacter*, *Erwinia* [*Dickeya*, *Pectobacterium* and *Pantoea*], *Ralstonia*, and *Xanthomonas*)-*CERX*, is unlikely to be laterally transferred and is amplifiable with genus-specific primers. The RIF marker was derived using comparative genomics of six complete genomes of *Xanthomonas* (3 species and 4 pathovars) and comprises 925 nucleotides of the *dnaA* replication initiation factor. *In silico* analysis revealed that RIF has equal or higher resolution than the ribosomal intergenic spacer (ITS) for differentiating strains of the same pathovar of *Xanthomonas*. Initial attempts to sequence the RIF and ITS markers of 360 *CERX* strains yielded a higher success rate (33%–97%) with RIF. Sequencing frameworks were constructed using the RIF marker with 111 *C. michiganensis*, 70 *Erwinia*, 220 *R. solanacearum* and 354 *Xanthomonas* strains. These frameworks have been used successfully to identify unknown bacterial strains associated with recent disease outbreaks, illustrating the value of RIF for the identification of plant pathogens.

Incidence and spatial distribution of *Rhizoctonia* and *Pythium* species determined with real-time PCR

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Populations of *Rhizoctonia* and *Pythium* are diverse in eastern Washington, with multiple species/anastomosis groups present throughout the region and within individual fields. Recent evidence suggests that species composition may be influenced by crop rotation. The Cook Agronomy Farm near Pullman, WA was established in 1999 to test direct-seed cropping systems on a field scale, with 369 GPS sampling locations spaced every 30 m. A three-year rotation was established to include winter wheat-alternate crop-spring wheat, with each rotation occurring every year. The alternate crops consisted of a winter and/or spring variety of pea, lentil, barley or canola. Soil samples were collected from about 115 sites from the spring wheat portion of the field following the alternate crop. DNA was extracted from each soil sample using a Barocycler™ and Mo-Bio Soil DNA kit, and quantified with species-specific primers and real-time PCR. *Rhizoctonia solani* AG-2-1 occurred more frequently in fields with a history of canola, with a higher frequency following spring canola (48% of sites) compared to winter canola (28%). *Rhizoctonia oryzae* was detected more often following winter alternate crops (39–44%) than spring crops (24–37%). Of four *Pythium* species examined, *P. irregulare* group IV and *P. rostratifingens* had the highest incidence of occurrence (66% and 67% respectively). These species of *Pythium* were also higher following winter canola and pea versus spring canola and pea.

Revisiting the taxonomy of *Candidatus* Liberibacter based on 16s rDNA sequencing from cultured and uncultured cells

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Due to the difficulty in distinguishing bacteria based on phenotypic characters, there has been a reliance on sequence data for recognizing species. While the gold-standard for bacterial taxonomy is DNA-DNA reassociation, many species are only differentiated using a single genetic locus, 16s rDNA. Because of this reliance, sequence similarity for this locus is often used for species delineation. Bacterial strains are recognized as separate species when similarity at the 16s rDNA locus is less than 99%, but higher taxonomic classification is not well-documented. Three species of *Candidatus* Liberibacter are recognized, *Ca*. L. asiaticus, *Ca*. L. americanus, and *Ca*. L. africanus. 16s rDNA sequences of the three groups were obtained from GenBank records and cultured cells. Similarity analysis showed there were three distinct taxa with *Ca*. L. americanus being 91 to 94% similar to the other two taxa. *Ca*. L. asiaticus and *Ca*. L. africanus shared 94 to 97% similarity. Additionally, samples acquired in Florida were sequenced for the 16s rDNA locus directly from plant material and from resulting cultures. We observed no difference between cultured and uncultured cells. From these results we suggest that *Ca*. L. americanus be placed in a new and different genus, while

Ca. L. asiaticus and *Ca*. L. africanus remain in Liberibacter until DNA-DNA reassociation and phenotypic assays can be conducted.

Genetic diversity of *Candidatus* Liberibacter asiaticus strains from Florida compared to worldwide populations

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In order to better monitor bacterial diseases, the population structures of the organisms must be understood. Huanglongbing (HLB) is an emerging disease of citrus in North and South America, but has been infecting citrus in Asia and Africa for many decades, perhaps since the 1910s. HLB is caused by several members of the genus *Candidatus* Liberibacter, with *Ca*. L. asiaticus being found in Asia, Brazil and Florida. To establish the population structure of *Ca*. L. asiaticus, 55 strains were collected from Florida (17), Brazil (8), and throughout Asia (30). Seven genes were used to assess polymorphisms; 6 conserved genes and 1 gene involved in flagellar development. The geographic variation was calculated between Florida and Asia and Brazil, within Florida, within Asia, and within Brazil. A genetic substructure was found between the geographic populations. The nucleotide diversity was also significantly higher in the Asian and Brazilian populations compared to the Florida population. The diversity of the Thai subset (12) of the Asian population was also higher than Florida. The lack of diversity in Florida populations of *Ca*. L. asiaticus suggests limited introductions to Florida in recent years.

Contans®, a fungal mycoparasite for control of *Sclerotinia* spp. in the soil from SipcamAdvan and Prophyta Biologischer Pflanzenschutz GmbH

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Phytopathology 99:S116

Contans® (*Coniothyrium minitans*) is a fungal mycoparasite active ingredient formulated for preventative application to field and greenhouse crop sites. The mycelia of *C. minitans* parasitizes the sclerotia resting bodies of *S. sclerotiorum, S. minor & S. trifoliorum* in the soil. The attack of *C. minitans* reduces sclerotia viability and survival, thus, minimizing attack on succeeding crops. Contans is exempt from EPA requirements for a food tolerance. Multiple university field trials demonstrated efficacy against *Sclerotina* spp. attack on legume, leafy vegetable and oilseed crops.

Use of plant host-derived RNAi targeted to parasitism genes to develop root-knot nematode-resistant tobacco

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Phytopathology 99:S116

The four major species of root-knot nematode (RKN), *Meloidogyne incognita, M. javanica, M. arenaria*, and *M. hapla,* have multiple crop host species and cause staggering economic losses worldwide. Proteins produced in specialized esophageal gland cells are secreted from the stylet of RKN to transform recipient host plant root cells into multinucleate giant-cells that are essential for nematode feeding. The secreted peptide encoded by the *M. incognita* 16D10 parasitism gene interacts with a plant cell transcription factor, and silencing of the 16D10 transcript in feeding nematodes using host-derived RNA interference (RNAi) makes *Arabidopsis thaliana* plants highly resistant to all four major RKN species. Two haploid lines of *Nicotiana tabacum*, TN90 a burley tobacco, and Hicks a flue-cured tobacco, were transformed with the 16D10-RNAi construct that was used previously in Arabidopsis. Since neither tobacco line contains any resistance gene to RKN, constitutive expression of 16D10-RNAi in these lines may provide broad-spectrum resistance to root-knot nematodes. Successful transformations of both tobacco lines have been confirmed and double-haploids have been recovered through midvein tissue culture of mature leaves. No off-target effects of 16D10 RNAi have been observed in the regenerated tobacco lines, and they are now being tested for transgene expression and resistance to root-knot nematodes.

Real-time PCR detection of *Puccinia pelargonii-zonalis* through greenhouse-grown geraniums

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Phytopathology 99:S116

Early detection of *Puccinia pelargonii-zonalis* on asymptomatic geranium tissues using a real-time PCR assay would allow for timely fungicide applications to limit pathogen spread and disease development in greenhouses. Primers (GRF and GRust-R2) were designed and screened for specificity against DNA of twenty fungal and two plant species. Sensitivity was tested against ten-fold serial dilutions of *P. pelargonii-zonalis* DNA and the detection threshold was 100 pg of template DNA and 10^3 urediniospores ml^{-1}. A greenhouse experiment was designed to follow disease spread through uninfected geranium plants using real-time PCR and direct visualization of urediniospores on leaf surfaces. Two sets of fourteen rows of geranium in 8-inch containers were placed on a bench in separate greenhouses. The first row was an inoculum source of four 5-week-old geranium cv Bright Red with sporulating rust lesions. Thirteen rows of uninfected geranium cv Red Maverick were evenly spaced over a 3 m distance adjacent to the inoculum source. To promote disease development, plants were misted through an overhead irrigation system. One leaf was sampled from each plant in each row weekly for 4 weeks. Each sample was viewed under a dissecting microscope (25x) and presence or absence of urediniospores was recorded. DNA was extracted from samples and subjected to real-time PCR. Urediniospores spread progressively throughout all 13 rows as indicated by direct visualization and by the signs of disease onset within 3 weeks.

Plum pox virus surveys in Oregon: 2000 to 2008

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Plum pox virus (PPV) is a quarantine pathogen within the USA and other countries. PPV was first reported in Pennsylvania in 1999, and in New York and Michigan in 2006. To maintain Oregon's free-from status for this virus and to meet the import requirements of Canada and others, the Oregon Department of Agriculture has conducted official PPV surveys since 2000. Surveys were conducted annually the first four years and then on a periodic basis. Samples were collected from Prunus trees within nurseries and cherry orchards. As specified in the official federal protocol for PPV, samples were collected at a rate of four leaves per tree. In total, from 2000 to 2003 and in 2006, 10,245 samples were collected from scion wood for testing using federally approved ELISA protocols. All samples were negative for PPV. In 2008, 1,000 samples were collected from nurseries only; half of the samples came from scion wood and half from rootstock. Again, all samples were negative for PPV. Based on these survey results, Oregon is free of PPV.

Management of Pythium root rot on tobacco seedlings with a non-ionic surfactant

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Pythium root rot (PRR) is a serious disease of tobacco transplants produced in float beds, a hydroponic system common in the southeastern U.S. Current control measures are sanitation and the preventive use of etridiazole (ETDZ); however, sanitary practices are not effectively employed and ETDZ is costly and relatively phytotoxic. We evaluated the number and timing of applications of Naiad, a non-ionic surfactant known to suppress Oomycetes, needed to provide control of PRR equivalent to ETDZ. Preventive treatments included Naiad (1000 ppm) applied once at seeding, 3 weeks after seeding (WAS), or 5 WAS. Two- and three-application treatments consisted of combinations of the aforementioned single applications. ETDZ was applied at 0.07 ml per L of float water at 3 and 5 WAS. All trays were inoculated with *Pythium* 1 day after the 3 WAS applications of ETDZ. Naiad also was applied after symptoms of PRR were first observed (FS); ETDZ was applied at FS at 0.1 ml per L of float water. Naiad applied at 3 and 5 WAS, or at S, 3, and 5 WAS, was comparable to ETDZ against PRR when applied preventively or curatively, and a similar number of useable transplants were obtained. Applications of Naiad after FS did not differ from the untreated control in terms of disease severity or numbers of useable seedlings. Naiad appears to be a suitable alternative to ETDZ for management of PRR when applied preventively. Additional work is needed to evaluate plant safety and compatibility with ETDZ in a control program.

Identification of haustorium specific genes of wheat leaf rust (*Puccinia triticina* Eriks.) that are expressed during early stages of infection

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Phytopathology 99:S116

Wheat (*Triticum aestivum* L.) is severely affected by the biotrophic fungus *Puccinia triticina* (leaf rust) which causes substantial yield loss annually. Although resistant varieties have been identified, the fungus tends to

overcome most new sources resistance very quickly. Disease resistance in plants is often controlled by the interaction between a single dominant plant resistance gene (R) and an effector/avirulence factor (Avr) from the pathogen. This interaction typically activates a hypersensitive typeresponse at the site of infection. In order to understand the resistance pathway and generate new alternatives for durable resistance, the goal of this research is to identify Avr genes from *P. triticina*. Leaf rusthaustoria were extracted by sucrose gradient and ConA affinity chromatography and cDNA libraries were generated. Considering the presence of signal peptide, 26 clones were selected as potential candidates and timing of expression wasevaluated by RT-PCR of infected leaf tissue and germinated spore samples. Twelve clones were selected as candidates and are being validated by transient expression experiments using co-bombardment with GFP into leaf tissue of leaf rust resistant isogenic lines. The results of this work will be discussed.

Differential hosts for *Triticum mosaic virus* and *Wheat streak mosaic virus*
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Triticum mosaic virus (TriMV) is a newly discovered virus isolated from wheat. This study was conducted to identify additional hosts of TriMV. Plants tested were mechanically inoculated with the 06-123 isolate of TriMV and plants were also separately inoculated with *Wheat streak mosaic virus* (WSMV) for comparative purposes. Maize (*Zea mays* L.) hybrids and lines were not infected with TriMV, however, 'N28Ht', 'Falconer', and 'Midland' were susceptible to WSMV. The nine barley (*Hordeum vulgare* L.) sources tested were infected with TriMV but 'Baronesse', 'Gallatin', 'Horsford Hay', and 'BZ-489-74' were not susceptible to infection with WSMV. Fifteen triticale [Triticale (x Triticosecale Wittmack)] sources were tested and all were susceptible to infection with TriMV, but two of the sources (ARCIA and NCPT01-1433) were not infected with WSMV. This demonstrates that maize, barley, and triticale can serve as differential hosts for TriMV and WSMV.

Radish cover crops as a means for *Rotylenchulus reniformis* management in cotton
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Preliminary field studies indicated that field plots with radish as a winter cover crop had significantly lower *Rotylenchulus reniformis* populations at cotton planting than fallow plots. Simulated cover cropping was carried out in the greenhouse to evaluate three radish (*Raphanus sativus* L.) cultivars before field trials began the following fall. Greenhouse trials indicated that the radish cultivars were not hosts for *R. reniformis* and sustained significantly lower ($P < 0.0001$) populations (317 per 500 cm^3) than cotton controls (20,850 per 500 cm^3). Cover crops were chemically terminated, allowed to dry for two weeks, and then replanted with cotton. All three radish cultivars prevented population rebound of *R. reniformis* populations (189 per 500 cm^3) over the cotton control (8011 per 500 cm^3) at 30 days after replanting. Field studies in naturally infested *R. reniformis* fields compared six radish cultivars, rye, and fallow plots. Mean numbers of *R. reniformis* per 500 cm^3 of soil per plot ranged from 6381 to 10,261 at 30 days after planting; populations of *R. reniformis* were significantly lower ($P = 0.01$) for the radish cv. Brutus than fallow plots. Based on the greenhouse trials, initial numbers of *R. reniformis* at planting should be lower in radish plots and may decrease the impact on the cotton crop.

Phenotypic and etiological differences between psyllid yellows and zebra chip diseases of potato
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Both potato psyllid yellows (PY) and Zebra chip (ZC) potato diseases are associated with the potato psyllid, *Bactericera cockerelli* (Sulc). Symptoms of both diseases are similar on potato plants but there is a difference in symptoms in tubers. ZC has recently been associated with a new species of *Candidatus* Liberibacter. Transmission studies were conducted to elucidate similarities and differences between the two diseases. Potato plants were exposed to both liberibacter-free and infected potato psyllids and later assessed for plant and tuber symptoms. The plants and tubers were also tested for liberibacter by PCR. In addition, potato plants exhibiting severe PY/ZC-like symptoms were collected from a commercial potato field heavily infested with the potato psyllid and tested for liberibacter. Results showed that ZC symptoms were associated with liberibacter, whereas the PY symptoms were not due to the presence of this bacterium in affected plants. Moreover, tubers from liberibacter-infected potato plants exhibited typical symptoms of ZC infection, but tubers from PY affected plants did not. Furthermore, results indicated that ZC infected plants tend to die quickly, in contrast to potato plants affected by PY. Although an association between liberibacter and ZC has been established, no pathogen was found to be associated with potato PY and mechanisms by which classical PY symptoms are induced by the potato psyllid remain unclear.

Loop-mediated isothermal amplification (LAMP) for rapid detection of *Rhodococcus fascians* on ornamentals
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Rhodococcus fascians (*Rf*) is of growing concern to ornamental growers due to its wide host range and the lack of effective control methods. Virulent strains of this gram-positive bacterium cause fasciation, leafy gall and shoot proliferation in susceptible host plants by interfering with the plant's hormone balance. Current detection methods, including polymerase chain reaction (PCR), are expensive and not readily transferable to growers. We have developed a LAMP assay to rapidly detect virulent *Rf* strains on plant tissue without the need for expensive equipment. A set of four primers were designed to detect a conserved, 191 bp region of the FasR virulence gene of *Rf*. A total of 20 virulent *Rf* isolates from a range of different geographical areas and hosts was tested and detected with our LAMP assay. LAMP products were run on a gel and sequenced, aligning with the *Rf* virulence gene in GenBank. Our primers did not detect 6 avirulent *Rf* strains or other bacterial species that are known to occur on plant surfaces, including *Agrobacterium tumefaciens*, another problematic gall-forming pathogen found in nurseries. This assay is able to detect the equivalent of 5×10^3 cfu's of *Rf* per LAMP reaction, which is comparable to PCR sensitivity. We conclude that our LAMP assay is a sensitive, specific, rapid and cost-effective method to detect *Rf* on plants, with potential application in nurseries.

Creation of a commodity based plant pathology identification tool: Lessons learned from building a Lucid tool for Citrus
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Detection and identification of crop pests and diseases are essential tasks in protecting agriculture. While regulatory officers and technicians are trained on a wide variety of commodities, they are not specialists that can quickly and confidently identify multitudes of diseases/pests on a vast number of commodities. Many current pest identification resources are difficult to navigate efficiently and are not readily available to all regulatory personnel. We explore the use and creation of a LUCID based pathology/pest identification tool. Development of commodity- based tools may drastically improve efficiency and confidence of users thus improving detection and identification rates. Citrus is an important crop in U.S. agriculture and citrus production coincides with geographic areas that are high-risk for the introduction and establishment of invasive pests and diseases. Lucid-based identification tools are both appropriate and needed in such economically important high risk commodities.

Training and implementation of distance diagnostics in regulatory agriculture to increase efficiency and reduce costs
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Between the 2006 and 2008 citrus seasons, disease samples submitted to plant pathologists working for the Citrus Health Response Program (USDA/APHIS/PPQ/CHRP) increased from 200 samples to over 1584 samples. To expedite diagnostic procedures and rule out negative samples, the CHRP pathology team integrated distance diagnostic procedures. While in-person pathologist diagnosis and laboratory tests were still employed to confirm a positive diagnosis, pathologists used digital images to quickly rule out negative disease samples. In addition, the CHRP pathology team maintained digital image standards through annual testing, training, and required quarterly image submissions from all CHRP technicians. From 2006 to 2008 the number of samples submitted to diagnostic laboratories that returned with a positive confirmation increased from 56% (in 2006 when technicians forwarded their own samples) to 92% (in 2008 when pathologists screened samples using distance diagnostics). Other benefits of the distance diagnostic program included: 1) CHRP technicians were better able to collect high suspects due to instant feedback from pathologists, 2) pathologists were better able to tailor training to meet technicians' needs, 3) fewer negative

samples were sent to diagnostic laboratories saving money and resources, 4) CHRP amassed an extensive database of citrus disease photos, and 5) pathologists were more quickly able to rule out negative samples benefiting stakeholders.

Pythium species causing green bean diseases in plastic greenhouses in southeast Spain

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A unique under plastic greenhouse intensive horticulture has been established along the Mediterranean coast of Spain in the last 30 yr with about 28,000 ha in Almeria and Granada. Green beans were cropped in an estimated area of 7,635 ha in 2005. Surveys to determine the causal agents of root necrosis and/or necrotic streaks of stems and wilting in this crop have been conducted in the region since the year 2000. Isolates of *Fusarium* spp., *Rhizoctonia solani*, *Thielaviopsis basicola* were obtained in acidified PDA and Pythium spp. in selective Corn Meal agar P_5ARP. Morphological and molecular (ITS rDNA) identifications of Pythium spp. were conducted to determine the identity of the obtained isolates. The species associated to the symptoms mentioned above are *P. aphanidermatum*, *P. irregulare*, *P. myriotylum*, *P. ultimum*, the recently described *P. solare*, *Pythium* sp. close to *P. macrosporum* and *Pythium* sp. close to *P. perplexum*. The pathogenicity of five of these species was evaluated under different conditions in several experiments in soil-less on plastic houses in Almeria. The most aggressive pathogens are *P. aphanidermatum, P. myriotylum* and *P. solare* that cause roots rots, stem necrotic streaks, wilt and death. The disease severity achieved by the two first pathogens was higher when the average daily temperatures ranged from 25–35°C. Pythium solare caused high disease severity at 20–30°C while *P. irregulare* and *P. ultimum* only caused root rot symptoms under 18–25°C.

Sequence diversity of badnaviruses and retrotransposons in pineapple in Hawaii

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Members of the genus Badnavirus (family Caulimoviridae) are DNA reverse-transcribing viruses that have been identified worldwide in many dicots and monocots including pineapple, Ananas comosus (L.) Merr. Oligonucleotides degenerate for conserved sequences in the reverse transcriptase/ribonuclease H (RT/RNase H) and protease regions of badnavirus open reading frame III (ORFIII) were used in PCR assays to identify badnavirus-like sequences from commercially-grown pineapple plants in Hawaii. Phylogenetic analysis with the complete ORF III from the pineapple badnaviruses we have identified in Hawaii and orthologs from other Caulimoviridae members shows the pineapple badnavirus from Hawaii is firmly embedded in the Badnavirus genus. Other sequences that contained RT/RNase H regions were also identified from pineapple using the degenerate primers. Further sequencing and phylogenetic analyses of these RT/RNase-containing contigs show they are closely related to retrotransposons such as Ty3-Gypsy in the family Metaviridae, or are potential integrants of the pineapple badnavirus in the plant genome. PCR assays with specific oligonucleotides that could detect and distinguish the Hawaiian pineapple badnavirus sequences from retrotransposon-like sequences were developed.

Resistance of onion varieties to foliar blight disease

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Foliar blight is a major bottleneck in the successful production of onion crop. Under the sub-tropics of Jammu, the disease was found to be caused by six pathogens, viz. Alternaria alternata, A. porri, A. tenuissima, Stemphylium vesicarium, Cladosporium allii-cepae and Colletotrichum circinans. Forty-five onion varieties/lines and a garlic variety were evaluated against the disease and data on disease intensity were recorded on 0–5 scale. None of the genotypes/varieties tested, showed resistant reaction, whereas, only four varieties, viz., Brown Spansl, NRCOG-227, Phule Safed and Nasik Dark Red showed susceptible reaction, in the year 2005. Arka Kalyan was the only variety with resistant reaction during the year 2006, whereas, Brown Spansl was the only variety with susceptible reaction. None of the varieties tested showed consistent behavior which showed that specific breeding programmes have to be carried out to find the most suitable variety.

Identification of wheat head scab pathogens infected by dsRNA

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Wheat scab (also known as Fusarium head blight) caused by fungi of *Fusarium* sp. was an important disease which can cause tremendous losses by reducing yield and quality in many small grains production regions all over the world. In this experiment, by single spore isolation from the diseased wheat seeds collected from different cultivating areas in China, 8 kinds of *Fusarium* sp. were obtained and identified based on cultural and morphological characters, which were *F. gramminearum*, *F. avenaceum*, *F. semitectum*, *F. oxysporum*, *F. tricinctum*, *F. lateritium*, *F. moniliforme* and *F. acuminatum*. Morphological changes of several isolates of *Fusarium* sp. were observed in the process of being cultured. The variation made the research work of identification of *Fusarium* sp. much more difficult than usual. The presence of double-stranded RNA (dsRNA) was detected within several isolated *Fusarium*. Pairs of specific primers of *Fusarium* sp. were designed and used for the identification of isolates infected by dsRNA. And then *Fusarium avenaceum* within dsRNA were recognized by morphology and PCR methods. The work was supported by university science fund for young scholars of BUA, No.06-ZD-ZK-02.

Cultivated conditions can affect the antagonism activities of cellulolytic enzyme produced by _Trichoderma_ sp.

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Phytopathology 99:S118

Trichoderma sp. are present in nearly all soils and root ecosystems. In addition to colonizing roots, many species in this genus are well known as biocontrol agents which can attack, parasitize or gain nutrition from fungi. Cellulolytic enzyme produced by *Trichoderma* sp. is a kind of inducing enzyme and can be affected by cultivated conditions easily. The culture fluid of *Trichoderma* sp. isolated in our prior research was extracted and then the antagonism function to 8 plant pathogenic fungi was tested. The fluids had antagonism activity to all 8 fungi tested by the method of toxic medium, including *Botrytis cinerea*, *Septoria apii*, *Verticillium dahliae*, *Sclerotinia sclerotiorum*, *Alternaria solani*, *Fusarium* sp., *Ascochyta lycopersici* and *Rhizoctonia solani.* The antagonism of the culture fluid against 8 pathogens was decreased markedly after being sterilization. Optimal culture medium prescription and cultivated conditions were compared as well to achieve higher activities rate in process of production of cellulolytic enzyme. The higher activities rate of cellulolytic enzyme secreted by *Trichoderma* sp. can be obtained by following cultivated conditions, the rate of wheat straw and bran is 1:1, the rate of solid and fluid is 1:3, 0.05% Tween-80 (the surface active) and then cultivated 96 h in 30°C. The work was supported by university science fund for young scholars of BUA, No.06-ZD-ZK-02.

Prediction models for potential yield losses caused by wheat stripe rust in the US Pacific Northwest

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Phytopathology 99:S118

Winter weather variations in the US Pacific Northwest affect wheat stripe rust epidemics caused by *Puccinia striiformis* f. sp. *tritici*. Previously developed forecasting models only estimate disease index at the flowering stage, which may not be indicative to disease damage. To develop models for predicting potential yield losses, correlation and regression analyses were conducted using the historical yield loss data and weather parameters: sum of daily temperatures (SDT), moving average temperatures (MAT), accumulated negative and positive degree days, rainfall, snow depth, and snow cover days (SCD) in periods of one, two, and three months, and whole winter season (Nov-Feb). The SDT of the whole winter season was more correlated with yield loss than the weather variables of one, two, and three months. Similar results were obtained for MAT and SCD. The SDT and SCD of December were correlated the best with yield loss among the monthly weather parameters, and can be used for early prediction. The prediction model using the sum of daily maximum temperature (Nov-Dec) estimates the yield loss more accurately than the rest of the variables. Different degrees of correlations

between yield losses and weather parameters allowed us to select best-fitting regression models that can be used to predict the yield loss in the following growing season during and just after the winter season.

Identifying resistance genes for eyespot of wheat in *Aegilops longissima*

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Eyespot, caused by the soilborne fungi *Oculimacula yallundae* (OY) and *O. acuformis* (OA), is an economically important disease of winter wheat. Currently, eyespot control in commercial wheat is based on two resistance genes (*Pch1* and *Pch2*). *Aegilops longissima* (2n = 2*x* = 14, S^lS^l) is a distant relative of wheat and potential donor of genes for wheat cultivar improvement, including disease resistance. Forty *Ae. longissima* accessions were evaluated for resistance to eyespot in a growth chamber assay by inoculating seedlings independently with Gus-transformed isolates of OY and OA. Approximately 30% of the lines were resistant and 30% were susceptible to both pathogens and 20% of the lines responded differently to OY and OA. Four accessions, PI 542196 (R), PI 604136 (R), PI 330486 (S) and PI 604117 (S) were selected to develop recombinant inbred populations for genetic analysis and molecular mapping of the resistance genes. Among 615 wheat microsatellite markers screened, 51% and 43% were polymorphic between the parents of crosses PI 330486 × PI 542196 and PI 604117 × PI 604136, respectively. Polymorphic markers are currently being used to estimate a linkage map and locate the resistance genes to chromosomal regions. This research provides the first evidence of eyespot resistance in *Ae. longissima*. The identification of molecular markers tightly linked to these genes will facilitate their transfer to wheat and broaden the genetic diversity of eyespot resistance.

Long-term survival and seed transmission of *Acidovorax avenae* subsp. *citrulli* in melon and watermelon seed

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Seed transmission of *Acidovorax avenae* ssp. *citrulli* (Aac) is a key factor in the dissemination of bacterial fruit blotch of cucurbits. In this study, we report seed transmission of Aac from 34-year-old watermelon seed (*Citrullus lanatus*) and from 40-year-old melon seed (*Cucumis melo*). The seed lots used for this work were held in refrigerated storage at 4C to 5C for 31 and 33 years, respectively, before being moved to freezer storage at –18C. Each seed lot was planted in a plastic tray and covered with a clear plastic bag supported by a wire frame. The plastic bags helped raise the humidity to near 100% and prevented cross-contamination between trays. Symptomatic plant tissue was tested with ELISA immunostrips, and bacterial isolations were made from positive plants. Pathogenicity testing was done by toothpick inoculations of both 'Edisto' melon and 'Crimson Sweet' watermelon seedlings. Isolates were further confirmed as Aac-positive by PCR. The longest previously reported survival for any seedborne bacterial pathogen was 24 years, for *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* from common bean seed. The fact that *Acidovorax avenae* ssp. *citrulli* can survive for 40 years or more on seeds indicates that the bacterium is highly tolerant of desiccation and aging. These findings suggest that *Acidovorax avenae* ssp. *citrulli* has the potential to survive as long as the seed is viable.

Development of multiplex SNP assays for multiple disease resistance in tomato

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Phytopathology 99:S119

Marker assisted selection (MAS) has become very important and useful in the selection of disease resistance genes in tomato (*Lycopersicon esculentum*). Single nucleotide polymorphism (SNP), because of its abundance and high-throughput scoring potential is becoming a powerful tool in genome mapping, association studies, diversity analysis, and tagging of important genes in plant genomics. The objective of this research was to develop multiplex SNP assays for MAS of multiple disease resistance genes in tomato. Gene-derived SNP markers were discovered for the seven genes, *Asc-1* of Alternaria stem canker (*Alternaria alternata* f. sp. *Lycopersici*) resistance, *I-2* of Fusarium wilt (*Fusarium oxysporum* f. sp. *Lycopersici*) resistance, *Mi1.2* of root-knot nematode (*Meloidogyne incognita*, *M. javanica*, and *M. arenaria*) resistance, *Sw-5* of tomato spotted wilt virus (TSWV) resistance, *Tm-2* and *Tm-2*^{2} of tomato mosaic virus resistance, and *Ve1* of Verticillium wilt (*Verticillium dahliae* and *V. albo-atrum*) resistance. One 10-SNP multiplex assay, consisted of one SNP for *Asc-1*, two SNPs for *I-2*, one SNP for *Mi1.2*, two SNPs for *Sw-5*, two SNPs for *Tm-2* and *Tm-2*^{2}, and two SNPs for *Ve1*, was developed and verified to be useful in identifying and selecting the seven resistance genes in different tomato germplasm.

Molecular markers for *Tm-2* alleles of Tomato mosaic virus resistance in tomato

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Marker assisted selection (MAS) has become very important and useful in the selection of ToMV resistance genes. Tomato mosaic virus (ToMV) is one of the most infectious viral diseases in tomato (*Lycopersicon esculentum*). The practical and effective method of disease control is using resistance genes. So far, three genes *Tm-1*, *Tm-2* and *Tm-2*^{2} conferring ToMV resistance have been reported and used in tomato cultivar regressions. The objective of this research was to identify *Tm-2* and *Tm-2*^{2} allele-specific PCR-based markers, CAPS (cleaved amplified polymorphic sequences) markers, and gene-derived SNP (single nucleotide polymorphism) markers for MAS in tomato breeding. One allele-specific PCR-based marker and one allele-specific CAPS marker were identified for *Tm-2* and *Tm-2*^{2}, respectively. Two gene-derived SNP markers were specially developed for the *Tm-2* locus. These markers are useful in identifying and selecting *Tm-2* and *Tm-2*^{2} resistance genes in different tomato germplasm.

Characterization of PPR1 and PPR2, genes encoding regulatory subunits of protein phosphatase 2A, in *Fusarium verticillioides*

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Phytopathology 99:S119

Fusarium verticillioides causes maize ear rot and stalk rot. The fungus also produces fumonisin B1 (FB1), a mycotoxin linked to disorders in animals and humans. A cluster of genes, designated *FUM* genes, plays a key role in the synthesis of FB1. However, our understanding of the regulatory mechanism of FB1 biosynthesis is limited. Previously, we demonstrated that Cpp1, protein phosphatase type 2A (PP2A) catalytic subunit, negatively regulates FB1 production. PP2A is a holoenzyme with a structural A subunit, a catalytic C subunit, and a regulatory B subunit. Significantly, there are two regulatory subunits in *F. verticillioides* genome, Ppr1 and Ppr2, which are homologous to yeast Cdc55 and Rts1, respectively. The aim of this study was to characterize the role of *F. verticillioides* PP2A regulatory subunits in FB1 biosynthesis and fungal development. We generated *PPR1* and *PPR2* gene-deletion mutants, and the homologous recombination with *HPH* gene was confirmed by PCR and Southern analyses. The *ppr2* mutant showed drastic decrease in growth rate and conidia production, and displayed intense violet pigmentation on V8 agar plates. We also observed precocious hyphal branching during conidia germination in *ppr2*. Significantly, when grown in defined liquid medium *ppr2* mutants produced lower level of FB1 when compared to that of wild type. Phenotypic characterization of *ppr1* mutants is in progress.

Bacteriophages of *Erwinia amylovora* from British Colombia, Canada

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Nineteen bacteriophages active against *Erwinia amylovora*, the causal agent of fire blight, were collected from apple and pear orchards in the Okanagan and Fraser valleys of British Colombia. The phages were isolated from the soil surrounding trees with a history of fire blight. To avoid a single-host selection bias, seven local bacterial host strains were used in the initial isolation and enrichment processes. Twelve survived the isolation, purification and storage processes. Based on the morphology of the plaques produced, the bacteriophages isolated were divided into five groups. Preliminary work in determining the morphology of the phages with transmission electron microscopy (negative staining) indicates classification as either *Myoviridae* or *Siphoviridae* of the order *Caudovirales*. The phages were classified in three groups according to host range. The first group effectively lysed 87% of the 18 *E. amylovora* hosts tested, the second group lysed 57% and the last group 31%. Their ability to control fire blight was tested by pear blossom assays using *Pantoea agglomerans* as a carrier for the bacteriophages, and four were tested on potted trees in a screenhouse. The molecular characterization of the phages with restriction endonuclase digestions is in progress.

Using bioinformatics in the characterization of novel putative virulence determinants of *Phytophthora infestans* expressed *in planta*

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Phytophthora infestans, the causal agent of potato late blight, has been extensively studied in the past few years in terms of the molecular strategies it uses to cause disease in plants. Recently, its genome has been extensively studied as well as the virulence factors it encodes. However, no high-throughput study has been conducted to determine the whole proteome expressed *in planta*. We designed and implemented a bioinfomatics pipeline to characterize pathogen's genes expressed during the host invasion. For this purpose we mined data contained in cDNA libraries obtained from *P. infestans* infected host plants. Several of the genes found have been previously reported as the Crinklers, the elicitin genes and pectate lyases among others. However, we discovered genes that have never been reported as *P. infestans* virulence factors like several types of transporters and other proteins that are putatively secreted by the pathogen. We will also discuss the degree of conservation of these genes among the genomes of other plant pathogenic oomycetes.

Survey of fungal, nematode and virus diseases in soybean fields in Alabama

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A soybean disease survey was conducted in Alabama in 2008. Evaluations were made on foliar, stem and root diseases, plant viruses, and plant-parasitic nematodes. Forty commercial fields were surveyed in August or September. A one acre section of each field was used for disease evaluations. Downy mildew (*Peronospora manshurica*) was found in 72.5% of the fields surveyed and was the most common foliar disease observed. Cercospora leaf blight (*Cercospora kikuchii*) and target spot *(Corynespora cassiicola)* were observed in 45% of the fields, respectively. Charcoal rot (*Macrophomina phaseolina*) was found in 12% of the fields surveyed and was the only root and stem disease observed. *Bean pod mottle virus* (BPMV) and *Tomato spotted wilt virus* (TSWV) were detected in 58% and 26% of the fields surveyed, respectively. This is the first report of TSWV on soybeans in Alabama. Reniform nematode (*Rotylenchulus reniformis*) was found in 32% of the fields surveyed and was the most common plant-parasitic nematode detected. Soybean cyst nematode (*Heterodera glycines*), root-knot nematode (*Meloidogyne* spp.) and lesion nematode (*Pratylenchus* spp.) were detected in 11–13% of the fields surveyed.

Genomic regions associated with resistance to tan spot of wheat

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Tan spot, a major foliar disease of wheat, is caused by an ascomycete *Pyrenophora tritici-repentis*. Linkage disequilibrium can be used to identify genomic regions associated with tan spot resistance. Association analysis utilizing population structure and additive genetic covariance between relatives was conducted on a historical set of 170 bread wheat lines developed at CIMMYT, Mexico with the genetic data generated with 813 DArT and 831 other markers. Tan spot disease reaction data was obtained by screening the 170 wheat lines with *P. tritici-repentis* race 1 isolate Ptr-1. Three experiments were conducted in the greenhouse with each experiment designed as randomized block design with two replicates. Two-weeks old seedlings were spore-inoculated and rated eight days later for disease reaction based on a 1 to 5 lesion type rating scale. Results reveal that genomic regions on short arm of chromosomes 1A, 1B, and 6B and long arm of chromosomes 4A, 6A, 2B, 3B, 5B, and 7B are associated with resistance to tan spot. Some of the genomic regions contributing to tan spot resistance have been previously identified; however, novel genomic regions on long arm of chromosomes 4A, 6A, and 7B, were identified in this study. Findings of this study reveal that CIMMYT wheat germplasm is likely to contain additional novel sources of resistance to tan spot.

Apple trees deficient in Fibrillin 6 are sensitive to biotic and abiotic stresses and exhibit decreased plastoglobule osmiophilicity

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Plastoglobules are compartments within chloroplasts that contain antioxidant compounds such as tocopherols, carotenoids, and plastoquinone. Here, we disrupted the function of the *Fibrillin 6* (*FBR6*) gene, which encodes a highly conserved protein component of the plastoglobule. *FBR6* RNAi knockdown (*FBR6* KD) plants were more susceptible than control plants to *Erwinia amylovora*, the bacterium that causes fire blight disease, in shoot inoculation tests. *FBR6* KD plants were also more sensitive to ozone, excess light, paraquat, and hydrogen peroxide. *FBR6* KD plants accumulated higher levels of peroxides following ozone treatment and superoxide anion during *E. amylovora* infection than did control plants. Transmission electron microscopy revealed that *FBR6* KD plant chloroplasts had fewer osmiophilic plastoglobules than control plant chloroplasts; plastoglobules are osmiophilic because their antioxidant contents reduce osmium tetroxide. This finding suggests that FBR6 may facilitate the accumulation of antioxidant compounds inside plastoglobules. Lower amounts of antioxidants in *FBR6* KD plastoglobules may affect the ability of the plant to deal with oxidative stresses. We propose that normal plastoglobule function has been disrupted in *FBR6* KD plants, causing increased sensitivity to oxidative stresses, thus leading to higher susceptibility to fire blight and higher sensitivity to excess light, oxidative compounds, and the herbicide paraquat.

The N-terminus of the *Erwinia amylovora* HrpN protein is dispensable for its secretion but is essential for its virulence and avirulence activity

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The HrpN (harpin) protein of *E. amylovora* is an essential virulence factor secreted via the type three secretion system (T3SS). HrpN is also responsible in part for *E. amylovora* avirulence in non-host plants such as tobacco. In a previous mutational analysis, an internal section between amino acids 189-254 was identified as essential for HrpN secretion by *E. amylovora* cells. In addition, the C-terminal portion of HrpN was found to be essential for its virulence and avirulence activity; in contrast, none of the N-terminal mutations examined affected HrpN virulence or avirulence activity. In this study, we created a series of deletions in the region of amino acids 2-200 of HrpN and tested the function of the mutant proteins in *E. amylovora*. Most of the mutant proteins were expressed and secreted by *E. amylovora* cells growing in culture, indicating that the N-terminus of HrpN is dispensable for its secretion via the T3SS. In contrast, most other proteins secreted via the T3SS are known to have N-terminal secretion signals. Our results also narrowed down the internal section of HrpN that is required for its secretion via the T3SS to amino acids 201-254. Interestingly, none of the HrpN mutant proteins with N-terminal deletions had detectable virulence activity on immature apple fruits or avirulence activity in tobacco leaves. This points to a critical role for the N-terminal section of HrpN in disease and avirulence processes, which was not revealed by previous mutagenesis studies.

PCR amplification of nematode genomic DNA after traditional or alternative storage methods

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Alternative methods forstoring nematode specimens prior to polymerase chain reaction (PCR) were tested against a standard method involving mechanical disruption in worm lysis buffer. Crude genomic DNA preparations were incubated with either an anhydrobiotic Sample Matrix or spotted onto a specially treated filter paper (FTA cards). Rehydrated nematode genomic DNA that had been stored in Sample Matrix gave more consistent PCR results than DNA spotted on FTA cards; DNA remained stable at room temperature or at –20C. Sample Matrix has been used successfully to ship nematode DNA at ambient temperature, thus facilitating exchange of specimens between laboratories.

Micro-budded citrus: A new production system for huanglongbing management

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Citrus micro-budding is a successful technique developed to propagate scion buds on small rootstocks, saving time and money. The cost of a micro-budded tree is approximately one-third that of a conventional nursery tree, and the cost of planting is almost an eighth. In addition, micro-budded trees are known to be precocious, producing fruit 1–2 years ahead of T-budded citrus trees. These reduced costs and early fruit production are important factors that would help manage plant disease control strategies. In addition, these features bring an opportunity to plant high density orchards for shorter-term cycles to circumvent plant disease pressure. Citrus greening or Huanglongbing (HLB)

is an extremely devastating bacterial disease in Florida. Texas and California have the HLB vector, but the HLB has not been detected, so far. Some micro-budded citrus trees are now commercially available in Texas. Results from current and previous plantings and economic models will be discussed, along with field performance of micro-budded trees under psyllid pressure.

The influence of resin components on virulent and avirulent strains of *Fusarium circinatum*

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Progeny obtained from a cross between inter-fertile stains of *Fusarium circinatum*, the cause of pitch canker in *Pinus* spp., were found to be incapable of causing disease on pines. Experiments were conducted to test the hypothesis that the progeny in question were avirulent due to their inability to tolerate monoterpene components of resin, which pines produce in response to injuries and infections. Growth and spore germination were evaluated in a saturated environment of volatile resin components: either limonene and β-pinene individually, or a mixture of resin components similar to that found in *Pinus radiata* (68% β-pinene, 30% α-pinene, 1% limonene and 1% myrcene). Spore germination was not significantly affected. Radial growth was inhibited by resin components, but more so in virulent than in avirulent strains. For *Fusarium subglutinans*, which was included as an avirulent control, both radial growth and spore germination showed significant inhibition, relative to virulent and avirulent strains of *F. circinatum*. None of the resin components tested induced significant differences in radial growth among isolates of *F. circinatum* that differed in virulence to pines. Thus, tolerance of resin appears to be necessary but is not necessarily sufficient for pathogenicity to pines, and quantitative differences in the level of tolerance are not predictive of differences in virulence.

Diverse and overlapping communities of the Botryosphaeriaceae on native and non-native trees in Southern Africa

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The Botryosphaeriaceae are widely distributed and important tree pathogens, but are also well known to cause endophytic infections in leaves, branches and stems of trees. In Southern Africa these fungi have mostly been characterized on non-native hosts of commercial interest. Preliminary studies have shown that some of the Botryosphaeriaceae occurring on these hosts also occur on related native vegetation. To better understand this pattern we characterized the Botryosphaeriaceae on pairs of related native and non-native trees in Southern Africa, including *Syzygium cordatum* (Waterberry) and *Eucalyptus* spp. in the Myrtaceae, *Sclerocarya birrea* (Marula) and *Mangifera indica* (Mango) in the Anacardiaceae, and African *Acacia* spp. and Australian *A. mearnsii* in the Fabaceae. More than 40 species in the Botryosphaeriaceae were identified, numerous of which have not been previously described. Only a few of these species overlap in occurrence on the related native and non-native tree hosts, while many only occur on one of these hosts, despite the evolutionary relationship of the hosts and their geographic overlap. Many of the species restricted to one host was also limited to a specific area. The species that do overlap on the different hosts, however, often appear to be amongst the most virulent and frequent, and are thus important in causing disease and for targeting in control programs.

Root-lesion nematode tolerance reactions among wheat and barley genotypes

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Root-lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) are widely distributed in the wheat-producing regions of the Pacific Northwest and significantly reduce wheat yields in Oregon. Comparisons of grain yields in aldicarb-treated and untreated soils were made to determine if differences in genotypic response could be identified among wheat and barley genotypes. Field experiments were conducted at two locations infested primarily either by *P. neglectus* or *P. thornei*. Phenotypic responses for tolerance to *Pratylenchus* sp. at each location differed ($P < 0.01$) among 45 genotypes of spring cereals examined over two years (6 comparisons; 2 trials × 3 replications/trial) but not among 22 and 45 genotypes of winter cereals examined over three years (9 comparisons). These tests described the phenotypic reaction for spring cereals at the 82% confidence level and it was predicted that 40 comparisons were required to attain the 95% confidence level. Up to 56 comparisons would be required to achieve the 95% confidence level for describing the lesion nematode tolerance reactions in winter cereals. These calculations indicated that this method was not practical for routine screenings of wheat and barley. Emphasis for reducing damage in *Pratylenchus*-infested soils should be focused upon minimizing the frequency of host crops and developing cereals with genetic resistance.

Fusarium crown rot tolerance reactions among spring and winter wheat genotypes

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Phytopathology 99:S121

Crown rot caused by *Fusarium pseudograminearum* is widespread and reduces yields of wheat in the Pacific Northwest (PNW). Observations of crown rot symptoms in traditional breeding and yield testing nurseries have been too variable to accurately describe tolerance differences among wheat cultivars. Experiments were established to determine if differences in genotypic responses could be more-accurately defined in inoculated field plots. Grain yields for spring and winter wheat genotypes were compared in inoculated and noninoculated plots in randomized complete block experiments at two locations over two years. Significant differences ($P < 0.01$) in tolerance were identified among spring but not winter wheat entries. PNW-adapted spring wheat genotypes exhibited a range of tolerance reactions comparable to Australian standards for tolerance and intolerance to *F. pseudograminearum*. Calculations revealed that spring wheat tolerance reactions could be defined at the 95% confidence level using 23 yield comparisons (experiments × replicates). However, more than 75 yield comparisons would be required to accurately define the crown rot phenotypic tolerance reaction for winter wheat entries, making this field screening method impractical for winter wheat in the PNW.

Logistic regression modeling of dollar spot epidemics using weather variables as inputs

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Dollar spot, caused by *Sclerotinia homoeocarpa*, is the most damaging disease of many turfgrasses in Oklahoma. A reliable dollar spot prediction model would be useful for making management decisions for high-value turfgrasses. Logistic regression was used to develop a model that input weather variables to predict probability of the occurrence of dollar spot on creeping bentgrass putting greens. Numbers of disease foci were determined daily in plots receiving no fungicide or treated preventatively or curatively with fungicide in the spring and fall seasons of 2008. Various on-site weather variables were recorded hourly. Weather data were transformed to 2-, 3-, 4-, and 5-day moving averages. Weather data and class variables (season and fungicide application) were used as independent variables and disease data as dependent variables in logistic regression analysis to identify best fitting models. Models using 5-day moving averages were better than models using other moving averages. Relative humidity was the only highly significant ($P = 0.0006$) weather variable. The best models also included season and fungicide application class variables. Temperature variables were not significant ($P = 0.60$), but minimum thresholds for disease symptom appearance were established to determine when to implement the predictive model. The model will be validated for use in determining the risk of dollar spot development to aid in fungicide application decision making processes.

Efficacy of bumble bee disseminated biological control agents (BCAs) for control of Botrytis blossom blight of rabbiteye blueberry

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Botrytis blossom blight caused by *Botrytis cinerea* may cause severe bloom and crop loss in rabbiteye blueberry, necessitating applications of expensive fungicides. Commercial bumble bees *Bombus impatiens* were tested as precise vectors of fungicidal biological control agents (BCAs) against blueberry blossom blight. The bees carried two BCA products: Prestop® *Gliocladium catenulatum* and Mycostop® *Streptomyces griseoviridis*. A single bumble bee hive and 4 flowering blueberry plants were confined within each of ten 1.5 m^3 Lumite®/PVC cages. A tubular V-shaped dispenser containing ~1 ml of test product affixed to each hive's entrance applied product onto the bodies of exiting worker bees. After collecting flowers and plating dissected floral parts onto agar, stylar infection rates were high for the BCAs: 100% for *S.*

griseoviridis and 70% for *G. catenulatum*. In trials in small blueberry fields, dispersal distances of UV-fluorescent dye and BCA stylar infection rate confirmed that two small bumble bee hives have the potential to effectively treat about one third of open blooms with BCA in as few as 8 days. Ten days after hive placement in the field, Botrytis inoculated flower clusters on bee-visited stems (Prestop treatment) had more white corollas (71%) and a lower disease incidence than unvisited flower clusters (52% white corollas). These results indicate that bumble bees can vector sufficient BCA to blueberry flowers to reduce floral damage caused by *B. cinerea*.

Resistance to *Cronartium ribicola* in whitebark pine – family variation and effect of inoculum density

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Phytopathology 99:S122

Whitebark pine (*Pinus albicaulis*) is a keystone forest species in many high elevation ecosystems in western North America. Its viability in many areas is threatened by several factors, including white pine blister rust, caused by the non-native, invasive pathogen *C. ribicola*. A petition in December 2008 by NRDC to list the species under the Endangered Species Act is pending. Genetic resistance offers the best option for survival and restoration of this species. For resistance testing, seedling progeny from field selections in natural stands are artificially inoculated with basidiospores by placing infected leaves of the alternate host, *Ribes* spp., above the pines. Sets of progenies from 18 field selections from OR and WA and one bulk seedlot from WY were inoculated at two spore densities (~1000 and ~5000 spores/cm^2) with a local OR population of rust. Needle infection and subsequent stem infection (SI) and mortality levels were very high at both spore densities, but variation in the latter two was evident among families. Survival four years after inoculation was similar across the inoculum densities (19.0 and 14.4%), and families varied from 0 to >50% survival. Families with higher survival generally displayed fewer stem infections, a lower percentage of trees with SI, and a higher percentage of trees alive with SI. Screening of hundreds of additional families of whitebark pine is now underway, and restoration plantings are being planned.

Family variation in *Phytophthora lateralis* resistance in Port-Orford-cedar: Greenhouse and raised bed testing

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Phytopathology 99:S122

Phytophthora lateralis is a non-native, invasive pathogen that kills Port-Orford-cedar (POC, *Chamaecyparis lawsoniana*) in both horticultural and forest settings. It has spread throughout much of the Pacific Northwest of North America and has heavily impacted both the horticultural and forest use of POC. Two short-term screening tests are used to examine genetic resistance in young seedling families: (1) a root dip test (RD) in the greenhouse (bottom 2 cm of roots exposed to the pathogen) and (2) an outdoor raised bed test (RB) in which seedlings are planted in soils infested with *P. lateralis*. 84 families were evaluated in a RD test, along with a subset of 45 families in a RB test; mortality was assessed over a period of nearly 3 years in the RD test and 2 years in the RB test. Overall mortality was slightly higher in the RB test, but many families ranked similarly, especially for percent mortality. The susceptible control reached 100% mortality in <120 days in both tests. Recent extension of the RD test from one-year to three-year duration has provided further clarification of differences among families. Two different patterns of resistance appear to be present among families in RD testing: (1) some families showed 50 to 100% survival and had little or no additional mortality after 12 months; (2) other families had additional latent mortality, with some individuals dying in the second or third year of the test.

Genus *Pestalotiopsis* species infecting *Vaccinium meridionale* in Colombia

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Phytopathology 99:S122

In a previous work the fungal genus *Pestalotiopsis* was identified as a pathogen of *Vaccinium meridionale*. However, since this genus is frequently isolated from plants aerial organs, and there is variability among symptoms produced by different isolates then there is a concern about the species involved. Using only morphological characters was not enough for species determination; consequently PCR using ITS4 and ITS5 markers was performed. Besides that, based on their aggressiveness selected fungal isolates were inoculated on detached leaves and plants. The PCR results showed that 3 different *Pestalotiopsis* species were present in our collection: *P. microspora*, *P. sydowiana* and *P. vismiae*. Although the 3 species induced foliar necrosis some differences were detected. *P. microspora* was the only specie inducing venial chlorosis, and colonized the leaves faster than the other two. The lesions produced by *P. sydowiana* expanded at a very slow rate, and were smaller than those produced by *P. microspora*. However, *P. sydowiana* was the only species affecting shoot apical bud. Lesions produced by *P. vismiae*, were randomly distributed on the leaf surface, and it was the only species that failed producing acervuli. Our results show that different species of the genus *Pestalotiopsis* are able to induced different symptoms on *V. meridionale*. Further studies are necessary in order to determine if their impact on *V. meridionale*'s health is also differential.

Impact of fungicide and insecticide application on infection of soybeans by *Phomopsis longicolla*, BPMV and SMV

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Phytopathology 99:S122

Soybean viruses, bean leaf beetles, soybean aphids and *Phomopsis* spp. all affect soybean seed quality and yield. However, the effects of management practices on interactions among these pests and pathogens are not well understood. To understand these effects, three experiments were established in 6 locations in Iowa during 2008. These consisted of fungicide-insecticide combination trials, bean leaf beetle and soybean aphid management trials. We evaluated the impacts of fungicides, insecticides and soybean varieties (susceptible and partially BPMV-resistant) on frequency of *Phomopsis* infection on seeds, virus-*Phomopsis* interactions and control of virus vectors. Stem and seed infection were evaluated by stem plating and blotter tests. Beetle-feeding damage, cumulative aphid days and soybean yields were compared among treatments. In fungicide-insecticide trials, the treatment that included the insecticide-fungicide combination resulted in lower *Phomopsis* incidence of soybean stems ($P \leq 0.0001$), fewer cumulative aphid days ($P = 0.01$) and a higher yield ($P \leq 0.0001$) than the simple insecticide or fungicide applications or nontreated plants. Treatments including insecticide application showed significantly fewer cumulative aphid days in soybean aphid management trials and less *Phomopsis* infection in stems ($P = 0.01$) and beetle-feeding damage ($P = 0.01$) in bean leaf beetle management trials. Seed infection by *Phomopsis* spp., BPMV, and SMV will be reported for each experiment.

Cellulase enzymes as a biocontrol mechanism for *Phytophthora cinnamomi* in mulching systems

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Phytopathology 99:S122

Phytophthora cinnamomi is a limiting factor in many perennial production systems. Wood-based mulches are currently used in avocado production and are being tested in Fraser fir production for reduction of Phytophthora root rot. Research with avocado systems has suggested a role of cellulase enzymes produced by mulch-inhabiting organisms in providing disease protection, through their direct effect on the cellulosic cell walls of *Phytophthora*. This mechanism, if confirmed, could provide improved control opportunities through enhancement of cellulase activity or direct application of cellulases. This work was undertaken to determine whether cellulase production in mulch could account for disease suppression in these systems. Standard curves were developed to correlate cellulase activity in mulches with concentrations of commercial cellulase formulations. *P. cinnamomi* was exposed to a range of enzyme concentrations, and data were collected on biomass and sporangia production. Cellulase activity levels in field-applied mulches were six to 39-fold higher than in surrounding soil. Preliminary trials indicate these levels may directly impact *P. cinnamomi* growth and sporangia production. Amendment of mulch with cellulytic fungi did not enhance disease suppression beyond that achieved with unamended mulch. Cellulase activity may be a primary factor accounting for the pathogen inhibition observed in Fraser fir mulching systems.

Adapting disease forecasting models to coarser scales: Global potato late blight prediction

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Phytopathology 99:S122

Many predictive models of plant disease rely upon fine-scale weather data collected in hourly increments, or finer. This data requirement is a major constraint when applying disease prediction models in areas of the world where hourly weather data are unreliable or unavailable. In response to the need to apply predictive models when only coarse weather data are available,

we developed a framework to adapt an existing potato late blight prediction model, SimCast. WorldClim offers freely available GIS climate data sets, providing worldwide temperature and precipitation data for current conditions and future scenarios predicted by climate change models. However, the temporal scale of the climate variables is a monthly average. We constructed a model that uses monthly weather averages to estimate SimCast results that would have been obtained based on hourly data. We applied this model with WorldClim current climate condition data to calculate the number of monthly non-systemic fungicide applications necessary for late blight disease control in a world map of potato growing areas. Model output was verified with survey data indicating fungicide applications necessary to manage potato late blight in Peru. We are using the model to calculate the impact of resistant cultivars on the need for fungicide applications relative to a susceptible cultivar, and to estimate potential environmental impacts.

Bacterial canker of sweet cherry – infection of horticultural and natural wounds, wound healing, and spread with contaminated pruning tools

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Phytopathology 99:S123

Bacterial canker, caused by *Pseudomonas syringae* pv. *syringae* (*Pss*), is a major disease of sweet cherry trees and is particularly serious in many parts of Oregon. A sweet cherry orchard, cv. Sunset Bing on Gisela 6 rootstock, was planted in 2006. Trees were inoculated with a suspension of 3×10^8 cfu/ml of *Pss* isolate KM406 throughout the 2006 and 2007 seasons at 7 times that corresponded to cultural operations that cause potential sites for infection. These sites included heading cuts, scoring cuts, dormant and summer pruning, leaf scars, and early and mid-winter low temperature injury. Studies also were conducted on the length of time needed for pruning wounds to heal and the potential spread of bacterial canker with pruning tools contaminated by cutting through active cankers. Infection occurred in all wound site types, and incidence ranged from 32 to 100%. Inoculation of heading cuts and leaf scars each resulted in 50% tree mortality. Canker length and tree mortality were least for freeze injury in early winter and dormant pruning wounds. Pruning wounds made in summer healed within 1 to 2 weeks. Pruning wounds made in winter required about 2 to 3 weeks for healing to occur. *Pss* was not spread by contaminated loppers when cutting was done in January or August.

Hypovirus mediated recovery of blight infected American chestnut trees in Michigan

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Phytopathology 99:S123

American chestnuts at six populations were monitored from 1996 to 2008. Two populations were experiencing epidemics of chestnut blight caused by Cryphonectria parasitica. Two other populations displayed signs of recovery caused by the invasion of hypoviruses that infected C. parasitica. Trees at the final pair of populations were initially disease-free, but blight entered one site in 1997 and in 1998 at the other. Survivorship was 67% in initially disease-free populations. However, all but one death was associated with blight infections that entered the population after monitoring began. 96% of the 251 large trees in recovering populations survived from 1996 to 2008. 27% of the 77 large trees in epidemic populations died over the 12-year period. Growth of large and small trees varied across years and among populations. Year effects were most likely due to annual variation in weather patterns, while local habitat differences presumably underlie differences among populations. Disease status had a significant effect on growth of large trees. Growth of large recovering and disease-free trees was small but positive over the period. In contrast, diseased trees displayed negative growth. Large stems of these infected trees often died and were replaced by smaller stump sprouts. Growth and survivorship of small trees (DBH < 10 cm) were not associated with disease classification. Our data suggest that hypoviruses can promote ecological recovery of American chestnut trees.

Spatial and temporal occurrence of large patch disease in Northwest Arkansas

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Phytopathology 99:S123

Zoysiagrass has become the most widely used turfgrass for high-end golf course tees and fairways. Large patch, caused by *Rhizoctonia solani* AG2-2 (LP), is the most destructive and wide-spread disease of zoysiagrass in the transition zone that includes Northwest Arkansas. The disease primarily attacks leaf sheaths in the spring and fall, causing irregular patches of necrotic turf up to several meters in diameter. The objective of this study was to determine the spatial and temporal distribution of large patch across seasons. Three 14 m × 7 m plots were permanently marked around areas where large patch disease was active. Patch locations in a plot area were recorded using a template divided into 100 cm^2. Mapping of large patch symptoms indicated that patches occurred in the same area from season to season and the diseased area increased over time even though fungicides were applied. Aerification at one course when disease was active likely caused numerous new patches to develop. Current management practices are inadequate and should consider the cumulative effects of disease over time. Furthermore, large patch was observed to develop earlier than anticipated in late summer, indicating the current recommendations for timing fall fungicide applications may be too late.

Effect of plant age and leaf maturity on the susceptibility to soybean rust caused by *Phakospora pachyrhizi*

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Phytopathology 99:S123

Soybean (cv William 82) was evaluated for the susceptibility to rust caused by *Phakospora pachyrhizi* on soybean leaves at different stages and ages of development grown in the greenhouse and field. Evaluations were conducted using detached leaves placed on water agar in petri plates in a growth chamber (72°C) and on leaves left attached to plants. Leaves from the 2nd, 5th and 7th nodes from plants established over 30 days were inoculated with a uniform suspension (4000 spores/ml) of urediniospores. After two weeks of incubation, leaves were evaluated for lesion number, number of pustules per lesion and overall rust severity. Assay results from attached and detached leaves from greenhouse and field plants for these three traits were positively correlated. The leaf node position and plant age significantly affected disease, whereas number of lesions and severity of the detached and attached leaf assays from greenhouse or field plants were highly correlated for all the nodes except the 5th node of greenhouse plants. Attached leaves from older plants were more susceptible to disease, possibly due to microclimate.

***Olpidium bornovanus*: A root pathogen?**

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Phytopathology 99:S123

Vine decline of cantaloupe, a disease characterized by a sudden and field-wide collapse of mature fruit-bearing plants, has historically been attributed to *Monosporascus cannonballus*, a soil borne, root-infecting ascomycete. In 2008, *Melon necrotic spot virus* (MNSV) was reported as the probable cause of vine decline of melon in the field in Guatemala. This virus is seed borne and transmitted (in a vector-assisted manner) by *Olpidium bornovanus*, a soil-borne, root-inhabiting chytridiomycete. Although the virus has not been reported to occur in the USA since its original discovery in a greenhouse in Riverside, California in 1979, our studies showed that the fungus is common in soil samples collected from commercial cantaloupe fields in both Arizona and California. Additionally, our greenhouse studies indicate that this obligate holocarpic fungus, while commonly regarded as a nonpathogenic parasite, is, in fact, a pathogen of cantaloupe. Extensive root rot and significant reductions (ca. 60%) in vegetative growth were recorded within 28 days following inoculation of plants with pure culture (zoospores obtained from a single sporangium) of the fungus. No virus was detected in *O. bornovanus* infected plants. The possible involvement of this fungus in vine decline of cantaloupe in the field is under investigation.

Germination of *Monosporascus cannonballus* ascospores in the rhizosphere: A host-specific response

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Germination of survival structures of soil-borne fungal and fungal-like pathogens is generally nonspecific and readily induced by root exudates from hosts as well as nonhosts. The specificity of ascospore germination of *Monosporascus cannonballus* in the rhizosphere of twenty-six species/cultivars of plants belonging to 8 families and 14 genera was examined. With the exception *Cucurbita maxima* (which has been employed as resistant rootstock for grafting of susceptible melons scions), ascospore germination and germling attachment to plant roots was highly specific and occurred only in genera and species of plants belonging to the Cucurbitaceae (six species/cultivars of *Cucurbita, Citrullus,* and *Cucumis*). Additionally, the

numbers of ascospore germlings attached to cultivars of *Cucumis melo* were minimally 3-fold higher (194–325 ascospore germlings/root system) than on root systems of all other cucurbit genera or species (3–60 ascospore germlings/root system). The latter indicates that qualitative and/or quantitative differences in root exudate(s) exists among various cucurbit species. No ascospore germination occurred in the rhizosphere of any of the other 11 plant genera and 13 species assayed, which included crops such as alfalfa, cotton, wheat, corn, sorghum, and broccoli that are commonly used in rotation with melons.

Detecting Phytophthora in recycled nursery irrigation water in East Texas

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Phytopathology 99:S124

Recycling irrigation water is a common practice in East Texas nurseries. Several different methods were evaluated in an attempt to develop a robust and sensitive method of evaluating the impact of Phytophthora on nursery production. Ponds were tested by either filtration of 1, 10 or 100 ml water samples, floating leaf baits in ponds for 24 hours, or placing leaf baits in 1-gallon water samples for 24 hours. Of the seven ponds tested in 2007, 47 of the 49 samples tested positive. An additional 6 ponds were added in 2008. Of the 44 samples tested in 2008 and early 2009, 31 were positive. Filter methods frequently estimated higher populations with lower quantities of water, suggesting inhibition of spore germination at higher volumes. Bacterial contamination became more apparent during the summer resulting in failure of the filtration methods. When colonies were isolated from BPARPH agar from leaf baits, directly inverted filters, or spreading of washed filters, 22%, 60%, and 88%, respectively, of the isolates showed evidence of bacterial contamination. Quantification using filter methods did not function well in the summer for this region. Baiting methods allowed for more successful recovery of isolates for speciation and was most effective at detecting the presence of Phytophthora in recycled nursery irrigation water.

The glassy-winged sharpshooter vector of *Xylella fastidiosa* harbors a phytoreovirus

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The glassy-winged sharpshooter (GWSS) vector of *Xylella fastidiosa* harbors a phytoreovirus species designated as *Homalodisca vitripennis* reovirus (HoVRV). Double-shelled isometric virus particles purified from GWSS adults resembled those observed in thin sectionsof GWSS salivary glands by transmission electron microscopy. Complete nucleotide sequences determined for 12 dsRNA segments indicated that HoVRV is a distinct virus species most closely related to the phytoreovirus *Rice dwarf virus* (RDV). Terminal nucleotide sequences of HoVRV positive-sense RNAs were similar to other phytoreoviruses with adjacent imperfect inverted repeats potentially able to base pair. Phylogenetic analyses confirmed placement of HoVRV in the genus *Phytoreovirus* sharing a most recent common ancestor with RDV. Yields of dsRNA recovered from individual GWSS adults indicated that HoVRV can replicate to high titer in the insect. Reverse transcriptase-polymerase chain reaction assays revealed that HoVRV infection of GWSS in California and the Carolinas was common. Currently, GWSS is the only known host of HoVRV; no plant host has been identified.

Impact of initial disease levels on development of strawberry powdery mildew epidemics and the benefits of clean stock plants

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Phytopathology 99:S124

Field trials at Ås, Norway and Geneva, NY, USA revealed a substantial impact of initial levels of powdery mildew (*Podosphaera macularis*) upon disease development in strawberry (*Fragaria* × *ananassa*). Five-row plots of 50 to 100 mildew-free plants (planting distance 0.45 × 1.2 m) were established each year within large grain fields with a minimum distance of 90 m between plots and were left untreated with fungicides. Beginning each season, plants at the center of each plot were inoculated with 0, 1, 10, or 100 mildew-infected leaflets. Uninoculated plots developed only trace levels of powdery mildew, despite nearby diseased plots and wild strawberries in both Norway and NY trials over 3 years. More rapid and severe disease development was observed in the inoculated plots; *e.g.*, mildew was observed on 31, 36, 311, and 912 leaflets in the 0, 1, 10, and 100 plots, respectively, in Norway in year 1 of the trial, and reached only 6 leaves per plot in the 0 plot in year 2. Spatiotemporal analyses were subsequently performed, but the practical impact of the trials was to demonstrate long-lasting benefits of clean, mildew-free planting stock in commercial strawberry production, even in areas where the pathogen is endemic and the environment is very conducive to disease.

Population of Bean pod mottle virus in Mississippi

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Phytopathology 99:S124

Bean pod mottle virus (BPMV; genus Comovirus, family Comoviridae) is the most widespread virus of soybeans in Mississippi. Recent, in-depth studies on this virus reported the emergence of recombinant/more severe strains of this virus in other regions of the USA, which prompted this study on the local population in Mississippi. Soybean samples were collected from production and research fields throughout Mississippi, ascertained for BPMV infections by ELISA, and submitted to further lab analyses via reverse-transcription polymerase chain reaction (RT-PCR). A few BPMV isolates from Arkansas, Missouri and Louisiana were also included for comparative purposes. Four sets of specific primers were employed to generate sequence data of the viral helicase, RNA-dependent RNA polymerase (RNA-1), movement protein and coat protein (RNA-2) of each isolate. Generated sequences were analyzed and compared using proper softwares. Phylogenetic trees were generated for all four genome regions. Representative isolates were selected from the pool of molecular data for their biological characterization on a set of herbaceous indicators.

Infection of soybean plants from a commercial field by more than one pathotype of *Phytophthora sojae*

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Phytopathology 99:S124

Management of *Phytophthora sojae* relies on the incorporation of resistant genes (*Rps* genes) into commercial soybean cultivars through plant breeding. This approach has not been entirely successful because new pathotypes of *P. sojae* that overcome resistant genes in these cultivars quickly develop. Several pathotypes have been found to co-exist within the rhizosphere of a single plant in Iowa. Layton and Kuhn (1990) showed in greenhouse studies that more than one pathotype of *P. sojae* can simultaneously colonize soybean, but this has not been demonstrated in the field. Four plants with Phytophthora root rot symptoms were collected from a field in Iowa in 2008. Four, 5, 2 and 2 isolates of *P. sojae* were recovered from plant I, II, III and IV, respectively. All isolates were mono-zoospored and each inoculated onto a differential set of 15 cultivars to determine pathotype. The isolates recovered from plants I, II, and III all belonged to a single pathotype with virulence formula 1b 1k 7. However, the isolates recovered from plant IV belonged to two pathotypes: 1b 1k 7 and 1k 7. Infection by more than one pathotype in a plant could result in outcrossing within the plant lesion that may lead to the development of new forms of virulence. The genetic variability of these isolates using microsatellite markers will be presented. Layton, A. C., Kuhn, D.N. 1990. Phytopathology 80: 602-606.

Purifying selection and biased codon usage at the mating locus in *Alternaria*

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Sexual reproduction in ascomycete fungi is controlled by a regulatory locus called *MAT1*. *MAT1* has two alternate alleles or idiomorphs, *MAT1-1* and *MAT1-2*, with highly dissimilar sequences. Many phytopathogenic fungi are considered asexual because they have no known teleomorph, yet still carry functional and expressed *MAT1* genes. The molecular evolution of *MAT1* was explored in *Alternaria,* a putatively asexual fungal genus. For each *MAT1* allele, nucleotide diversity, codon usage and nonsynonymous vs. synonymous (dn/ds) ratios were examined among species. *MAT1-1* had 20% more variable sites and 25% more pairwise nucleotide differences compared to *MAT1-2*. Significant differences ($P > 0.001$) were observed between *MAT1-1* and *MAT1-2* in GC content at the third codon position (GC3) and effective codon usage (Nc). Mean values were 0.571 and 48.497 for *MAT1-1* and 0.616 and 46.064 for *MAT1-2*. Dn/ds ratios at each codon revealed a background of strong purifying selection for both *MAT1* alleles. However, 7 codons in *MAT1-1* were under positive selection compared to only one codon in *MAT1-2*. Our analysis demonstrates that both *MAT1* alleles in *Alternaria* are under

strong purifying selection, but also that *MAT1-1* and *MAT1-2* alleles may be subject to differing selection pressures. The observed purifying selection suggests that *MAT1* has a functional role in asexual fungi, however this role is currently not known.

Characterization of adult-plant resistance in soft red winter wheat to stripe rust

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Phytopathology 99:S125

Stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., has become an important disease of soft red winter wheat in the eastern United States. The objective of this research was to characterize the adult-plant resistance to stripe rust in contemporary cultivars and breeding lines. Seedlings of 50 lines with low to moderate levels of stripe rust in the field were evaluated for resistance to races PST-3 and PST-100. Twenty lines susceptible to both races in seedling were inoculated at heading and evaluated for adult-plant resistance to PST-100 in growth chambers at low (10 to 18°C) and high (12 to 28°C) temperatures. Severity on flag leaves 21 days after inoculation was approximately twice as great at low than at high temperature and averaged 0 (six entries) to 43% (susceptible check) diseased leaf area. Flag-1 leaves were more resistant than flag leaves. Severity on spikes was approximately three times greater at low temperature and averaged 2 to 30% glumes diseased. Resistance in spikes was not always associated with resistance in leaves. These lines appear to have diverse types of adult-plant resistance, some of which provide almost complete protection against the predominant race. Additional experiments with both races are being conducted in the field at two locations and in growth chambers at low and high temperatures to determine the role of temperature in the expression of resistance and whether any of the resistances are race specific.

Cultivar selection for bacterial root rot in sugar beet

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Phytopathology 99:S125

Bacterial root rot of sugar beet caused by *Leuconostoc mesenteroides* subsp. *dextranicum* is a disease problem recently described in the United States, which has frequently been found in association with Rhizoctonia root rot. To reduce the impact of bacterial root rot on sucrose loss in the field, storage piles, and factories, studies were conducted to establish an assay for identifying host resistance. In 2006 and 2007, 21 commercial cultivars were grown in a commercial field, hand dug, and tested in a petri dish laboratory assay. Root slices were inoculated with *L. mesenteroides*, incubated at 30°C, and the diameter of the rotted area was measured after 72 and 96 h. The cultivars were tested in a randomized complete block design with 4 replications. When averaged over both studies, root rot after 96 h in the commercial cultivars ranged from 14 to 37 mm, while the least significant difference was 5 and 7 mm in 2006 and 2007, respectively. The cultivar ranking between studies was correlated at 72 ($r = 0.47$, $P = 0.03$) and 96 ($r = 0.43$, $P = 0.05$) h. The assay allowed for reliable cultivar separation regardless of whether 1, 2, 3, or 4 roots were used per replication. The assay should allow host resistance to bacterial root rot to be improved in sugar beet.

Efficacy of new formulations of Milsana®, conventional and organic Regalia™, in controlling cucumber powdery mildew (*Sphaerotheca fuliginea*)

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A biofungicide based on *Reynoutria sachalinensis* extract, Regalia™, controls powdery mildew and other diseases on many crops by inducing systemic resistance in plants and by inhibiting conidia germination of pathogens. Greenhouse studies were conducted on cucumber powdery mildew (*Sphaerotheca fuliginea*) to compare the efficacy of the new commercial formulations, conventional and organic Regalia™, with the old formulation, Milsana®. Results indicate that Regalia™ in a single application at 0.5% (v/v) provides better control (94.5–99.5% colony reduction) of powdery mildew than Milsana® (83.2–92.4% colony reduction). This could be explained by a higher effective concentration of active compounds in the improved formulations since good correlation between the concentration of the main active compound, physcion, and disease control has been demonstrated in earlier greenhouse studies. In addition to greater efficacy, enhanced chemical and physical properties such as increased solubility, greater suspensibility, increased bloom and homogeneity upon dilution, and compatibility with other pesticides make Regalia™ an effective and efficient new biofungicide.

Vector transmission of *Pineapple mealybug wilt associated virus-2* by *Dysmicoccus neobrevipes* and *Pseudococcus longispinus* in Hawaii

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Pineapple is an economically important tropical fruit crop grown for the fresh fruit and processed product markets. Yield is affected when plants are infected by pineapple mealybug wilt associated viruses (PMWaVs) which are transmitted by two pineapple mealybug species: *D. brevipes* (Cockerell) and *D. neobrevipes* (Beardsley). Although it is well established that these two species are vectors of PMWaVs, transmission parameters such as acquisition and retention have not been evaluated. It is also not known if *P. longispinus* is a vector of PMWaV-2 or if it is involved in the etiology of mealybug wilt of pineapple (MWP). PMWaV-2 was detected in viruliferous mealybugs and infected pineapple plants using two-step reverse transcription-polymerase chain reaction assays. Transmission experiments showed that *D. neobrevipes* achieved 100% transmission efficiency after a 3-day acquisition access period (AAP). *D. neobrevipes* remained viruliferous for up to 3 days after AAP when sequentially transferred to healthy plants at 24-hour intervals over a 7-day period. Maximum retention of infectivity for PMWaV-2 in viruliferous *D. neobrevipes* was 24 hours. *P. longispinus* was also shown to be a vector of PMWaV-2, but required a longer AAP (> 4 days) with lower transmission efficiency (20–50%) than *D. neobrevipes*. MWP symptoms developed in *D. neobrevipes*-infested pineapple plants but were absent in plants exposed to viruliferous *P. longispinus*.

Extracts of *Ascophyllum nodosum* induce systemic disease resistance in *Arabidopsis thaliana* and enhance disease resistance in several vegetable crops

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Commercial extracts of the brown seaweed *Ascophyllum nodosum* have been shown to promote disease resistance on vegetables in greenhouse and field studies. *Fusarium solani* symptoms were suppressed by extracts of *Ascophyllum* in a 2008 watermelon trial in Upper Marlboro, MD. By mid-July, there were more dead plants in the untreated plots ($P < 0.05$, orthogonal contrasts) than in the *Ascophyllum* extract treatments. By late July, 30% of the watermelon plants were dead from this disease in the non-seaweed treated plots vs. 10% in *Ascophyllum* extract treatments. *Alternaria radicina* and *Botrytis cinerea* were suppressed in greenhouse carrot trials. The mechanism of this resistance is largely unknown. The mechanism of induced resistance in wild type Col-0 *Arabidopsis thaliana* plants to *Pseudomonas syringae* pv. *tomato* was investigated following root applications of a commercial *Ascophyllum* extract. Disease symptom development on the leaves was reduced, suggesting that the seaweed extract triggers a systemic resistance in the plants. The extracts also induced disease resistance in a transgenic *Arabidopsis* line (NahG) that does not accumulate salicylic acid. However, the disease resistance was compromised in *jar1* (jasmonic acid resistance 1) mutants. These combined results indicate that commercial extracts of *Ascophyllum* can induce a jasmonic acid dependent systemic disease resistance in *A. thaliana* and enhance disease resistance in vegetable crops.

Diagnosis of plant viruses using FTA Classic Card technology

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Phytopathology 99:S125

In recent years, the practical application of FTA Classic Card technology has been demonstrated for a range of studies including the detection of viral, bacterial and parasitic pathogens. In the present study, we evaluated the usefulness of FTA cards for the collection, shipment and identification of viruses in different crops. Plant samples suspected for virus infections were collected from tomato, chili pepper, cucumber, yardlong bean, cassava and weed hosts in farmer's fields in India, Indonesia and Nigeria, pressed on FTA

cards and shipped to a central location. A simplified method was optimized for eluting the captured nucleic acids from the FTA Cards. The nucleic acid extracts were subsequently used for RT-PCR or PCR amplification of virus-specific genomic segments. A comparison of sequences obtained from cloned DNA fragments with corresponding sequences in the GenBank revealed the presence of *Bean common mosaic virus*, *Chilli veinal mottle virus*, *Papaya ring spot virus*, *Tomato spotted wilt virus*, *Peanut bud necrosis virus*, *Cucumber mosaic virus*, *African cassava mosaic virus* and *East African Cassava mosaic Cameroon virus*. These results illustrate the practical value of FTA card technology in disease surveys and other downstream applications for the diagnosis and molecular characterization of a broad range of plant viruses.

Etiology of almond brown line disease in Northern California

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Phytopathology 99:S126

During Spring 2008, a disease outbreak consisting of declining and dead almond (Prunus dulcis) trees (cv. Winters) grown on plum rootstock 'Marianna 2624' occurred in Northern California. Symptoms included drooped, rolled up pale-green leaves, and stunted growth. Many of the affected trees had snapped at the scion/rootstock union. Stripping of the bark of diseased trees revealed development of a necrotic brown line at the bud union typical of Almond brown line (ABL) disease described previously in California. In earlier studies, budding experiments using chip buds from Peach yellow leafroll phytoplasma-infected peaches reproduced this disease in almond trees on 'Marianna 2624' rootstock, but buds from ABL-affected almonds did not. A study was undertaken to establish the etiology of the agent associated with the disease and develop a molecular assay for detection of the agent. Nucleic acid extracts from the leaves of one affected almond tree, when subjected to amplification by polymerase chain reaction using primers specific to 16S-23S rRNA spacer region of apple proliferation phytoplasma group, yielded a product of 1.6 kb. This positive tree had developed only a partial girdling at the graft union. In contrast, fully girdled trees failed to yield the 1.6-kb product. Sequence analysis of the cloned 1.6-kb product indicated that the agent associated with the ABL disease was pear decline phytoplasma.

The grass symbiont, *Epichloë festucae,* visualized in endophytic and pathogenic states by GFP expression with epifluorescence and confocal microscopy

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Phytopathology 99:S126

Epichloë festucae, a grass-symbiotic (endophytic), ascomycete was transformed with a green fluorescent protein (GFP) reporter gene to aid in visualizing its growth in one of its host grasses, meadow fescue (*Lolium pratense = Festuca pratensis*). Transformants showing bright expression of GFP in vegetative hyphae on culture medium were selected for the experiments. These were inoculated into apical meristems of endophyte-free *L. pratense* seedlings by microsurgery, and the establishment of symbiosis observed by epifluorescent and confocal microscopy. The fungus was efficiently transmitted in new tillers of the plants. Hyphae tended to grow vertically beneath and into the shoot apical meristems. This growth pattern is important for stable symbiosis, allowing the fungus to extend into newly formed leaves and buds. We expect that similar growth into flower primordia promotes latent infection of ovules, hence, seeds, for vertical transmission of the symbiont. Stromata (which cause choke symptoms on the host), formed by untransformed *E. festucae* isolates, were mated with the GFP-expressing transformants to allow visualization of the sexual reproduction process of the fungus. However, no obvious expression of GFP was observed in cryosectioned stromata and perithecia, which may indicate little gene expression by the paternal parent during mating.

Structure-function analysis of the flagellin receptor Arabidopsis FLS2: Glycosylation, cysteine pairs and FLS2-FLS2 association

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Phytopathology 99:S126

FLS2 is a transmembrane receptor kinase of *Arabidopsis thaliana* that activates defense responses upon binding of bacterial flagellin or the flagellin-based peptide flg22. Using immunoprecipitation from plants expressing two different tagged versions of FLS2, we show that the receptor is present in FLS2-FLS2 complexes before and after plant exposure to flg22. Conserved Cys pairs flank the large leucine-rich repeat (LRR) domain in FLS2 and many other LRR receptors. Expression of mutant forms of FLS2 in *fls2* plants demonstrates that the Cys pair N-terminal to the LRR is required for normal FLS2 stability and function while the Cys pair C-terminal to the LRR is not. Even after restoration of mutant protein abundance by expression from a CaMV 35S promoter, the N-terminal Cys pair is required FLS2-FLS2 association, for flg22 binding, and for flg22-dependent defense signaling. Other FLS2 mutations reveal that flg22 binding capability is not required for FLS2-FLS2 association. Interestingly, a truncated form of FLS2 containing only the cytoplasmic kinase domain without the extracellular LRR domain has a dominant-negative effect on wild-type FLS2 function. The FLS2 extracellular domain contains multiple N-glycosylation sites, but is quite insensitive to the disruption of these sites. In contrast, the related receptor EFR can be rendered non-functional by disruption of single glycosylation sites.

Comparison of aflatoxigenicity of corn kernel and soil populations of *Aspergillus flavus*

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Aspergillus flavus is an ascomycete fungus which infects corn, peanuts, cotton and tree nuts and produces carcinogenic aflatoxins. Aflatoxin contamination of corn is a persistent problem in Louisiana and in some years is severe throughout the state. The objective of this research is to determine if the soil population of *A. flavus* consists of both saprophytic and facultative parasitic strains. Five soil samples and 10 corn ears were collected from each of seven corn fields throughout Louisiana. In addition, Francis Deville of Monsanto Company collected 2, 4, 6, and 7 soil samples and corn ears from four additional fields in Louisiana. *A. flavus* was isolated from soil dilutions (280 cultures) and corn kernels (608 cultures) on *A. flavus/parasiticus* agar selective medium. All single spore isolates were grown on rice and aflatoxin B1 was extracted and quantified with high performance liquid chromatography. The mean aflatoxin B1 was 2314 ± 302 ppb and 9464 ± 675 ppb for corn and soil isolates respectively. Analysis of variance was performed on log transformed toxin levels. The amount of aflatoxin B1 was significantly higher (p-value 0.0004) in the soil isolates than the corn isolates. The aflatoxin B1 in the *A. flavus* soil and corn kernel populations indicates a difference between these two populations and there may be differing abilities for strains of *A. flavus* to parasitize corn. Further understanding of these differences could help find a better control of aflatoxin contamination in corn.

Evaluation of integrated management strategies for Fusarium head blight of soft red winter wheat in Missouri

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The University of Missouri has cooperated in a multi-state, multi-year study funded by the U.S. Wheat & Barley Scab Initiative to evaluate the benefits of crop rotation, host resistance and fungicide application for Fusarium head blight (*Fusarium graminearum*, FHB) and deoxynivalenol (DON) management. Trials were established in adjacent fields with either standing corn residue or soybean residue. Within each residue plot, the experimental design was a split plot with five replicates. Wheat varieties and fungicide application served as the whole-plot and sub-plot factors, respectively. In each plot, percent FHB incidence, percent FHB severity, FHB index, percent Fusarium-damaged kernels, yield and test weight were quantified. Grain samples were submitted for DON analysis. For the 2007 trials, mean FHB and DON levels in the corn residue ranged from 0.12 to 38% and 0.25 to 5.6 ppm, respectively, while in the soybean residue FHB ranged from 0.0 to 8.0% and DON levels from 0.25 to 2.0 ppm. In 2008 the mean FHB and DON levels in corn residue ranged from 10.0 to 47.5% and 3.7 to 18.7 ppm, respectively, while in the soybean residue FHB incidence ranged from 5.9 to 32.5% and DON levels from 0.6 to 4.7 ppm. The impact of residue on both FHB and DON levels was significant in both years. FHB was not severe in 2007 and differences between varieties and fungicide applications were not significant. In 2008 FHB was severe and in both residue types the effects of varieties and fungicides were statistically significant.

Colonization of corn (*Zea mays*) by the pitch canker pathogen, *Fusarium circinatum*: Insights into the evolutionary history of a pine pathogen

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Fusarium circinatum is the causal agent of pitch canker, a disease that affects pines in native forests, landscape plantings and plantations. *Fusarium circinatum*

shows a close relationship to the corn-infecting species *F. subglutinans*, based on cross fertility and phylogenetic analysis. *Fusarium subglutinans* is unable to infect pines, suggesting that the common ancestor was not a pine inhabitant. Alternatively, both species might share a common ancestor associated with grasses and if so, *F. circinatum* may have retained some capacity to colonize grass species, such as corn. To test this hypothesis, corn (*Zea mays*) was inoculated separately with *F. subglutinans* and *F. circinatum*. Emergence and survival were monitored over four weeks, after which plants were assayed for extent of colonization. Overall, *Fusarium circinatum* did not inhibit corn emergence or survival, but inoculated plants had 18% lower stem fresh weights compared to controls and the fungus was recovered from roots of all inoculated plants and 70% of stems. Plants inoculated with *F. circinatum* had a 30% higher rate of emergence and survival and a 30% lower incidence of stem infections than plants inoculated with *F. subglutinans*. These results document that *F. circinatum* can colonize corn seedlings, which is consistent with derivation of this species from a grass infecting ancestor. Furthermore, this raises the possibility that grasses could serve as a reservoir of inoculum for susceptible pines.

Fuzzy pedicel: A new disease of banana

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Recently, a transnational banana company attempted to develop single fingers as a product in the US. Product development was impeded by a new problem, "fuzzy pedicel," in which fungal mycelia grew on the cut pedicel surface and made fruit nonmarketable. We identified fungi from 32 symptomatic fruit. *Sporothrix* sp. was most common on the pedicel surface (72%), followed by *Fusarium* spp. (6%) and sterile fungi (22%), whereas *Sporothrix* sp. (72%), *Fusarium* spp. (19%), *Pestalotiopsis* sp. (16%), and *Nigrospora* sp. (22%) were recovered from 2–4 mm beneath the pedicel surface. Isolates of *Fusarium* and *Sporothrix* were characterized morphologically and phylogenetically with, respectively, EF1α and ITS DNA sequences. *Fusarium* isolates were in the *Gibberella fujikuroi* or *Fusarium incarnatum–esculentum* species complexes which contain well-known banana crown rot pathogens. In contrast, the *Sporothrix* isolates were related to previously described environmental taxa; to our knowledge, *Sporothrix* had not been reported previously on banana. In experiments with locally grown fruit of 'Grand Nain,' thiophanate methyl controlled fuzzy pedicel in fruit inoculated with the *Fusarium* spp., but was only slightly effective against *Sporothrix* (20–50% reduction). Thus, the *Sporothrix* colonists may have originated where benzimidazole fungicides are used to control post-harvest crown rot. Fungicides with other chemistries would probably be needed to manage this problem.

***Triticum mosaic virus*: A distinct member of the family *Potyviridae* with an unusually long leader sequence**

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The complete genome sequence of *Triticum mosaic virus* (TriMV), a member in the family *Potyviridae*, has been determined to be 10,266 nucleotides excluding the 3'-polyadenylated tail. The genome encodes a large polyprotein of 3,112 amino acids with the 'hall-mark proteins' of potyviruses including a small overlapping gene, PIPO, in the P3 cistron. The genome of TriMV has an unusually long 5'-nontranslated region of 739 nts with 12 translation initiation codons and three small open reading frames, which resemble those of the internal ribosome entry site containing 5'-leader sequences of the members of *Picornaviridae*. Pair-wise comparison of 10 putative mature proteins of TriMV with those of representative members of genera in the family *Potyviridae* revealed 33–44% amino acid identity within the highly conserved NIb protein sequence, and 15–29% amino acid identity within the least conserved P1 protein, suggesting that TriMV is a distinct member in the family *Potyviridae*. In contrast, TriMV displayed 47–65% amino acid sequence identity with available sequences of mature proteins of *Sugarcane streak mosaic virus* (SCSMV), an unassigned member of the *Potyviridae*. These data together with phylogenetic analyses of polyprotein, CI, NIa, NIb, and CP sequences of representative species of six genera of the *Potyviridae* suggest that TriMV and SCSMV should be classified in a new genus, and we propose the genus *Trimovirus* (Triticum mosaic virus) in the family *Potyviridae* with TriMV as the type member.

Multilocus sequence analysis of *Sclerotinia homoeocarpa* populations from turfgrasses

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Sclerotinia homoeocarpa is the fungal pathogen responsible for dollar spot disease on turfgrasses. This pathogen infects all turf species and is found worldwide. *Sclerotinia homoeocarpa* was first described by F.T. Bennett in 1937. Bennett described the fungus as producing both apothecia and sclerotial structures, both of which are not seen today. This has resulted in a scientific debate where most believe the fungus belongs to the family *Rutstroemiaceae* due to the production of substratal stromata. Isolates of *S. homoeocarpa* were obtained from turfgrass species collected in the United States, Europe and Asia. Four loci (ITS, beta-tubulin, Elongation Factor, and Calmodulin) were amplified using PCR and then sequenced via cycle sequencing. Isolates of *S. sclerotiorum*, *Rutstroemia paludosa* and *R. cuniculi* were also included for comparison. All isolates analyzed thus far are distinct from *S. homoeocarpa* type-isolates described by Bennett in 1937. The results obtained to date indicate that genetic diversity among isolates is dependent on host species rather than geographic location, with isolates from warm- and cool-season turfgrasses separating into distinct clades. Host species is clearly a major factor that determines genetic diversity in populations of *S. homoeocarpa* causing dollar spot in turfgrasses. Additional methods such as mitochondrial gene analysis or microsatellites are needed to provide further resolution into the genetic diversity of *S. homoeocarpa*.

Genetic diversity among strains of *Erwinia psidii*, the causal agent of guava bacterial blight

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Bacterial blight of guava caused by *Erwinia psidii* is a serious disease in Brazil and it has not been reported in any other country. It was first detected in São Paulo state in 1982 and has recently emerged in Distrito Federal (DF) and three states. Little is known about the genetic diversity of *E. psidii.* In this study, 42 bacterial strains isolated from guava trees from DF (33), São Paulo (7), Espírito Santo (1) and Paraná (1) were characterized. All strains showed the same biochemical and nutritional characters as described for *E. psidii* (Rodrigues Neto *et al.* Fitopatol. Bras. 12:345-350. 1987). Pathogenicity tests with detached shoots resulted in wilt, browning of leaves and veins and, sometimes, bacterial exudate, one to twelve days after inoculation, suggesting variability in aggressiveness among the strains. Genetic diversity was evaluated by rep-PCR (REP, ERIC and BOX-PCR), which revealed a mean similarity of 87% among the DF strains, collected in different orchards and years. Similarities of 91 to 100% was observed among 36 strains from São Paulo (6), Espírito Santo (1) and Paraná (1) states, type strain IBSBF435 (ICMP 8426, NCPPB 3555), one strain from DF collected in 1997, and 27 strains from DF collected between 2001 and 2004. Based on rep-PCR results, *E. psidii* populations have little genetic diversity.

Rapid and early detection of *Erwinia amylovora* in pear and apple orchards using loop-mediated isothermal amplification (LAMP)

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Fire blight is frequently an inoculum-limited disease, but weather-based prediction models for fire blight assume the pathogen is present. To improve disease risk assessment, we developed a rapid pathogen detection protocol that utilizes the method of 'loop-mediated isothermal amplification' (LAMP) to target and amplify DNA of *Erwinia amylovora*. The protocol involves sampling bulked, 100-flower cluster samples (one per hectare) and processing the sample wash with LAMP, which requires 1–2 hr to complete. The method reliably detects a single, epiphytically colonized flower in a sample of 100 clusters (~600 flowers). In three experimental orchards inoculated with *E. amylovora*, positive LAMP reactions were attained from nine of nine 100-flower cluster samples; pathogen populations in the floral washes ranged from 1–9 × 10^4 CFU per ml as determined by dilution plating. In commercial orchards located in the Rogue and Hood River valleys, *E. amylovora* was detected by LAMP in flower samples from four orchards, all of which developed fire blight; pathogen populations in the positive washes ranged from 6 × 10^2 to 4 × 10^3 CFU per ml. In another four orchards, all floral washes were negative for *E. amylovora* by LAMP and by dilution plate, and no disease was observed. Overall, detection in commercial orchards coincided with full bloom during high disease risk periods as determined by a weather-based prediction model.

***Cylindrocarpon* species associated with apple trees in South Africa, and the development of a molecular quantification technique from roots**

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Species of *Cylindrocarpon* have a worldwide distribution and are generally considered to be weak or minor pathogens. However, they can sometimes

have a significant economic impact on some hosts especially in synergism with other pathogens. *Cylindrocarpon* species have been shown to play a role in the apple replant disease complex. Very little is known about *Cylindrocarpon* species associated with apple trees in South Africa. Therefore, a survey was conducted in the main apple producing regions of South Africa, which identified four species including *C. macrodidymum*, *C. destructans, C. liriodendri* and *C. pauciseptatum*. *Cylindrocarpon macrodidymum* was the most prevalent species. Pathogenicity tests on apple seedlings revealed that isolates within each species contained pathogenic isolates that varied in virulence. The most virulent isolates were identified among some of the *C. destructans* and *C. macrodidymum* isolates. A SYBR Green™ real-time PCR method was developed for quantification of all four species using *Cylindrocarpon* genus specific primers on pure culture DNA. High resolution melting analyses of amplified PCR products allowed differentiation of *C. pauciseptatum* from the other three species. The real-time PCR method will be used to investigate whether a correlation exists between *Cylindrocarpon* DNA concentration in roots from the pathogenicity trial, and the amount of seedling stunting observed for each isolate in the trial.

Encapsidation of *Soybean dwarf virus* RNAs

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Encapsidation of viral RNAs by structural proteins is a highly specific process and an essential step in the life cycle of most RNA viruses. The RNAs packaged by one soybean (Wisc4) and two red clover (ClAgt1 and ClIL2) isolates of *Soybean dwarf virus* (SbDV) were compared in a preliminary study. Virions purified from SbDV-infected soybean and *Nicotiana benthamiana* tissues were fractionated on 10–40% sucrose density gradients. ELISA using a monoclonal antibody to SbDV detected single peak of virions near the bottom of the gradients. RNA was extracted from each fraction, separated on agarose gels, blotted to nylon membranes and hybridized with a 3'-proximal biotinylated probe. ELISA-positive virions from the bottom of the gradients encapsidated genomic and small subgenomic RNAs in soybeans systemically infected with ClAgt1. In *N. benthamiana*, leaves agroinfiltrated with ClIL2 and Wisc4, only genomic RNAs were detected in virions near the bottom of the gradients. The large subgenomic (Lsg) RNA was detected near the top of sucrose gradients in ELISA-negative fractions from soybean plants infected with ClAgt1 and *N. benthamiana* infiltrated with Wisc4, but not ClIL2. These results suggest that the Lsg was either encapsidated in a different type of ribonucleoprotein complex or was double stranded. The observation that only the Lsg survived heat treatment of virions supported the latter possibility. Hence, the packaging of SbDV RNAs may be isolate and/or host specific.

Grafting watermelon for managing southern root-knot nematode, *Meloidogyne incognita*

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Grafting watermelon scions on cucurbit rootstocks is widely used in Asia, the Middle East, and Europe for managing root diseases. The loss of methyl bromide from the U.S. market has stimulated interest in using grafting for disease and root-knot nematode (RKN) management in watermelon. Five wild watermelon (*Citrullus lanatus* var. *citroides*) germplasm lines, four bottlegourd (*Lagenaria siceraria*) cultivars, one squash (*Cucurbita moschata* × *C. maxima*) hybrid, and one commercial wild watermelon (*C. lanatus* spp.) cultivar were evaluated as rootstocks for 'Tri-X 313' seedless watermelon in a field infested with southern RKN *Meloidogyne incognita* in Charleston, S.C. in 2008. Three wild watermelon lines had significantly less root ($P < 0.05$) galling than non-grafted 'Tri-X 313', and the squash hybrid and bottlegourd rootstocks. The squash hybrid and bottlegourd rootstocks exhibited severe galling (80 to100% of root system). Galling was moderately severe for non-grafted 'Tri-X 313' (56%). The commercial wild watermelon rootstock had 39% root galling. Root galling for germplasm lines derived from *C. lanatus* var. *citroides* ranged from 32 to 42%. *Citrullus lanatus* var. *citroides* germplasm lines may provide a source of resistance that will be useful in developing root-knot nematode resistant rootstocks for watermelon.

Induced resistance in flowers and its effectiveness in suppressing flower-infecting fungi

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Flowers are attractive infection courts for many specialized and unspecialized plant pathogens. Optimal defense theory proposes that flowers, being ecologically valuable tissues with a direct impact on plant reproductive fitness, should be protected by constitutive rather than inducible defenses. On the other hand, since constitutive resistance could deter pollinators and since flowers are ephemeral, it has also been suggested that flowers should invest in the less costly induced resistance to protect themselves. To clarify these conflicting theories, we first tested whether expression of the ISR and SAR marker genes *PINII* (proteinase inhibitor) and *PR4* (pathogenesis-related protein), respectively, in tomato flowers can be induced by methyl jasmonate (MeJA), benzothiadiazole-S-methyl ester (BTH), and 2,6-dichloroisonicotinic acid (INA). Applying MeJA or BTH to the entire plant resulted in overexpression of *PINII* and *PR4* in pistil tissue by 4.5 and 5.8-fold respectively. This was not significantly different ($P > 0.05$) from an average of 2.6 and 3.9-fold when the plants, but not the flowers, were treated with MeJA and BTH, respectively, suggesting that the resistance is systemic. Preliminary data with INA application suggest a similar trend for *PR4* induction. The effectiveness of induced resistance in protecting tomato flowers from infection by *Botrytis cinerea* is being investigated.

IR-4 Project - Fungicide Registration Update

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In 2008 the IR-4 Project obtained new uses of 41 chemicals on many food crops with a total of 999 new chemical uses being registered. New fungicide uses established on food crops in 2008 or to be established in early 2009 include boscalid, chlorothalonil, cyazofamid, cyprodinil, fenbuconazole, fenhexamid, fludioxonil, fluopicolide, myclobutanil, propiconazole, pyraclostrobin, tebuconazole, tetraconazole, thiabendazole, and triflumizole. The Ornamental Horticulture Program submitted 12 data packages to registrants on efficacy and crop safety. The Biopesticide Program funded 30 research proposals (6 Early, 15 Advanced, and 9 Demonstration) to provide data to support expansions of biopesticide labels. Downy mildew of basil was identified as a new problem and cyazofamid and mandipropamid residue studies initiated. Post-harvest projects were initiated for tomato and figs. IR-4 is exploring use of quarternary ammonium products directly on crops. The chlorothalonil risk cup was expanded and residue studies on citrus, guava and lychee initiated in 2009. An international residue program with mandipropamid and difenoconazole on tomato is underway to help determine if data from around the world can be used to establish national residue tolerances. New residue data for Streptomycin use on tomatoes is being developed in 2009. Efficacy trials continue to identify products that aid in the control of Fusarium wilt of watermelon. New crop group established for edible fungi.

Black walnut mortality in Colorado caused by the walnut twig beetle and thousand cankers disease

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Since 2001 widespread mortality of black walnut (*Juglans nigra*) has been reported in Colorado, USA. Affected trees initially show a yellowing and thinning of leaves in the upper crown, followed by twig and branch dieback and ultimately tree death. We report that this mortality is the result of a combination of an expanded geographic range of the walnut twig beetle (*Pityophthorus juglandis*), its aggressive feeding behavior on black walnut, and extensive cankering caused by an unnamed *Geosmithia* fungus associated with the beetle. *Geosmithia* was consistently recovered from the bodies of *P. juglandis* and this insect apparently introduces the fungus into healthy trees during gallery formation. This is the first report of *Geosmithia* as a pathogen of black walnut. We propose the name thousand cankers to describe this disease because mortality is the result of bark necrosis caused by an enormous number of coalescing branch and trunk cankers. Thousand cankers disease is eliminating black walnut along the Front Range of Colorado and poses a grave risk to this species in its native range in eastern North America should the insect/*Geosmithia* complex be introduced.

Determination of an etiological agent causing a novel foliar disease of zoysiagrass

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A new foliar disease was observed on 'Zeon' zoysiagrass fairways at a golf course in Tomball, TX, following Hurricane Ike in October, 2008. Disease

symptoms exhibited prominent elliptical black lesions along the margin of the leaves and leaf sheaths. Copious amounts of dark-gray mycelium were evident on the plant surface after incubation of the diseased sample at 22°C with high humidity for 3 days. The purpose of this study was to identify the etiological agent of this novel foliar disease of zoysiagrass and confirm pathogenicity using Koch's postulates. None of the eighty-four isolates from symptomatic leaves produced a sporocarp in culture. Therefore, identification was accomplished through molecular means. Genomic DNA was extracted from a pure culture. The internal transcribed spacer region of ribosomal DNA was amplified by PCR using ITS1 and ITS4 primers and was sequenced. The resultant DNA sequence was best matched with isolate NK03-1 of Exserohilum sp. (GenBank Accession no. AB245152.1) with 99% identity. In Koch's postulates, foliar lesions were produced on zoysiagrass plants 21 days after zoysiagrass was seeded into soil infested with Exserohilum sp. The lesions were the same as those observed on the original diseased zoysiagrass sample from Texas. Exserohilum sp. was successfully reisolated. Confirmation of Exserohilum sp. as the etiological agent of a novel foliar disease of zoysiagrass was achieved.

Interactions between fungal and bacterial biocontrol agents

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In a practical context, the mixing of microbial agents (strains or species) that have different environmental requirements and different pathogen targets could have profound effects in terms sustainability and efficacy of disease management. In a preliminary attempt to identify any antagonistic reactions, pairings between selected commercial, fungal and bacterial biocontrol agents were studied in culture and on plants. The growth of *Clonostachys rosea* (Endofine) and *Gliocladium catenulatum* (Prestop) was inhibited by *Streptomyces lydicus* (Actinovate), *S. griseoviridis* (Mycostop) and *Bacillus subtilis* (Serenade) but not *Pseudomonas fluorescens* (BlightBan A506) and *Pantoea agglomerans* (BlightBan C91). Growth of *Pseudozyma flocculosa* (Sporodex) was inhibited by all the bacterial biocontrol agents tested. These data indicate that there is a significant risk in mixing fungal biocontrol agents with bacterial agents, particularly those known to produce antifungal compounds.

TurfFiles decision aids for diagnosis and management of turfgrass diseases

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Two internet-based decision aids have been developed to assist turfgrass professionals and homeowners in the diagnosis and management of disease problems. The Disease Identification program (http://www.turffiles.ncsu.edu/diseaseID/) contains information on 26 diseases commonly found in North Carolina. This program guides a user through the diagnosis by a process of elimination based on turf species, month(s) when symptoms are present, stand symptoms, plant symptoms, and fungal signs. Links to detailed descriptions of each disease, including image galleries, are available to the user at any time during the process. The Disease Management program (http://www.turffiles.ncsu.edu/diseasemgmt/) returns a list of active ingredients, ranked according to efficacy, based on the user's input of turfgrass species and diseases present. Up to 5 diseases can be selected at one time, and the active ingredients are sorted based on average efficacy against the selected diseases. The user may then select up to 5 active ingredients to be applied, and a new page is generated for each active ingredient that includes product trade names, application rates and intervals, application instructions, label precautions and restrictions, and recommended strategies to prevent fungicide resistance. Information sheets on each disease are also available, which summarize disease symptoms, factors that foster disease development, cultural control methods, and recommendations on the proper use and timing of fungicide applications.

Response of spring dead spot caused by *Ophiosphaerella korrae* and *O. herpotricha* to fertilization programs and preventive fungicide applications

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Field studies were conducted in Raleigh, NC on 'Tifway' bermudagrass that was artificially inoculated with the spring dead spot (SDS) pathogens *Ophiosphaerella korrae* (OK) and *O. herpotricha* (OH). Inoculation was performed in Oct 2005 using rye grain infested with each species. Fertilization and preventive fungicide treatments were applied in 2006 and 2007, and SDS symptoms induced by each species were evaluated in 2007 and 2008. Nitrogen source significantly impacted SDS development, but OH and OK responded differently. Ammonium sulfate and sulfur-coated urea suppressed OH as compared to calcium nitrate and urea. On the other hand, calcium nitrate significantly suppressed OK when compared to urea, ammonium sulfate, and sulfur coated urea. Fall applications of potassium chloride, dolomitic lime, gypsum, or elemental sulfur exhibited no effect on either SDS pathogen. Preventive applications of azoxystrobin + propiconazole, fenarimol, myclobutanil, or tebuconazole provided effective control of both species in 2007 and 2008. Propiconazole provided significant control only in 2007, and azoxystrobin did not suppress SDS in either year. While OH and OK were controlled effectively by several fungicides, these species responded very differently to nitrogen sources. Further research is needed to clarify the mechanisms by which SDS is influenced by nitrogen source. Fall applications of potassium and other nutrients may not be beneficial in spring dead spot management.

Effect of timing the initiation of fungicide programs for control of spinach white rust

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The effect of timing the initial fungicide application for calendar-based (7-d) and weather-based (advisory) programs for control of spinach white rust (*Albugo occidentalis*) was evaluated over 3 trials. The first application of the 7-d program and the accumulation of infection periods that serve as spray thresholds for the advisory program began at early (first true leaf), middle (7-d after first true leaf), and late (14-d after first true leaf) spinach growth stages. The number of aplications per trial declined from 6 to 4 for the 7-day program and from 3 to 1.5 for the advisory program as initiation was delayed from early to late. Disease incidence (DI = leaves with rust) was 60% and disease severity (DS = leaf area with rust) was 20% in the untreated control. Only the main effects of fungicide, spray program, and initiation timing were significant (P = 0.05). Pyraclostrobin (DI = 5.1, DS = 0.4) was more effective than azoxystrobin (DI = 17.3, DS = 2.1) or zoxamide (DI = 20.5, DS = 3.2). Disease control was better for the 7-d program (DI = 9.8, DS = 1.4) than the advisory program (DI = 22.2, DS = 2.8). For the 7-d program, disease control for the early (DI = 6.2, DS = 0.7) and middle (DI = 6.2, DS = 0.7) timings was better than for the late timing (DI = 16.8, DS = 2.1). For the advisory program, disease levels did not differ among the early (DI = 16.3, DS = 2.1), middle (DI = 23.6, DS = 2.3), and late timings (DI = 23.5, DS = 3.8). Calendar and advisory programs can be initiated after the first true leaf stage as only small differences in disease severity were apparent.

Characterization of the cultivable endophytic bacterial communities associated with roots of HLB pathogen infected and non-infected citrus plants

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Citrus Huanglongbing (HLB) is one of the most destructive diseases on citrus. The diversity of endophytic bacteria found in association with the roots of Huanglongbing (HLB) pathogen infected and non-infected citrus plants was investigated as part of larger study to assess possibility and practicality of using beneficial bacteria to manage HLB. Fifty four morphologically distinct isolates were obtained from surface sterilized roots of infected and asymptomatic citrus plants. We used a detailed approach for screening novel isolates by conducting qualitative and quantitative assays for traits related to mineral nutrition (phosphate solubilization, siderophore production), development (phytohormone synthesis), health (production of antibiotic and lytic enzymes), induction of systemic resistance (salicylic acid production) and stress relieve (production of 1-amino-cyclopropane-1-carboxylate deaminase). For all of these criteria, we observed that asymptomatic plants harbored a significant greater diversity of potentially beneficial bacterial strains. Some of the bacterial isolates from asymptomatic plants were found to possess genes related to antibiotic synthesis and nitrogen fixation. From an assessment of the molecular and physiological characteristics of these bacteria, a short list of isolates are being tested for the possibility to be used to control HLB disease or delay symptom development.

Fusarium wilt of *Gerbera jamesonii* caused by *Fusarium oxysporum* f. sp. *chrysanthemi* and *Fusarium oxysporum* f. sp. *tracheiphilum*

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Gerbera is one of the top ten cut flower crops in America, Europe, Asia and Australia. In United States 100,598,000 gerbera stems (98% in California) were produced in 2004 and the acreage devoted to this crop continues to increase. Recently different outbreaks of *Fusarium oxysporum* wilt were

observed in Italy, Spain and Brazil in conventional and in soilless cultivation system. Six isolates of *F. oxysporum* obtained from infected gerbera plants originating from Italy, Spain and Brazil were tested on different cultivars of host species in pathogenicity tests showing different virulence. The random amplified polymorphic DNA (RAPD) technique was used to determine membership of a different *forma specialis* of *Fusarium oxysporum* of tested isolates comparing them to representatives of the *formae speciales chrysanthemi* and *tracheiphilum*. A close genetic relationship was observed among most of the new isolates from *G. jamesonii* confirming a clonal origin with isolates obtained from different ornamental crop in previous works. They shared RAPD markers with the tested representatives of the *forma specialis chrysanthemi*. Some isolates obtained from *G. jamesonii* in Brazil and Italy were placed in a different cluster, which included representative isolates of *forma specialis tracheiphilum* identifying, for the first time, this pathogen in *G. jamesonii.*

Molecular diversity of *Xanthomonas axonopodis* pv. manihotis in three different agroecological regions in the Caribbean region of Colombia
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Xanthomonas axonopodis pv. manihotis (*Xam*) is the causal agent of cassava bacterial blight and the most important bacterial problem in this crop. A study carried out in Colombia ten years ago to assess the population dynamics of *Xam* showed a strain migration process between different agroecological regions, with a prominent diversity index in the Caribbean region. Aiming to establish the current status of the population structure of *Xam* in the Caribbean region of Colombia, three agroecological zones were selected. Bacterial samples were collected during the second semester of 2008 and the first semester of 2009. Total DNA was extracted from all strains and AFLP analysis was performed with EcoRI/MseI and MseI/PstI primer combinations. The distribution of haplotypes was determined and a clustering analysis established the relationship between haplotypes, as well as their distribution in the Caribbean region. Additionally, virulence-associated genes were sequenced to determine their degree of variability and to assess the presence and nature of selection exerted by the host. The results confirmed haplotype migration between the three different agroecological regions. This could be the result of deficient cultural practices, which represent a potential risk for smaller cassava growers. This study shows the current condition of populations of *Xam* in the Caribbean region of Colombia and it could be used for improvement of the existing bacterial blight control practices.

A tandem affinity purification strategy to isolate proteins interacting with type III secretion chaperones
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Type III secretion systems (T3SSs) are essential to the virulence of many gram negative bacterial pathogens and are used to inject a battery of bacterial proteins, type III effectors, into host cells. Although the molecular function of many type III effectors is still unclear, we know that collectively they enable the pathogens to cause disease. A subset of effectors needs the aid of other accessory proteins, type III chaperones (TTCs), for secretion from the bacterial cell. How TTCs facilitate secretion of their cognate effectors is still unclear. To aid in the elucidation of the roles TTCs play we devised a tandem affinity TTC purification strategy to isolate *in vivo* interacting proteins form the plant pathogen *Pseudomonas syringae* DC3000. To establish the strategy we used a known TTC-effector pair, ShcA/HopA1, and initially employed the widely used TAP tag. We have successfully isolated HopA1 from *P. syringae*, using another tandem affinity tag fused to ShcA. This tag consists of a FLAG peptide followed by a calmodulin binding peptide and an AcV5 epitope. We plan on using this strategy to identify other bacterial proteins interacting with the ShcA TTC.

Transmission ecology of *Grapevine Leafroll-associated Virus 3*
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Grapevine leafroll disease is caused by grapevine leafroll-associated viruses (GLRaVs). Within this virus complex, GLRaV-3 is probably the predominant species in the world. GLRaV-3 has been shown to be transmitted from vine to vine by many mealybugs and soft scales. The introduction of the vine mealybug (*Planococcus ficus*) in California and other regions of the world may result in increasing disease incidence of established GLRaVs. We examined the tissue specificity for GLRaV-3 transmission by *P. ficus* under greenhouse conditions and monitored the seasonal progress of GLRaV-3 in chronically infected vines in a vineyard using quantitative RT-PCR. Our results indicate that there were no significant differences in transmission rate whether mealybugs acquired or inoculated the virus from different tissues. GLRaV-3 was detected in chronically infected vines during early growing season. In spite of high infection rate at all sampling dates, virus populations within plants increased during the summer. The results suggest that GLRaV-3 may be acquired from vines more efficiently during the summer, independent of vector host tissue preference, although further research is necessary to confirm this hypothesis. In addition, the invasion of *P. ficus* in California and other regions posts a threat to increase incidence of grapevine leafroll disease.

Evaluating the role of the *Ralstonia solanacearum* GspC protein in type II secretion substrate specificity
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The type II secretion (T2S) system in *Ralstonia solanacearum* secrets multiple plant cell wall-degrading enzymes and other proteins important for pathogenesis. GspC is an essential component of the T2S system, and is one of two proteins thought to help determine substrate specificity. The N-terminal half of GspC is conserved, but the C-terminal half usually has one of three protein-protein interaction domains. However, in *R. solanacearum* GspC lacks a predicted C-terminal protein-protein interaction domain, and this may contribute to its secreting more proteins than other bacteria. To investigate the role of GspC in the T2S system, we deleted *gspC* in *R. solanacearum* and tested various plasmid-borne genes for their ability to restore secretion of polygalacturonase and endoglucanase enzymes. The wild type and a C-terminally truncated form of *R. solanacearum* GspC, and wild-type *Cupriavidus metallidurans* GspC restored secretion of both enzymes almost to wild type levels. However, wild-type *Burkholderia thailandensis* GspC, which naturally lacks a C-terminal domain, partially restored secretion of polygalacturonase but did not restore secretion of endoglucanase. These results suggest that general recognition of secreted proteins by the *R. solanacearum* T2S system lies in both the secreted protein and GspC recognition. These insights could have broad ramifications, because the T2S system is a critical function for many plant and animal pathogens.

Inheritance of cold temperature tolerance in mycelium of *Phytophthora infestans*
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Temperature influences spore germination, mycelial growth rate, inoculum production and survival of *Phytophthora infestans*, the causal agent of potato late blight. Isolates of *P. infestans* differing in mating type, temperature tolerance, resistance to metalaxyl and genotype were selected as parents for crossing experiments. The objective of this study was to evaluate the inheritance of cold temperature tolerance *in vitro* in the progeny isolates. The mycelium of parental and progeny isolates of *P. infestans* was exposed to –5°C for 5 days (cold exposure) *in vitro* and cold tolerance was analyzed using a digital imaging technique. Cold tolerance was quantified using the percentage relative average reflective intensity (%RARI). This was derived from the average reflective intensity (ARI) of images of the isolates collected after exposure to cold followed by incubation at 18°C for 25 days compared with negative and positive controls. Out of 24 parental isolates tested, 15 were tolerant, three sensitive and six intermediate in tolerance to cold exposure from which nine crosses were performed. The number of oospores germinated *in vitro* varied greatly among the nine crosses tested. In most crosses, more than 50% of the progeny retained cold tolerance phenotypes identical to their parents. The effects of cold temperature on mycelial survival and sporangia germination could have important implications for epidemic development and disease management.

Phenotypic and genotypic characteristics of *Phytophthora infestans* from mating: Determination of inheritance or recombination
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Late blight of potato, caused by *Phytophthora infestans*, remains a threat to the production of high quality potatoes. Crosses were carried out between

isolates that were highly variable for temperature tolerance and identified as tolerant, sensitive or intermediate in tolerance of exposure to –5°C for 5 days. Single oospore isolates of *P. infestans* produced *in vitro* from the selected nine crosses between different temperature tolerance were exposed to several other phenotypic characteristics and their survival was assessed. In this project mating type is used as a biological tool for enhancing the mutation in the pathogen. To determine if temperature tolerance is a genetically inherited trait or occurs as a result of physiological adaptation, the progeny isolates of *P. infestans* from these crosses were assessed by using the phenotypic and genotypic characteristics of progeny such as mating type, isozyme analysis, Simple Sequence Repeats, metalaxyl sensitivity, virulence and pathogenicity tests. Additionally, DNA content of nuclei and nuclear condition of a few isolates was examined with laser flow cytometry to clarify interpretation of inheritance patterns. These data illustrated the variation in different characteristics of the pathogen after recombination or mutation has occurred. Oospores formed at the beginning of epidemics could therefore contribute to the emergence of novel phenotypes within a growing season as well as between seasons.

Epidemiological analysis of multi-virus infections of watermelon in experimental fields in Southwest Florida

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Whitefly-transmitted Squash vein yellowing virus (SqVYV) and *Cucurbit leaf crumple virus* (CuLCrV) have seriously impacted watermelon production in west-central and southwest Florida in recent years. We monitored the progress of SqVYV and CuLCrV and whitefly density in 2.5 acre experimental fields of 'Fiesta' located in Immokalee, FL over the course of 3 growing seasons. Symptoms of CuLCrV were always found before SqVYV and were present as soon as 5 weeks after planting. Symptoms of SqVYV consistently appeared 7 weeks after planting and in 2 of 3 seasons the planting fully collapsed from disease by week 12. The largest number of whiteflies was typically found in the weeks preceding rapid collapse of plants. Preliminary analyses indicated that the degree of association between the two diseases was not greater than would be expected from random arrangement of the two viruses, and that SqVYV was distributed randomly at low incidences, but became more aggregated as disease incidence increased. These results are an indication that the viruses are being introduced independently by whiteflies, although the whiteflies may be emigrating from the same source, with secondary spread being dominated by within-field populations of whiteflies. This is conceivable based on results in which it was discovered that the distribution of the two viruses in individual watermelon plants was somewhat spatially separated. Additional field surveys are in progress to verify and extend these findings.

Response of processing tomato varieties to TSWV under Fresno County, California conditions

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Tomato spotted wilt virus has caused substantial losses in California processing tomatoes. Use of available TSWV-resistant varieties is limited because few have yield or quality comparable to commercial standards. The objective of this study was to compare susceptibility of processing tomato varieties to this virus. In three 4 replication randomized complete block trials, 13 mid-maturity processing tomato varieties were evaluated at the University of California West Side Research Center in Fresno County in 2008. Entries included AB 2, AB 8058, H 2005, H 2601, H 4007, H 8004, H 9780, HM 6898, NDM 5578, NUN 672, PX 1723, SUN 6368 and UG 4305. Trials were transplanted on 16 Apr and 13 May and one was direct seeded on 13 May. The number of plants expressing TSW-symptoms was recorded one to three days before harvest in each one bed by 21.3 meter long plot. Representative samples were tested with TSWV immunostrips. Highest percentage of TSW-symptomatic plants was 20, 16 and 11 in 16 Apr and 13 May transplant, and 13 May direct seeded trial, respectively. Variety AB 8058 has genetic resistance to the virus (SW5 gene) and incidence was very low. Incidence in Sun 6368, without SW5 gene, was similar to AB 8058. Other entries with consistently low incidence included H 2005, H 4007 and UG 4305. Knowledge of relative susceptibility of processing tomato varieties is important for those making management decisions regarding TSWV in this production area.

The transmission and management of Tobacco mosaic virus in a greenhouse environment

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Tobacco Mosaic Virus (TMV) is a troublesome virus in greenhouse production. Experiments were carried out to investigate the ease with which the virus spreads from infected greenhouses plants to non-infected plants through workers clothing, cutting tools, and contaminated greenhouse benches. Two tobacco cultivars (*Nicotiana tabacum* cvs White Burley and Samsun NN were used as indicator plants. Two commonly used clothing materials (Cotton and Polyethylene fibre, Tyvek) for greenhouse dustcoats and latex for gloves were tested as vehicles for transmission of the virus. Workers contact on plants were simulated by applying various contact grades on infected and then healthy plants. The slightest brushing of the materials against infected plants and to healthy ones caused a disease incidence of >70% for cotton, 60% for Latex, and 30% for Tyvek. However, cleaning of these materials using various cleaning agents in a simulated laundry condition eliminated the virus. The efficacy of various disinfectants used in cleaning greenhouse cutting tools depended on the concentration and time of exposure. A simple dip into a 2% dry milk solution, 3% Menno-Florades (MF) or 14.6 g/liter of trisodium phosphate (TSP) eliminated the virus. The potential of these disinfectants to clean contaminated workers clothing, cutting tools and benches may allow reuse of these resources hence cutting down on production costs.

Soybean vein necrosis virus: A new threat to soybean production in Southeastern United States?

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A new soybean disease was identified in 2008 in Tennessee. Symptomatic soybeans were exhibiting vein clearing along the main veins that turned necrotic as leaves matured. Eventually, large portions of the leaves became necrotic. The symptomatic tissues were probed with antisera against 16 known soybean viruses, but none was found associated with the observed symptoms. Double-stranded RNA was extracted from infected plants and used as template for shotgun cloning. The majority of the clones corresponded to a new tospovirus that was given the provisional name of soybean vein necrosis virus (SVNV). Many tospoviruses cross-react in immunological tests, but SVNV did not react with antibodies against impatiens necrotic spot virus or tomato spotted wilt virus. These observations prompted us to further characterize the virus. SVNV shows minimal similarity to characterized members of the genus *Tospovirus* as it shares about 50% amino acid identities in the polymerase of L RNA and about 45% and 40% in the proteins of the M and S RNAs, respectively. Although a molecular detection test is currently available, however, we are working towards the development of an immunological-base assay that allows us to extend screening of soybean fields in Tennessee and the surrounding states for the presence and prevalence of SVNV in the Southeastern United States.

New viruses found in fig exhibiting mosaic symptoms

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It has been established that fig mosaic (FM) is caused by one or more viruses. The hypothesis that more than one virus can cause FM is due to the different intensity and symptom patterns that is often observed on infected plants. There has been limited information on the identity of the causal agent(s), a knowledge gap we aim to close. We have identified at least four new viruses in FM trees (FM virus 1-4). Sequence information of the polymerase region of FMV-1 indicates that is related to European mountain ash ringspot-associated virus and is probably the same virus identified recently and given the name Fig mosaic associated virus. FMV-2 is a typical badnavirus, closely related to *Citrus yellow mosaic virus*. FMV-3 and -4 belong to the genus *Closterovirus* in the family *Closteroviridae* and are different from the recently identified Fig leaf mottle associated virus-1. Detection protocols have been developed for these four viruses. A survey of FM trees showed that FMV-1 is present in all samples whereas FMV-2, -3, -4 were found in subsets of the samples. Transmission trials are under way for the latter three new viruses to identify vectors and better understand their involvement in disease development and severity. Mechanical inoculations onto herbaceous hosts resulted in several plants that developed severe virus-like symptoms but were not infected with FMV-1, -2, -3, -4, indicating that the number of viruses found in FM plants will continue to increase.

Genetic linkage map of *Phaeosphaeria nodorum*, the causal agent of stagonospora nodorum blotch disease of wheat

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A genetic linkage map of the fungal pathogen *Phaeosphaeria nodorum*, the causal agent of stagonospora nodorum blotch disease of wheat, was created. A total of 152 ascospore-derived progeny from a single pseudothecium, which resulted from a cross of two opposite mating type isolates, Sn37-1 and S-81-B13B, was analyzed with AFLP, RAPD, ISSR, EST-derived microsatellite primers and sequence tagged site markers developed from specific genes. The genetic linkage map consisted of 276 molecular markers, and included markers developed from five genes [Glyceraldehyde 3-phosphate dehydrogenase (*gpd*), malate synthase (*Mls1*), mannitol 1-phosphate dehydrogenase (*Mpd1*), mating type (*MAT1*) and RNA polymerase II (*RPB2*)], which were assigned to 21 major linkage groups (LGs). The total length of the 21 major LGs was 1932.1 centiMorgans (cM). The idiomorph mating type gene (*MAT1*) loci was placed in LG 2 and was closely linked to RAPD marker A4-680. On the other hand, 24 molecular markers and 4 gene loci [β-glucosidase (*bgl1*), histidinol dehydrogenase (*Hdh2*), mannitol 1-phosphate dehydrogenase (*Mpd2*), and xylanase (*Xyl 10-2*)] were dispersed in 11 minor LGs. This is the first genetic linkage map reported for this important foliar pathogen of wheat. The availability of a genetic linkage map of this organism would be an important tool to investigate quantitative trait loci (QTL) of biologically important phenotypes and for positional cloning.

Defense gene induction in soybean seeds after infection with *Cercospora kikuchii* and *Diaporthe phaseolorum*

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Phytopathology 99:S132

We measured the expression levels of seven pathogen defense genes in detached soybean seeds after controlled inoculation with mycelial suspensions of *Cercospora kikuchii* (CK), causal agent of purple seed-stain and leaf blight and *Diaporthe phaseolorum* var. *meriodinales* (DPM), causal agent of seed decay and stem canker in order to gain insight into intrinsic basal host defense in developing seeds. Expression of defense genes in pathogen and mock-inoculated green seeds, harvested 35 days after flowering, was monitored over a 48 h post-inoculation period during which both pathogens, but *D. phaseolorum* more rapidly, colonized the seeds. Gene expression measured by RT-qPCR and normalized to soybean actin revealed that six genes were upregulated by CK and four genes were upregulated by DPM. Three of the seven genes, pathogen stress protein 10 (PR10), chalcone synthase 1-6 (CHS), and matrix metalloproteinase (MMP) were upregulated by both pathogens. β 1-3 endoglucanase (PR2) was not upregulated by CK and PR1, PR3 (chitinase I), and LOX5 (lipoxygenase 5) were not upregulated by DPM. LOX5 was induced earlier at 24 h by CK treatment. Results show that the pattern and magnitude of induction of these defense genes in soybean seeds was different for *Cercospora* and *Diaporthe*, but both pathogens elicited a remarkably high expression (55 to 161 fold over the control) of the metrix metalloproteinase (MMP) gene by 48 h post inoculation.

Aflatoxin contamination in peanuts: Evaluation of risk guidelines

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Peanut (*Arachis hypogea*) is an important crop throughout the world. Yield and quality of peanuts are often affected by a number of pests and diseases, including aflatoxin contamination. Aflatoxins are naturally produced by *Aspergillus flavus*-type fungi and are strictly regulated due to their carcinogenicity. Aflatoxin contamination of peanut can occur in the field (pre-harvest) especially if severe late season drought occurs. Pre-harvest aflatoxin contamination can be prevented with irrigation, however, this option is not universally available. Other recommendations for minimizing the risk of aflatoxin contamination of peanuts include providing appropriate amounts of calcium and other soil nutrients to the plants, managing insect pests and nematodes, and by harvesting peanuts at the proper physiological maturity. In the current project we are compiling the various factors that contribute to the risk of preharvest aflatoxin contamination of peanut. For this effort, we sampled peanut fields across Alabama at three times (within 2 weeks of planting, mid season during pod fill and within 2 weeks of digging). Nematode populations were determined from all soil samples; soil calcium, other nutrients and cation exchange capacity were determined from mid-season samples; and aflatoxins were assayed from pod samples collected at or near harvest. Weather data were also collected. From these data a risk score for aflatoxin contamination was calculated for individual field sites and will be presented.

Targeted lignin modification confers tolerance to fungal pathogens in alfalfa

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Lignin modification benefits the biofuel, pulp and paper, and forage industries through improving the accessibility of cell wall polysaccharides to chemical, microbial or enzymatic digestion. However, lignin reduction is suggested to negatively impact plant defense against pathogens, and down-regulation of cinnamate 4-hydroxylase (*C4H*), an early enzyme in the lignin pathway, resulted in increased susceptibility to fungal pathogens in alfalfa. Surprisingly, down-regulation of caffeoyl CoA 3-*O*-methyltransferase (*CCoAOMT*) and caffeic acid methyltransferase (*COMT*) resulted in increased tolerance to *Phymatotrichopsis omnivora*, a destructive fungal pathogen against which there is no known genetic resistance in alfalfa. Wild-type, *C4H*, *CCoAOMT* and *COMT* down-regulated lines were further challenged with several other economically important fungal pathogens of alfalfa. *CCoAOMT* and *COMT* down-regulated lines were also more tolerant to *Colletotrichum trifolii* than the wild-type alfalfa. However, *CCoAOMT* and *COMT* down-regulated lines were neither more tolerant nor more susceptible than the wild-type to other pathogens that were tested. These results suggested that the tolerance conferred by down-regulation of *CCoAOMT* and *COMT* is not common to all fungal pathogens. The induced tolerance of *CCoAOMT* and *COMT* down-regulated lines may result from increased accumulation of monolignols and/or redirection of flux towards the flavonoid pathway.

Towards identification of genes controlling nonhost resistance of *Nicotiana benthamiana* and *Medicago truncatula* to switchgrass rust

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Switchgrass is one of the feedstock crops selected as a dedicated bio-energy crop for cellulosic ethanol production. Diseases could become important factors once a switchgrass variety is grown in monoculture over a long period of time and could negatively impact biomass production and feedstock quality. Rust disease of switchgrass, caused by Puccinia emaculata, is one of the growing concerns in Oklahoma and other parts of the United States. Rust fungi can cause disease in switchgrass but not in many other nonhost model plants including *Nicotiana benthamina* and *Medicago truncatula* due to the phemomenon of nonhost resistance. Initial characterization of nonhost interactions with *N. bentahamiana* and *M. truncatula* has shown that rust spores germinate and form very long hyphae with few appressoria. No direct penetration of the fungus and epidermal cell death associated with attempted penetration was observed, and both nonhost plants showed pre-invasive resistance to switchgrass rust. Consistently, the nonhost interactions were not associated with high accumulation of reactive oxygen species and major changes in gene expression at early stages. However, accumulation of pathogenesis-related proteins was noticed, 7 days post-inoculation. Current progress and initial results from reverse/forward genetic screens to identify genes involved in pre-invasive resistance will also be presented.

Comparison of seed treatments for control of soybean seedling diseases in field soil at three temperatures

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Seedling diseases on soybean frequently reduce seed germination, seedling emergence, stand, vigor, and yield. Environmental conditions shortly after planting play a large role in seedling disease severity. The objectives of this research were to evaluate seed treatment fungicides and identify seedling pathogens in field soil at temperatures typical of early, conventional and late planting. Three cultivars were treated with six fungicide treatments or left untreated and planted in soil from two fields collected in April, May and June. Tests were conducted in growth chambers at 21°C (April soil), 25°C (May soil) or 28°C (June soil). After 18 days, the tests were rated for stand, root rot and plant growth (fresh and dry weight), and isolations were made from the roots and seeds. Seed treatments resulted in greater stands than the control at all three temperatures for the cultivar Archer but not the other cultivars. Archer had the lowest seed quality of the three cultivars. Reduction in root discoloration occurred with some seed treatments at 28°C for the Hope soil. In all temperatures and soils, Pythyum spp. followed by Fusarium oxysporum were the most frequently isolated pathogens from roots. Pathogencity tests of these isolates are being conducted and Pythium isolates are being identified to species.

Susceptibility of *Juglans* and *Carya* species to *Geosmithia*; a cause of thousand cankers disease

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Thousand cankers disease of black walnut (*Juglans nigra*) is caused by an unnamed species of *Geosmithia* that is vectored by the walnut twig beetle (*Pityophthorus juglandis*). Black walnut is extremely susceptible to the disease and has been eliminated from several Colorado municipalities. The beetle and cankers caused by *Geosmithia* have been observed on *J. hindsii* and *J. californica* throughout California and are associated with mortality of both species near Sacramento. The disease was found on a single *J. regia* tree in Colorado, although cankers have not yet been observed in *J. regia* orchards in California. Similarly the beetle and fungus were found on shaded, senescing branches of *J. major* in Arizona, but did not appear to be causing tree decline. *Juglans major* is thought to be the native host of the walnut twig beetle. An assessment of susceptibility of these and other species is being conducted by artificial inoculations in greenhouse and field studies. In preliminary studies, cankers caused by *Geosmithia* developed on *J. microcarpa* and *J. mandshurica* but not on *J. cinerea*, *J. ailantifolia*, or *Carya illinoensis*

***Pythium apinafurcum* sp. nov.: Its morphology, molecular phylogeny, and infectivity for plants**

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During survey of the *Pythium* species in soils of Japan, high-temperature growing *Pythium* strains were isolated from an uncultivated field soil in Wakayama Prefecture. The all six strains showed similar morphology each other, and had complexly branched secondly hyphae, globose non-proliferating sporangia and smooth surface oogonia which have one or two oospores per oogonium. The combination of these characteristics differentiated these strains from the other known species of *Pythium*. Phylogenetic analyses based on sequences of the D1/D2 region of the large subunit ribosomal DNA showed that the all *Pythium* strains were clustered in a single clade which distantly related from the other known clades of the genus. We described these strains as a new *Pythium* species, *Pythium apinafurcum* sp. nov., based on morphology, and molecular phylogeny. The *P. apinafurcum* strains infected non-symptomatically to the roots of seedlings of bermudagrass, cabbage and cucumber in a pot inoculation test.

Characterization of an ATP/ADP translocase in the citrus huanglongbing bacterium, *Candidatus* Liberibacter

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Citrus huanglongbing (HLB), a disease currently threatening the citrus industry worldwide, has been associated with three different species of Alphaproteobacteria known as *Candidatus* Liberibacter. A complete genome sequence was recently obtained via metagenomics for *Ca.* L. asiaticus (Las), the prominent species of the bacteria found within the United States. Because of its rapid spread and devastating effects, efforts are underway to decipher the genetic information found within the genome of this obligate-intracellular pathogen for targets, which may be used to control this deadly disease. One putative protein target encoded by Las is that of an ATP/ADP translocase. This enzyme has been shown to directly import ATP into a cell from its surroundings, thus allowing the bacteria act as an energy parasite on its host. The ATP/ADP translocase identified in Las contains the 12 transmembrane helices typical of this class of proteins and has an isoelectric point of 9.3. Although the bacterial translocase has been characterized in other intercellular parasites such as *Chlamydia trachomatis* and *Rickettsia prowazeki*, it has not been characterized in a plant bacterial pathogen. Here, we have analyzed the use of this system as a possible target to combat the *Ca.* Liberibacter species.

Physical methods for postharvest control of *Cryptosporiopsis perennans*

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This research was carried out to study the colonization of *Cryptosporiopsis perennans* [(Zeller & Childs) Wollenweber (1939)] on fruit surface and to assess the efficiency of physical methods for its control in apples. Scanning electron microscopy of apples inoculated with *C. perennans* showed colonization of lenticels in 'Maxi Gala', and between lenticels in 'Fuji Kiku'. Conidium of *C. perennans* in aqueous suspension were assessed for their susceptibility to heat exposure [at 28°C for 15 seconds (control), and at 45, 50 and 55°C for 15 and 30 seconds], and to UV-C radiation (at doses between 0.018×10^4 and 3.00×10^4 ergs.mm^{-2}, besides the control, without UV-C irradiation). The number of colony forming units was reduced in more than 99% when the pathogen suspension was treated at 50 and 55°C, during 15 and 30 seconds, as well as when treated with UV-C radiation, at doses between 0.75×10^4 and 3.00×10^4 ergs.mm^{-2}. Apples 'Fuji Kiku' inoculated with *C. perennans* were treated with UV-C radiation, at doses between 0.375×10^4 and 1.500×10^4 ergs.mm^{-2}, and subjected to hot water (50°C) spraying during 15 and 30 seconds, in a commercial packing line. In both experiments control fruits received water spraying at 28°C for 15 seconds. Spores collected on fruits treated with different doses of UV-C radiation and times of hot water (50°C) spraying exhibit germination inferior to 94%.

Addition of food preservatives to hydroxypropyl methylcellulose-lipid edible coatings to control postharvest penicillium molds of citrus fruit

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New hydroxypropyl methylcellulose (HPMC)-lipid edible composite emulsions containing food additives or GRAS compounds with antifungal properties were developed. Film disks containing sodium salts of parabens, potassium sorbate (PS), or sodium benzoate (SB) were the most effective to inhibit *in vitro* the pathogens *Penicillium digitatum* and *Penicillium italicum*, the causal agents of citrus postharvest green and blue molds, respectively. Selected coatings were tested *in vivo* on 'Valencia' oranges and 'Ortanique' mandarins to determine their curative (fruit coated after fungal inoculation) and preventive (fruit coated before fungal inoculation) antifungal activity. In general, the curative activity after incubation at 20°C for 7 days was higher on oranges than on mandarins. On coated oranges, coatings prepared with the mixture PS+SB reduced disease incidence and severity up to 85 and 95%, respectively, with respect to uncoated controls. On coated mandarins, the incidence of green and blue molds was reduced by about 65 and 80%, respectively, by the application of a PS+SP (sodium propionate)-based coating. The tested coatings did not provide any preventive activity against both molds. These (HPMC)-lipid edible coatings effectively preserved fruit quality during cold storage and showed promise as nonpolluting commercial alternatives to conventional citrus waxes.

Effect of acibenzolar-*S*-methyl on the management of early blight and target spot of tomato

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Acibenzolar-*S*-methyl, the active ingredient of Actigard (Syngenta Crop Protection, Greensboro, NC), is an elicitor of plant defenses. While labeled for tomato, usage is currently limited to the control of bacterial leaf spot (*Xanthomonas* spp.) and bacterial speck (*Pseudomonas syringae*). In 2008, two field trials assessed the performance of Actigard (8 weekly applications at 0.75 oz per acre) when integrated into a standard spray program that included weekly applications of copper sulfate (2.1 lbs a.i. per acre) mixed with either mancozeb (1.5 lbs a.i. per acre) or chlorothalonil (1.5 lbs a.i. per acre). The addition of Actigard reduced the severity of early blight (*Alternaria solani*) and target spot (*Corynespora cassiicola*) by 22 to 44% over the standard spray program alone, and by 31 to 60% compared to the non-treated plots. In the spring trial, plots treated with Actigard yielded 336 more cartons (25 lbs) of marketable tomatoes per an acre than those receiving the standard alone, and 1,179 cartons more per an acre than the non-treated plots. No yield improvement was observed in the fall trial, due to the late development of disease in the season. Results demonstrate the benefit of including Actigard as part of an overall spray program to manage common foliar diseases caused by bacterial and fungal pathogens of tomato.

Effect of acibenzolar-*S*-methyl on bacterial leaf spot of shrub roses caused by a *Xanthomonas* sp.

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Shrub roses have gained popularity in modern landscapes due to their low maintenance and resistance to many of the diseases that plague the other modern and old garden roses. Recently, a *Xanthomonas* sp. was identified on

shrub rose varieties 'Knockout' and 'Double Knockout' that causes a bacterial leaf spot. This disease can be problematic during vegetative propagation and nursery production during the summer months in Florida. Acibenzolar-*S*-methyl, the active ingredient of Actigard (Syngenta Crop Protection, Greensboro, NC), is a plant defense elicitor with demonstrated efficacy in the control of several bacterial diseases of vegetables. Greenhouse and nursery trials were established to test the effect of Actigard on the severity of bacterial leaf spot on 'Knockout' and 'Double knockout' roses. While lower rates of 0.25 to 0.5 oz of Actigard per 100 gallons were effective at reducing disease severity on potted roses and those in propagation liners, higher rates of 0.75 to 1.0 oz gave the best results. Multiple applications of Actigard (1.0 oz/100 gal) prior to disease development improved bacterial leaf spot control over single applications. Results demonstrate the potential of expanding the use of Actigard to manage diseases of ornamental and nursery species.

Bell pepper endornavirus: Host range, sequence and effect on cellular signaling

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Endornaviruses are dsRNA viruses found in the kingdoms Plantae, Fungi and Chromista. Plant endornaviruses do not appear to cause symptoms and are transmitted vertically. Bell pepper endornavirus (BPEV) has been found infecting many symptomless bell pepper (*Capsicum annuum*) cultivars. The virus was detected also in *C. chinense* and *C. frutescens* by reverse transcription PCR using virus specific primers and by electrophoretic analyses of purified dsRNA. Partial sequence of the BPEV was obtained and analyzed. Amino acid motifs typical of RNA-dependent-RNA polymerase (RdRp), helicase, and a region between the RdRp and the helicase with similarities to UDP glycosyltranfereases were present. Innate immune response is the first line of defense against invading pathogens. In experiments, using a cell-based reporter assay, we tested the effect of BPEV on retinoic acid-inducible protein (RIG-I) and melanoma differentiation-associated protein 5 (MAD5), two receptors for mammalian response to RNA viruses. Preliminary results indicate that the signaling by the RIG-I receptor is inhibited by purified BPEV dsRNA.

Flower infections with *Xanthomonas campestris* pv. *campestris* can result in internal seed infection

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Xanthomonas campestris pv. *campestris* (Xcc) is a seed borne pathogen that causes black rot, a destructive disease of *Brassica* species. Internal seed infections play a major role in disease outbreaks, as they cannot be controlled. We studied when during plant development infections of plants with Xcc can result in internally colonized seeds. In 2007 and 2008, vernalized cauliflower plants of three varieties were placed in tunnels in the Netherlands and inoculated during different stages of plant development, the 8-leaf stage, during forming of secondary stems and during flowering. At harvest time, we found a systemic infection in more than 50% of all inoculated plants, irrespective when they were inoculated. Xcc was found both in the stem base and in the secondary stems at the top. However, only in flower inoculated plants, Xcc was found in disinfected seed, indicating the risks of flower infections. Therefore, we also studies the potential role of pollinating insects in the transmission of Xcc. Flies (*Calliphora vomitoria*) could harbor 10^5 cfu per fly which could survive up to 5 days externally. Artificially inoculated flies, released eight times during blooming of cauliflower plants, resulted in relatively high percentage of internally infected seeds. Xcc was frequently detected on insects collected from a heavily blackrot diseased Brassica crop by TaqMan PCR, but was rarely isolated. The potential role of insects in the epidemiology of Xcc will be discussed.

Antifungal activity of a new broad spectrum bio-fungicide in the controlling of plant diseases

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The global search for enviromentaly safe and residue free plant production solutions has become a major target in the plant protection industry. A new organic fungicide Timorex Gold (TG) containing 23.8% tea tree oil (TTO), effective against broad spectrum of plant pathogenic fungi, while being safe to plant tissue, was recently introduced. TTO is an essential oil steam distilled from the Australian plant *Melaleuca alternifolia*. TTO contains over 100 components, mostly terpenes and their alcohols and is an effective antiseptic, fungicide and bactericide, and has many uses in the natural health and cosmetics industries. In non plant pathogens it disrupts the permeability barrier of living organisms membrane structures. Its use against plant pathogens has not been investigated. In vitro tests showed that TG at 0.001–0.01% inhibited spore germination and at 0.01–0.1% inhibited mycelial growth of phytopathogenic fungi. A single spray of TG as a prophylactic treatment at 0.2–0.5% effectively controlled powdery and downy mildews in cucumbers and grapes and suppressed the existing colonies on mildewed cucumber, tomato or pepper leaves. It reduced sporulation of *Plasmopara viticola* fungus on infected grape tissue. Field trials conducted in 2006–09 showed that TG at 0.5–1% controlled wide range of foliar diseases. TG is an effective product, enviremntaly safe and residue free altenrative against fungal diseases in organic and IPM agriculture and for anti-resisrance program.

Quinoa cultivar resistance to *Peronospora farinosa* f. sp. *chenopodii*

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Quinoa (*Chenopodium quinoa*) is a major source of food in mountainous areas of the Andes. Downy mildew (*Peronospora farinosa* f sp. *chenopodii*) is the major pathogen problem encountered in quinoa cultivation and can cause 100% crop loss. Downy mildew is an obligate parasite that thrives when conditions are cool and wet, which occurs frequently in some Andean regions. With resistance to *P. farinosa* quinoa can increase food supplies and nutritional intake of subsistence farmers. Recombinant inbreed lines have been advanced to the F2:8 generation by single seed propagation. These immortalized lines came from crosses: Chucapaca × NL-6, KU-2 × 0654, L-P × 0654 and NL-6 × 0654. Field evaluations were made in 2008 and 2009, resistance to mildew was based on percentage of severity in replicated F_2 populations at different locations within replicated trials. The same F_2 populations were tested for resistance in a growth chamber with humid conditions that are ideal for *P. farinosa*. Field studies indicate that some populations from the parent NL-6 have are more resistant than the other populations and are have good potential for continued selection. Growth chamber studies show similar results but the resistance does not with stand all the disease pressure.

Peracetic acid treatment of fresh market grapes for post-harvest *Botrytis cinerea* control

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Botrytis cinerea, causal agent of bunch rot, is a common pathogen of stored table grapes. Growers manage bunch rot with multiple fungicide applications in the field and SO_2 during storage. Organic table grape growers have few in-season treatments available and cannot use SO_2 for postharvest management. An organically approved, pre-harvest disinfestation that decreases post-harvest losses would be beneficial for both organic and conventional table grape production. The disinfectant peracetic acid (PAA), which also contains acetic acid and hydrogen peroxide, was applied as 0.05% PAA 1-day pre-harvest to an organic and two conventional Thompson seedless (TS) vineyards. In the organic TS vineyard, rot after 1 month in storage was significantly reduced from 10.4% (fresh weight) in the untreated control to 4.3%; there were no significant differences between a PAA application 1-day pre-harvest and an application the day of harvest. In both conventional TS vineyards, a field application of 0.05% PAA significantly reduced post-harvest rot. In the TS Madera vineyard, a 1-day pre-harvest PAA application reduced rot after two months storage from 7.2% in the untreated control to 1.9%, and after three month storage, from 14.2 to 5.2%. Similarly, in the TS Fresno vineyard, a 1-day pre-harvest PAA spray reduced rot after 37 days in storage from 8.7 to 2.2%. The data suggest that some infections occur post-harvest and that PAA could be a feasible option for *B. cinerea* management.

Evaluation of biofumigant plants and organic amendments for suppressiveness of root rot of *Quercus* spp. caused by *Phytophthora cinnamomi*

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Root rot caused by *Phytophthora cinnamomi* is the most serious disease of Mediterranean *Quercus* spp. in southern Spain. A strategy for disease control in natural agroecosystems is soil amendments. We examined the effects of fumigant plants (*Brassica carinata, B. juncea, Cistus albus, C. ladanifer, Diplotaxis* sp., and *Phlomys purpurea*) and fresh and composted animal

manures (bovine, chicken, pig, and sheep) on mycelial growth on culture media, inoculum potential in artificially infested soil, and root infection of the susceptible host *Lupinus angustifolius*. Inoculum potential was assessed as inoculum density and colonization of *Eucalyptus* leaf pieces in soil bioassays. All treatments reduced mycelial growth of *P. cinnamomi* and complete suppression was reached by all doses of *B. carinata* and chicken manure. Most treatments also reduced inoculum potential of infested soil. Incidence of root infections in *L. angustifolius* seedlings was reduced (> 81%) by all organic amendments. The three most effective treatments under controlled conditions (*Brassica* spp., chicken manure and pig slurry) are being tested at 1.3 Kg per m^2 in two experimental fields with young trees of *Quercus rotundifolia* growing in soils highly infested by *P. cinnamomi*.

The effect of storage temperatures on the growth and virulence of *Penicillium expansum* and *Penicillium solitum*

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Penicillium expansum and *Penicillium solitum* cause blue mold of apples which is an economically important postharvest disease. These two fungi differ in their virulence but both can cause significant losses and their control is necessary during storage. Therefore, we evaluated the effect of storage temperatures (0, 5, 10 and 20°C) on conidial germination, mycelial growth, decay on apples, and activity of polygalacturonase (PG) *in vitro*. Conidial germination and mycelial growth of both pathogens occurred at 0, 5, 10 and 20°C and were temperature sensitive. *P. expansum* and *P. solitum* differed in pathogenicity on fruit as *P. expansum* caused decay at 10 and 20°C, while *P. solitum* induced symptoms only at 20°C. Virulence of *P. expansum* and *P. solitum* also differed as lesions caused by *P. expansum* were larger at all temperatures. No lesions developed on apples stored for 21 days at 0 and 5°C. However, both fungi caused decay on apples when moved from low temperature and allowed to incubated at 20°C. *In vitro* polygalacturonase activity was detectable at 0, 5, 10 and 20°C and was temperature-responsive. Nevertheless, symptoms did not develop at low temperatures because PG may not have been produced or could not overcome host defenses. Results obtained from this study show that fungal growth occurs at common storage temperatures, that purified PG from *P. expansum* and *P. solitum* is active at 0, 5, 10 and 20°C, and that both fungi remain viable in inoculated apple fruit following incubation at low temperature.

Molecular characterization and fungicide sensitivity profiling of *Monilinia laxa* from a cherry orchard in western NY

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Blossom blight and fruit rot caused by *M. fructicola* are a considerable threat to stone fruit production in New York. To date, the presence of *M. laxa* has yet to be confirmed in this region. In late July 2008, a potential outbreak of *M. laxa* was reported after *Monilinia* sporulation was observed on blighted twigs, spurs, leaves and fruit on 'Surefire' tart cherry trees at a commercial orchard in Niagara County, NY. Eleven symptomatic shoots were removed from each of the three diseased trees, and three *Monilinia* isolations were made from each shoot. Sequencing of the internal transcribed spacer (ITS) regions and the b-tubulin gene confirmed the presence of 9 *M. laxa* out of a total of 33 single spore *Monilinia* isolates. Comparison of the ITS regions and b-tubulin gene of the NY *M. laxa* strain demonstrated a high degree of similarity to *M. laxa* strains from Spain and California. Sensitivity of all isolates to the DMI fungicide fenbuconazole, and the QoI fungicide pyraclostrobin, was assessed *in vitro* using mycelial relative growth assays. Percent relative growth of *M. laxa* isolates at discriminatory doses of 0.3 µg/ml fenbuconazole and 1.1 µg/ml pyraclostrobin ranged from 0.0–11.2 and 9.9–36.9%, respectively, whereas *M. fructicola* isolates from the same collection ranged from 9.0–36.9 and 24.4–39.7% respectively. The presence of *M. laxa* in NY may present new challenges for stone fruit producers in the region.

Tracking the timeline of the progression of verticillium wilt infection in tissue of susceptible and tolerant plants of *Mentha longifolia*

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The threat of verticillium wilt, caused by *Verticillium dahliae*, is a serious concern of the U.S. mint industry. Peppermint (*Mentha* ×*piperita*) is susceptible to this fungus, while spearmints (*M. spicata*, *M. gracilis*) are less so. Commercial mint species are polyploid, presenting difficulties as genetic research subjects. Therefore, we are using the diploid mint species, *Mentha longifolia*, as a research model. The U.S. mint industry would benefit from discovery in mint of genes that confer tolerance to infection, so as to prevent widespread crop loss. Knowing the pathogen's location inside the plant during the stages of infection is a step towards identifying genes that might confer tolerance to infection. A GFP strain of *V. dahliae* obtained from Lynda Ciuffetti at Oregon State University was used to infect tolerant and susceptible *M. longifolia* accessions. Confocal microscopy was used to track fungal invasion and dissemination throughout the host plant. The progression of infection in root and vascular tissues was examined at two day intervals post-inoculation. Equally extensive root infection was observed in both the susceptible and the resistant *M. longifolia* accessions. By eight days post-inoculation, the fungus was observed in stem tissue of susceptible plants. Further studies will elucidate the fungal progression into the vascular tissues, and RNA isolation from infected tissues may help determine which genes confer tolerance to *V. dahliae* infection.

Screening for verticillium wilt resistance in diploid and octoploid strawberry germplasm

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Phytopathology 99:S135

Verticillium wilt is a chronic disease problem for strawberries grown in perennial cropping systems. Identification of both resistant and susceptible accessions would provide an opportunity to study the genetic basis for resistance and isolate potentially useful resistance genes. To assess the level of resistance in available germplasm and cultivars, we screened and rated 51 *Fragaria* (strawberry species) accessions for response to root-dip inoculation with a strawberry *Verticillium dahliae* isolate. These accessions included diploid (2x) species *Fragaria vesca*, *F. iinumae*, *F. viridis*, and *F. nipponica*, as well as octoploids (8x) *F. virginiana, F. chiloensis* and *F.* ×*ananassa* (the commercially cultivated strawberry). Appearance of inoculated plants relative to water-dipped controls was rated four weeks after inoculation according to a three-point scale on which 1 = healthy, 2 = moderate disease symptoms, 3 = dead plant. Considerable variation in response to inoculation existed at both the diploid and octoploid levels. Substantial within-species variation provided opportunity for performance of resistant × susceptible crosses within the diploid model species *F. vesca* and in octoploid *F. virginiana*, an immediate ancestor of the cultivated strawberry, as a step toward identifying verticillium resistance genes.

Surveillance and identification of fungal pathogens associated with corn in Puerto Rico

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Phytopathology 99:S135

Pioneer Hi-Bred has corn winter nurseries in the southeast area of Puerto Rico. During 2008–2009 in experimental plots located near Salinas, the incidence and severity of fungal pathogens were monitored. For morphological identification observations of the type of lesion, spore shape and or mycelial appearance on PDA were documented. For molecular identification, DNA extractions and ITS sequencing were conducted at the University of Puerto Rico. The fungal identities (%I) were obtained by submitting the sequences to the NCBI Gen Bank. Southern corn leaf blight, *Bipolaris maydis* (99% I), was the most prevalent disease in the three locations with incidences up to 100% and moderate severity. Symptoms included oval or rectangular spots and were observed in vegetative or reproductive stages of the crop. Common smut, *Ustilago maydis* (99%I), caused galls on the ears during the dent stage in Juana Diaz with incidences under 1% and moderate severity. Southern rust, *Puccinia polysora* (93%I) was found in the three locations during the crop's reproductive stages. Pustules (0.5–2 mm) were circular or ellipsoidal and developed mostly on the upper surface of the leaves. Susceptible germplasm showed incidences up to 100%, however the severity was low. Two different inbred lines each containing the Rpp9 gene showed no sign of sporulation. This suggests the new race of *P. polysora* observed in the southeastern USA in 2008 by Dolezal et. al. is not currently distributed in Puerto Rico.

Development of a real-time PCR diagnostic protocol for Fusarium wilt of palm

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Phytopathology 99:S135

Fusarium oxysporum f. sp. *canariensis* is the causal agent of Fusarium wilt of Canary Island date palm. Fusarium wilt is an important disease that results in the loss of mature palm specimens. Management options involve removing

infected trees as quickly as possible to try to prevent spread to other palms. Rapid and accurate detection is vital to the management of this disease. We evaluated and improved a previously-published PCR detection protocol. Our goal was to differentiate *Fusarium proliferatum,* which is a common saprophyte, from the Fusarium wilt pathogen. Commercial DNA extraction kits were compared with the previously published extraction technique. Additional conventional PCR primer sets were developed and multiplexed with the previous set to distinguish between the two species in one reaction. Real-time PCR primers and probes also were evaluated as part of a new detection protocol for the Cepheid SmartCycler system. The new extraction methods and PCR protocols were evaluated and found to reliably detect *F. oxysporum* f. sp. *canariensis*. The updated protocols distinguished between *F. oxysporum* f. sp. *canariensis*, *F. proliferatum*, and non-*canariensis F. oxysporum* isolates. At the conclusion of the project the new protocols were incorporated into a standard operating procedure (SOP) for diagnosticians, based on the template developed by the National Plant Diagnostic Network.

Label-free detection of soybean rust spores using photonic crystal biosensors

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Soybean rust, caused by the fungus *Phakopsora pachyrhizi*, is one of the most devastating foliar diseases affecting soybeans grown worldwide. The disease was reported for the first time in the United States in 2004. Early spore detection, prior to the appearance of visible symptoms, is critical to effective fungicide management strategies. We have developed a subtractive inhibition assay that involves the use of specific antibodies (both mono and poly, referred to as mAb and pAb respectively) and a photonic crystal biosensor for label-free detection of soybean rust spores. In this assay, the spores and antibodies were mixed, spore-bound antibodies removed by centrifugation, and the remaining unbound antibodies quantified using the biosensor. A detection limit of 2.5 × 105 spores/ml was achieved using an antibody concentration of 0.01 mg/ml (both mAb and pAb). The results were compared to a negative control consisting of corn rust spores that showed the highest signal, meaning no antibody was bound to them. This may be the first step in reaching the goal of developing an economical and field-deployable detection system.

Serenade biofungicide (strain QST of *Bacillus subtilus*): A new tool for control of stem rot (*Sclerotinia sclerotiorum*) in Canola

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Sclerotinia slcerotiorum is a serious soil borne fungus that causes yield-robbing Stem Rot Disease in Canola. Serenade® biofungicide, a strain of *Bacillus subtilis* (QST 713), shows great promise for the management of this disease. Two formulations of Serenade – a wettable powder (MAX) and an aqueous suspension (ASO) – were tested in the field in 2007 and 2008. Each trial was established as a randomized complete block design with four replicates and plots 3 m × 7–10 m. All materials were applied with CO_2 sprayers, employing flat fan nozzles operating at 275Kpa and spray volumes ranging from 46 - 94 L/Ha. Applications were made at approximately 30% bloom. Disease incidence and severity were evaluated at 5–7 seeks after treatment. Yields were also recorded. Serenade displayed rate-related control of stem rot and associated yield increases. The best results were achieved within the rate ranges of 5.0 - 7.5 L/Ha for Serenade ASO and 1.25 - 1.88 Kg/Ha for Serenade MAX. The aforementioned rates resulted in disease control and yields equal to or better than the commercial standard, Rovral®, at 3 L/Ha. A combined analysis of variance across field trials showed the aforementioned rates of the products to significantly (P = 0.05) reduce disease incidence and severity. Only Serenade treatments provided significant improvement in yields. Future efforts will employ more field trials, further refine use rates and make comparisons with a larger array of commercial standards.

Ground-level circumference of loblolly pine saplings is not a significant factor in fusiform rust infection

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The objective of this study was to measure relationships between ground-level circumference of 2 ½ year-old loblolly pine saplings and gall formation. We chose the Arrowhead seed orchard, plantation 133, in Bleckley/Pulaski Counties, part of the Georgia Forestry Commission, because pine rust infection was particularly high in this location. Forty eight families and 20 replications per family were used. We measured diameter at ground level and gall number per tree for 960 trees after 2 ½ years and later counted number of galls on the same trees after 8 year's growth. Our hypothesis is that higher infection by the fusiform rust fungus at 8 years is correlated with larger circumference size at 2 ½ years. There did not appear to be a relationship between the circumference of saplings and the number of galls formed at 8 years. Only saplings measuring 18 cm after 8 years had more galls than any other size class. Circumference of 2 ½ -year old saplings with galls already present at ground level did not seem to differ from those with no galls when measured later at 8 years. Stem circumference and infection of 2 ½ year –old seedlings is not a predictor of rust infection after 8 years.

Foliar biofilms of *Burkholderia pyrrocinia* FP62 on geraniums

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Biofilm formation on foliar surfaces is commonly associated with plants in water-saturated environments (e.g. tropics or modified environments). On most leaf surfaces bacteria are thought to reside in aggregates with limited production of an exopolysaccharide (EPS) matrix. However, the biocontrol agent, *Burkholderia pyrrocinia* FP62, was observed to form structures resembling biofilms that covered several leaf cells in the absence of surface water. The development of these biofilms occurred on geranium plants that were inoculated with a suspension of washed FP62 cells and housed in a greenhouse with bottom watering. Leaf samples were examined with both scanning electron microscopy (SEM) and environmental SEM (ESEM) at 0, 1, 3 and 7 days post inoculation. The ESEM was used in conjunction with SEM to identify artifacts associated with the chemical fixation of SEM samples. At Day 0, bacterial cells were randomly distributed on the leaf surface. By Day 1, EPS and other attachment structures were present; aggregates were observed in regions where inoculum collected (e.g. trichomes and cellular junctions). On Day 3, the aggregates are compact and beginning to cover the plant cell surfaces. By Day 7, aggregates had coalesced to cover multiple plant cells. The bacteria were encased in EPS, visible as an opaque layer or as collapsed strands. These observations indicate that bacterial biofilms are able to develop in unsaturated conditions on the surface of geranium leaves.

The relationship of biofilm production to biocontrol activity of *Burkholderia pyrrocinia* FP62

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Foliar biocontrol agent (BCA) efficacy is often inconsistent due to poor colonization and survival on plant surfaces. *Burkholderia pyrrocinia* FP62, a superior leaf colonist and BCA of *Botrytis cinerea*, forms unsaturated biofilms on plant surfaces. To determine the relationship between biocontrol activity and biofilm formation a mini-Tn*5* mutant library was created and screened for biofilm deficiency in liquid. Mutant 55B1 has a transposon insertion in a region homologous to a *trmE* GTPase gene. 55B1 was complemented by marker exchange (55B1-C). Geraniums were inoculated with suspensions of washed FP62, 55B1 and 55B1-C cells. Inoculated plants were housed in a greenhouse with bottom watering for 0, 1, 3, and 7 days. Leaf populations were measured by sonicating excised leaves in phosphate buffer and plating washates on 5% yeast extract broth agar. Leaf samples were collected pre- and post-sonication, chemically fixed and viewed with scanning electron microscopy (SEM). In another experiment, 1 or 7-day treated plants were challenged with *B. cinerea* for 5–7 days before rating for percent disease severity. Biocontrol activity was reduced in 55B1 and restored in 55B1-C. SEM revealed that despite similar culturable populations, significantly more FP62 and 55B1-C cells remain attached to the leaf surface between plant cell junctions and trichome bases. These findings suggest that biofilm production is vital to the biocontrol activity of FP62.

Evolution of lodgepole pine phytochemical defenses to combat diseases

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It has been hypothesized that pines grown in climates more favorable for disease development have evolved to produce greater amounts of defense-associated compounds, such as phenolics and terpenes, to become more resistant to pathogen attack. In a lodgepole pine (*Pinus contorta* var. *latifolia*) seed orchard consisting of clones collected from provenances across British Columbia, trees showed a gradient of foliar, canker, and gall disease severity,

with certain clones remaining much healthier than others. Phytochemical analyses revealed that increases in needle lignin and tannin levels were associated with reductions in foliar disease severity, and increases in phloem monoterpene content resulted in greater resistance to cankers. An analysis of the origins of the trees used in this study revealed that clones from wetter climates, which are favorable for foliar pathogen spread and infection, had not only higher levels of lignin and tannin in the needles, but also were more resistant overall to the foliar diseases. However, associations between the origin of clones and bark phytochemical profiles were less clear. These results show that pines from populations exposed to climates where foliar disease pressures are likely greater have evolved to produce greater amounts of defense compounds in their needles. Therefore, such pines should be utilized in breeding programs that are designed to increase quantitative resistance to disease.

Races of *Puccinia striiformis* identified in the United States in 2008

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Puccinia striiformis f. sp. *tritici* (PST) and *P. striiformis* f. sp. *hordei* (PSH) cause stripe rust on wheat and barley, respectively. To monitor virulence changes in the pathogen populations, isolates obtained from stripe rust samples collected through collaborators from 18 states in the US were tested on 20 wheat and 12 barley differential genotypes for identifying PST and PSH races, respectively. Ten previously existing PSH races were detected and a new race, PSH-82 (virulent on only Topper and I 5), was identified, of which PSH-33 was predominant. A total of 33 PST races were detected with PST-138 (with all PST-127 virulences except on Produra) as a new race. The most frequent races were PST-114 (virulent on Lemhi, Heines VII, Moro, Produra, Yamhill, Stephens, Lee, Fielder, Tres, Express, *Yr8*, *Yr9*, Clement, and Compair), PST-100 (all virulences of PST-114 except for those on Moro and Tres), PST-116 (all PST-114 virulences plus virulence on Paha), PST-101 (all PST-100 virulences plus virulence on Chinese 166), and PST-98 (all PST-100 virulences except for virulence on Yamhill). PST-127, first detected in California and Washington in 2007, had a big increase in frequency in Washington in 2008. PST-127, virulent on all 20 wheat differential genotypes except for Moro (*Yr10*, *YrMor*), Yr5, and Tres (*YrTr1*, *YrTr2*), is the most virulent race identified to date. More attention should be paid to PST-127 in wheat breeding programs.

High-throughput genetic analysis and association mapping to identify novel genes for resistance to stripe rust in spring wheat germplasm

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Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (PST), is a destructive disease worldwide. Only a few effective resistance genes are available. To identify more effective resistance genes, we crossed 100 spring wheat genotypes selected from our several years' germplasm evaluation with 'Avocet Susceptible'. F_2 plants of 92 crosses were tested using the most predominant and most virulent races, PST-100 and PST-127, respectively under controlled greenhouse conditions and in the field under natural infections in 2007 and 2008. From the tests, 78 F_2 populations were identified to be segregating, of which 27 of the parental genotypes were still resistant in all tests, 41 were resistant in the field but susceptible in the greenhouse seedling tests, and 7 were resistant in the greenhouse seedling tests but susceptible in the field tests. Of the 27 parent genotypes resistant in all tests, 9 were identified to have a single gene each, 17 to have 2 genes, and 1 genotype to have 3 genes. Of the 41 parental genotypes, 11 had a single gene, 27 had 2 genes, and 5 had 3 genes each. Of the 7 parental genotypes, 2 had a single gene and 5 had 2 genes each. The simultaneous bulk segregant analysis (BSA) and association mapping using resistance gene analog polymorphism (RGAP) markers showed that most of the genes are different from each other and many should be different from previously reported stripe rust resistance genes.

Molecular signature of *Erwinia amylovora* virulence

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Erwinia amylovora, the causal agent of fire blight, is considered as a genetically homogeneous species based on physiological, biochemical, phylogenetic and genetic analysis. However, high virulent strains of *E. amylovora* such as Ea273 are isolated in nature. It is well known that the exopolysaccharide amylovoran and type III secretion system (T3SS) are two major virulence factors in *E. amylovora*. In this study, we compared *E. amylovora* virulence by measuring major virulence gene expression and production. Four strains (Ea273, Ea110, Ea1189, and CFBP1430), widely used in studies of *E. amylovora* pathogenesis, were analyzed. The expression of amylovoran production (*amsG*) and T3SS (*dspE* and *hrpL*) genes was much higher in Ea273 and Ea110 than that in Ea1189 and CFBP1430. Consistent with gene expression data, Ea273 and Ea110 elicited severe hypersensitive response on tobacco plants within short period of time than that of Ea1189 and CFBP1430; furthermore, amylovoran production was much higher in Ea273 and Ea110 than that in Ea1189 and CFBP1430. However, these strains had a comparable growth in immature pear fruits. Our results demonstrate that these strains could be separated into two major groups. Our results also indicate that, although *E. amylovora* strains of different origin cause similar diseases, their molecular signature for virulence differs. Therefore, we propose that we could use these molecular signatures to differentiate them, which may have taxonomical and evolutional implications.

Evaluation of fungicide treatments for control of soilborne pathogens of American ginseng

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American ginseng (*Panax quinquefolium* L.), a perennial herb, is valued for its root. Soilborne pathogens *Cylindrocarpon destructans* and *Phytophthora cactorum* cause damping off and root rot of American ginseng which lead to yield loss and replant problems. Successful ginseng production depends on the control of soilborne pathogens in the early stages of the crop and requires effective fungicides. Fungicides were tested for efficacy against either *C. destructans* or *P. cactorum* on seedlings in the greenhouse. Seedlings inoculated with *C. destructans* were dipped into a conidial solution prior to transplanting and treatment. Seedlings inoculated with *P. cactorum* were drenched with a sporangial solution 24 hrs after fungicide applications. Results showed that plants treated with Captan 80WDG and Quadris 2.08SC had the lowest disease severity and plant death (%) caused by *C. destructans* and Topsin 4.5L and Cannonball 50WP treatments limited plant death to ≤50%. Reason 500SC prevented plant death caused by *P. cactorum* and Dithane 75DF, Revus 250SC, Presidio 4SC, Kocide 2000DF and Acrobat 50WP treatments limited plant death to 12.5% by the final rating. Other fungicides, Iprodione 4L, Inspire 250EC, Inspire Super 336EW, Cabrio 20EG, Quadris Top 325EC, A15909A 263.8SE, Tanos 50DF, Moncut 70DF (*C. destructans*), and Tanos 50DF, Phostrol 6.69SC, Captan 80WDG, Ridomil Gold 2EC and Aliette 80WDG (*P. cactorum*), did not significantly reduce disease.

Isolation and characterization of *Phytophthora capsici* from irrigation ponds in southern Georgia

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A simple method was developed to improve the efficiency of recovering *Phytophthora capsici* from fruits used as baits in irrigation ponds. In contrast to direct isolation on agar plates, infected fruit tissues were used to inoculate stems of pepper seedlings, and infected pepper stems were used for isolation. When isolating through inoculation of pepper stems, the frequency of recovering *P. capsici* from infected eggplant and pear fruits increased significantly compared with direct isolation on agar plates. *P. capsici* was isolated from 7 out of 9 irrigation ponds evaluated, with most of the ponds containing both A1 and A2 mating types. All *P. capsici* isolates were pathogenic on squash plants and some isolates were resistant or intermediately sensitive to mefenoxam. Simple sequence repeats (SSRs) were identified through bioinformatic mining of publicly available expressed sequence tags (ESTs) of *P. capsici* and thirty-one pairs of SSR primers were designed and used for analysis of genetic diversity of *P. capsici* isolates from irrigation ponds. Genetic groups were correlated with their pond origins but not mating type, mefenoxam sensitivity, or aggressiveness on squash. This is the first report indicating contamination of *P. capsici* in irrigation water sources in Georgia and the first report of developing SSR markers of *P. capsici* that may contribute to more comprehensive analyses of genetic diversity of this important pathogen.

Differential gene expression in incompatible interaction between wheat and stripe rust fungus revealed by the cDNA-AFLP technique

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Puccinia striiformis f. sp. *tritici* is a fungal pathogen causing stripe rust, one of the most important wheat diseases worldwide. The overall objective of this study is to dissect whole gene expressions that are transcriptionally regulated in wheat in response to the stripe rust pathogen attack using cDNA-AFLP techenique. cDNAs from total mRNA were isolated from inoculated resistant wheat plants (cv. Suwon 11) with avirulent pathotype CY23 at 12 time points post-inoculation. The results indicated about 52, 992 transcript derived fragments (TDFs) generated using 64 primer pairs. Of the total TDFs, 2, 437 (4.6%) displayed altered expression patterns after inoculation with 1,787 up-regulated and 650 down-regulated. A total of 300 TDFs were selected for sequencing and 255 of them produced reliable sequences (>100 bp), of which 113 (44.3%) had known functions involving in energy, metabolism, signal transduction, defense, transcription, protein metabolism and transport. In particular, a large number of genes encoding signal molecules were found to be activated or suppressed as early pathogen responsive genes and potential disease resistance-related genes. Comparative analyses of the TDFs between incompatible and compatible interactions at the protein level showed that majority of these TDFs were shared by the interactions, and only 81 were expressed in the incompatible interaction, 20 of the 81 TDFs were chosen to further validate the expression patterns using qRT-PCR.

Cytochemical localization of reactive oxygen species and peroxidase in the incompatible and compatible interaction of wheat –wheat stripe rust fungus

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The subcellular localizations of O_2^- accumulation, H_2O_2 accumulation and peroxidase activity have been studied by cytochemical methods in the interaction between the wheat cv. 'Suwon 11' and two races of *Puccinia striiformis* f. sp. *tritici* (avirulent and virulent). O_2^- accumulation could be seen in the tonoplast, the plasma membrane and the cell wall of mesophyll cells adjacent to hyphae and necrotic host cells, and main distribution of O_2^- production was in the tonoplast of host cells. The accumulation of H_2O_2 was observed mainly in the cell wall and the plasma membrane of mesophyll cells adjacent to hyphae and necrotic host cells, as well as in the tonoplast of some host cells and in the intercellular space. Accumulation of H_2O_2 was also observed in the cell wall of hyphae; peroxidase was mainly located in the cell wall and the plasma membrane of host cells. The results clarified the distribution of O_2^-, H_2O_2 and peroxidase in wheat and stripe rust fungus interactions and indicated that the subcellular localization of O_2^- accumulation, H_2O_2 accumulation and peroxidase activity in the compatible interaction were similar to the incompatible one, but there were striking differences in the content of ROS accumulation and peroxidase activity in different interactions.

Sequence analysis of the genes of two isolates of Grapevine leafroll-associated viruses from Liaoning Province in China

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The dormant cutting of grapevine cv. Venus Seedless originated from Xingcheng region in Liaoning province with typical leafroll symptom was detected for *Grapevine leafroll-associated virus* (GLRaV) by RT-PCR. The results showed that two complete sequences of major coat protein (CP) gene of GLRaV-2 (GenBank accession number FJ786017) and GLRaV-3 (FJ786016) were obtained, which indicating that the grapevine plant detected was co-infected with GLRaV-2 and GLRaV-3. The genes encoding CPm (minor capsid protein), P19 and P24 located in 3′ genomic RNA of GLRaV-2 Liaoning isolate (LN) were then cloned and sequenced. The nucleotide sequence of GLRaV-3-LN CP was 942 bp in length, and sequence analysis showed that the identities of nucleotide sequence and deduced amino acid sequence among GLRaV-3-LN and other previously reported isolates including ten overseas isolates and two domestic isolates ranged from 89.8% to 91.8% and 94.9% to 97.4%, respectively. The nucleotide sequences of CP, CPm (FJ786018), P19 (FJ786019) and P24 (FJ786018) of GLRaV-2-LN were 597 bp, 672 bp, 486 bp and 618 bp in length, respectively. The relative sequence analysis showed that the nucleotide identities of CP, CPm, P19 and P24 among GLRaV-2-LN and other isolates reported ranged from 88.3% to 100%, 78.9% to 100.0%, 75.1% to 99.4% and 87.5% to 99.5%, and the identities of deduced amino acids ranged from 92.9% to 100%, 89.2% to 100.0%, 73.9% to 100.0% and 89.3% to 99.0%, respectively.

Caught in the act: A field gone suppressive for common scab?

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Potato varieties are evaluated for resistance to common scab (CS) in fields with high CS disease pressure. Occasionally, disease pressure naturally declines in a CS nursery; this is termed disease suppression. We have data on severity of potato CS in a scab nursery in Maine for 6 years between 2001 and 2007. In 2007, the mean CS incidence and severity, as judged both by area affected and type of lesions, was markedly lower than in earlier years. We isolated Streptomyces, the cause of common scab, from scabby potatoes in all 7 years between 2001 and 2007. The ratio of presumably pathogenic isolates (containing genes for biosynthesis of the pathogenicity determinant thaxtomin) to isolates lacking this pathogenicity determinant shifted from about 4:1 to 1:1 between 2004 and 2007, suggesting that the new strains successfully competed with pathogenic Streptomyces in the potato rhizosphere. The competitors belong to different Streptomyces species and grow more rapidly than pathogenic strains. These species have potential for biocontrol of CS.

Characterization of a co-inhabitant of uredinia of Asian soybean rust

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Phakopsora pachyrhizi, causal agent of Asian soybean rust, has been documented to cause yield losses of up to 100% in subtropical soybeans. We had been studying the dynamics of urediniospore production on field-grown infected leaves. In 2007, an apparent mycoparasitic fungus was observed colonizing urediniospores within rust sori. Scanning electron microscope observations revealed intense colonization of uredinia and apparent trophic growth of hyphae from uredinium to uredinium and between urediniospores within a sorus. Inoculation of this fungus onto field-grown rust-infected leaves resulted in a significant reduction in production of hyaline urediniospores within 7 days of inoculation. The fungus colonized rust pustules within 5 days, but it failed to establish on noninfected leaf surfaces. Colonized spores turned dark brown and did not germinate. Implications of these interactions will be discussed.

Inhibition of *Xylella fastidiosa* polygalacturonase to produce Pierce's disease resistant grapevines

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Xylella fastidiosa is a xylem-limited, gram-negative bacterium that causes Pierce's disease of grapevines. X. fastidiosa possesses a single polygalacturonase (PG) gene and it was shown that if the PG gene was disrupted the resulting PG-mutant was non-pathogenic in grapevines. We are using phage panning technology to identify peptides or recombinant single chain fragment variable (scFv) antibodies that can bind to and inactivate X. fastidiosa PG. E. coli recombinant X. fastidiosa PG has measurable enzymatic activity in vitro; however most of the recombinant protein is localized in insoluble inclusion bodies and enzymatically inactive. For this reason we synthesized 2 peptides that are likely involved in PG substrate cleavage and binding. The peptides were used as panning targets and monoclonal phage raised against the peptides also bound to full length X. fastidiosa PG. These candidate inhibitory monoclonal phage will be used in X. fastidiosa PG activity assays to determine if they can inhibit the activity of the enzyme. The ultimate goal of this research is to identify peptides that can inactivate the enzymatic activity of X. fastidiosa PG and express those peptides in transgenic grapevines rootstocks to inhibit or retard the movement of X. fastidiosa in grapevines, thus providing a novel form of resistance against Pierce's disease.

Contamination of the spinach phylloplane by *Escherichia coli* O157:H7: Do house flies play a role in dissemination?

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A 2006 multistate outbreak of illness caused by *Escherichia coli* O157:H7 was linked to consumption of bagged spinach. The objective of this study was to investigate the potential of house flies, *Musca domestica* (Diptera: Muscidae), associated with cattle manure, to transfer *E. coli* O157:H7 to spinach. Flies were exposed to four substrates: autoclaved manure mixed with GFP-tagged *E. coli* O157:H7, GFP-tagged *E. coli* O157:H7 lawns grown on LB ampicillin agar plates, manure mixed with PBS and LB ampicillin agar plates. Exposed flies were caged on spinach plants for 18 hrs. Both leaves and fly labellae (mouthparts) were examined by fluorescence microscopy for GFP-tagged bacteria on their surfaces. Leaf tissues also were tested by PCR for the *E. coli* O157:H7 *eae* gene, and used for bacterial isolation. Fifty percent of the leaves caged with flies exposed to manure mixed with *E. coli* had fluorescing bacterial rods on their surfaces, whereas 100% of the leaves exposed to flies exposed to *E. coli* lawns were visually positive. The fluorescent colonies obtained from the plants exposed to flies acquiring *E. coli* O157:H7 from both manure and lawns were PCR positive for *E. coli* O157:H7. Labellae of flies exposed to *E. coli* O157:H7 lawns showed GFP-tagged bacteria attached to the pseudotracheae. These results suggest that flies are possible vehicles for the transfer of live *E. coli* O157:H7 to the spinach phylloplane under experimental conditions.

Effects of soil pH on Rhizoctonia damping-off of sugar beet and disease suppressiveness caused by antagonistic soil microorganisms

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Effects of soil pH on damping-off of sugar beet by *R. solani* (AG2-2 IIIB) and soil suppressiveness against the disease were studied by comparing disease incidences in pasteurized and non-treated soils after pathogen infestation. Soil pH of 9 cultivated soils ranged from 4.53 to 7.19, and no correlation was observed between soil pH and disease incidence in non-treated soils. Suppressiveness, the difference in disease incidences between a non-treated soil and its pasteurized equivalent, was also not correlated to pH. When an alkaline soil was acidified progressively with H_2SO_4, disease suppression markedly declined, increasing disease incidence in the non-treated soil. Addition of $CaSO_4$ to this soil did not enhance the disease. The opposite was observed when soil pH of an acidic soil was raised progressively by adding $Ca(OH)_2$. Addition of $CaCl_2$ to the soil decreased soil pH, enhancing the disease. Incorporation of dried peanut plant residue into a pasteurized, neutralized soil infested with *R. solani* markedly suppressed the disease, lowering the incidence to the level in the non-treated soil. The identical treatment was much less effective in a pasteurized acid *R. solani*-infested soil, and even enhanced the disease in the same non-treated soil. This indicates that indigenous disease suppressiveness operating in these soils through the activity of antagonistic soil microorganisms can be weakened or nullified in acidic soils since the antagonism is restrained at low soil pH.

Trichothecene production and sporulation by *Myrothecium verrucaria* in response to substrate composition

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Myrothecium verrucaria (IMI 361690) is a candidate bioherbicde for use against kudzu and several other weed species. This fungal strain also produces macrocyclic trichothecene mycotoxins, which hinder its safe production and application. The safety of this biological control agent during production and handling would be improved if an inoculum could be produced without concomitant accumulation of macrocyclic trichothecenes. Sporulation and trichothecene production by *M. verrucaria* was evaluated on standard potato dextrose agar (PDA) and a series of complex and defined media. Sporulation on PDA and on agar media with nitrogen supplied as ammonium nitrate or potassium nitrate was more than ten-fold greater than sporulation on the medium with ammonium sulfate as the nitrogen source, indicating a possible inhibitory role of sulfate on sporulation. Accumulation of macrocyclic trichothecenes was strongly affected by the media composition, with higher levels often associated with higher carbon content in the media. A defined, glucose and ammonium nitrate based medium was identified that yielded the best combination of spore production and minimal trichothecene levels. These results support the hypothesis that accumulation of trichothecenes by this fungus may be independent of sporulation and demonstrates that the bioherbicide can be readily produced without high levels of trichothecene contamination.

Symptoms and distribution of Squash vein yellowing virus in vining cucurbits

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Squash vein yellowing virus (SqVYV) has been identified as the casual agent of watermelon vine decline in south Florida, a disease which is characterized by foliar chlorosis, necrosis and wilt, followed by plant death. Symptoms of wilt and death induced by SqVYV in watermelon have not been observed on any other known host species, all of which are in the family *Cucurbitaceae*. To examine the infection phenotype across a broader range of cucurbits, more than 30 vining cucurbit types and cultivars in 15 species were inoculated with SqVYV and subsequently rated for visual symptoms of virus infection. Virus distribution within plants was determined by nucleic acid hybridization of tissue blots on nylon membranes. Vein yellowing induced by SqVYV was observed on leaves of many cucurbits inoculated but this symptom did not progress to wilt or death in most of the cucurbits analyzed except watermelon. Differences in disease severity were also noted and cucumbers displayed transient symptoms of some upper, non-inoculated leaves followed by apparent recovery. Nucleic acid hybridization of vine and petiole sections confirmed virus presence in symptomatic tissue, and also showed an uneven distribution of the virus within infected plants. The systemic wilt and death that is induced by SqVYV infection of watermelon appears to be infrequent in other cucurbits.

Prediction of DON with Fusarium head blight incidence, severity, index, and *Fusarium*-damaged kernels in winter wheat

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Deoxynivalenol (DON) is a mycotoxin produced by *Fusarium graminearum*, causal agent of Fusarium head blight (FHB) of wheat. Accurate prediction of DON in grain is important in making timely decisions regarding marketing and end-use. In this study, the ability of FHB incidence (inc), severity (sev), and index; and the percentage of *Fusarium*-damaged kernels (FDK) to predict DON was compared. The winter wheat cultivars Jagalene, Harry, 2137, Hondo, Alliance, Infinity, Goodstreak, Karl 92, Wahoo, Millennium, Wesley, and Overley were planted following corn in Oct 2007. Cultivars were arranged in randomized complete blocks with 4 replications. In addition to natural inoculum, plots were inoculated with 1×10^5 spores/ml of *F. graminearum* at early anthesis and were not irrigated. FHB inc and sev were measured 21 days after inoculation and used to calculate index. FDK and DON were measured after harvest. The best predictor of DON measured in grain from heads with visible FHB was index ($R^2 = 0.52$, $P = 0.0082$) and the worst predictor was FDK ($R^2 = 0.21$, $P = 0.1308$). When Harry (known to show low sev but high DON) was omitted from analysis, the best DON predictor was index ($R^2 = 0.83$, $P < 0.0001$) and the worst predictor was FDK ($R^2 = 0.14$, $P = 0.2581$). The results from this study suggest that FHB index is a better predictor of DON measured in grain from heads with visible FHB than inc, sev, or FDK.

Gene expression profile changes of tomato in response to infection by potato purple top phytoplasma

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Phytoplasmas are phloem-restricted, cell wall-less bacteria that cause numerous diseases in agriculturally and economically important plant species worldwide. Although remarkable progress has been made in phytoplasma identification, classification and epidemiology, knowledge about pathogen-host interactions involved in phytoplasma pathogenesis and host plant defense mechanism is still lacking. To gain an insight into molecular events that lead to disease development in susceptible plants, we used Columbia Basin potato purple-top (PPT) phytoplasma and its alternative host, tomato, as an experimental system. Real-time reverse transcriptase-polymerase chain reactions (qRT-PCR) were performed on RNA samples extracted from tomato plants in the course from grafting inoculation to first appearance of characteristic “big bud” symptom. Special attention was focused on genes encoding signal transduction network components and stress-response proteins such as protein kinases, transcriptional factors, and pathogenesis-related proteins. The qRT-PCR results were compared with the microarray

data obtained in our previous study. As indicated by the expression profiles of pathway marker genes, disease induction in tomato by PPT infection may involve both salicylic acid and jasmonic acid signaling pathways.

Supramolecular structure and genomic island of type III secretion system in plant growth-promoting *Pseudomonas fluorescens* 2P24

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Pseudomonas fluorescens 2P24 is a plant growth-promoting bacterium isolated from wheat take-all decline soil. This strain produces several antifungal compounds, such as 2,4-diacetylphloroglucinol (2,4-DAPG), hydrogen cyanide and siderophore(s). Recently, a needle complex homologous to the type III secretion apparatus was purified from *P. fluorescens* 2P24 in successive centrifugal steps. Electron microscopy revealed that the supramolecular structure was composed of two distinctive substructures: an extracellular filamentous needle and a membrane-embedded base with ca 400 nm and 30 nm lengths, respectively. The basal part was constituted by a rod channel connecting an outer and an inner ring. A total of 25 predicted open reading frames (ORFs) residing within a 21 kb gene cluster for assembling the type III secretion system (TTSS) were cloned from *P. fluorescens* 2P24. With the exception of lacking *hrpR* gene, the arrangement and orientation of the ORFs in strain 2P24 bears strong similarity to *Pseudomonas syringae hrp/hrc* genes. In both animal and plant pathogens, the TTSS is an essential component of pathogenicity. However, *P. fluorescens* 2P24 is nonpathogenic and does not elicit the hypersensitive response in any host plant tested. So, it is interesting to investigate the function of TTSS in *P. fluorescens* 2P24.

Preliminary study on fermentation process of *Bacillus amyloliquefaciens* BJ-6

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The strain *Bacillus amyloliquefaciens* BJ-6 was isolated from the rhizosphere of an apricot tree in Beijing, China. It showed strong antagonistic activity to 20 species of plant pathogens tested. The strain could produce antifungal substances during the fermentation process. In order to increase the yield of antagonistic substances, the fermentation medium and conditions were optimized by single factor experiments and orthogonal experiments in flask. The results showed that optimum fermentation medium formula for antifungal substances was maltose 20 g, peptone 100 g, bran 10 g, $CaCO_3$ 10 g, Na_2HPO_3 2 g, KH_2PO_3 1 g and water 1000 ml. The favorable fermentation condition was determined, i.e. the age of inoculum 10 h, inoculation amount 1%, fermentation temperature 30°C–35°C, rotate speed 210r/min, fermentation time 80 h, and initial pH value of medium 9. On this basis, the strain was cultured in 5L fermentor. Biochemical methods were used to detect alive bacteria count, protein produced amounts, consumption amounts of carbon and nitrogen source. It showed that the best fermentation time is 96 h and the best supplementary time of carbon and nitrogen source is 8 h–12 h. During the fermentation process, pH and dissolved oxygen concentration have been recorded automatically by fermentor. (PXM2008-014207-055164, KF2007-003, KM200710020003)

Recognition of total protease activity of antagonists as an indicator of biocontrol efficacy against Meloidogyne root-knot of tomato

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Twenty-six bacterial strains that were shown being antagonistic towards some fungal and bacterial pathogens of tomato were evaluated in this study for their biocontrol potential against the plant root-knot nematode *Meloidogyne incognita* Chitwood. These strains were tested for their *in vitro* protease and chitinase activities, and their effects on the mortality of juvenile at stage 2 (J2) and egg hatching rate of the nematode. Twelve strains causing J2 mortality of over 40% were chosen for further greenhouse experiments, and one of the remaining strains was randomly selected as a control. The biocontrol efficacies of the 12 chosen strains in greenhouse experiments ranged from 29.50% to 63.83%. The actual biocontrol efficacies against Meloidogyne root-knot of the antagonistic strains were highly correlated to their *in vitro* protease activities ($r = 0.92$), but not correlated to their chitinase activities and inhibition on J2 mortality or egg hatch. According to these results, we concluded that *in vitro* protease activity could be an indicator for promising nematode biocontrol efficacies of antagonistic strains. This conclusion was further supported by the field experiments with antagonistic strains *Bacillus* sp. AR156 and GJ24 on tomato plants. Thanks to a grant-in-aid for science research from the Chinese 863 high-tech Program (2006AA10A211).

Impact of fumigation on *Pythium* species associated with forest tree nurseries of Oregon and Washington

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Pythium species cause damping off of conifer seedlings in forest tree nurseries. Identification of the species responsible for the disease has been traditionally based on morphology. However, newer DNA-based identification methods may allow more accurate identification and assessment of soil populations. Field trials were established at three nurseries (2 in OR, 1 in WA) to assess: 1) the impact of fumigation on *Pythium* soil populations; and 2) the rapidity of species reestablishment after fumigation. Six fumigant treatments (including a conventional methyl bromide treatment and a nonfumigated control) were applied according to a randomized complete block design with four blocks at each nursery. Soil samples were collected from each treatment plot before and after fumigation and *Pythium* populations were assessed by dilution plating onto PARP, a semiselective medium for pythiaceous species. Isolates were identified on the basis of DNA sequence from the ITS region and confirmed with morphological characteristics. One month after fumigation, populations in nonfumigated control plots were greatest at nursery B (8 CFU/g soil), intermediate at nursery A (5 CFU/g soil), and least at nursery C (1 CFU/g soil). All fumigant treatments reduced soil populations by at least 68%. Three species (*Pythium macrosporum*, *P. irregulare* and *P. dissotocum*) were predominate at all three nurseries. Analyses of *Pythium* populations will continue through 2009.

Incidence, distribution, and genetic variations of '*Candidatus* Liberibacter sp.' associated with zebra chip of potato in North America

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The presence of '*Candidatus* Liberibacter solanacearum' (CLs) and '*Candidatus* Liberibacter psyllaurous' (CLp) were confirmed in potato plants affected with zebra chip/zebra complex (ZC) disease throughout potato production regions in Texas in 2005–2008, in seed tubers produced from Wyoming in 2007, and potato production regions in Colorado, Kansas, Nebraska, and Mexico in 2008 using CLp/CLs specific primers in PCR assays. The PCR assays were capable of detecting 0.19 to 1.56 ng of total DNA per reaction, depending on the primer set used. The overall efficiency associated with the detection of true positives was generally less than 60%. Detection efficiencies were much higher for below ground portions of plants, such as stolons and tubers, as opposed to leaves, leaf petioles and above ground stems. The '*Candidatus* Liberibacter' species detected in all samples divided into two clusters sharing similarity of 99.8% in their partial 16S rDNA sequences and 99.3% in their partial ISR-23S rDNA sequences. Genetic variations in the 16S rDNA region consistently matched with those of the ISR-23S rDNA region. In this partial 16S-ISR-23S rDNA region, there were total 8 SNPs among the CLp/CLs 'isolates' investigated in this study. Preliminary studies suggest silverleaf nightshade (*Solanum elaeagnifolium* Cav.), wolfberry (*Lycium barbarum* L.), black nightshade (*S. ptychanthum* Dun.), and jalapeno pepper (*Capsicum annuum* L.) as potential reservoir hosts for the ZC bacterium.

Another 'extracellular polysaccharide' functioning in plant defense: Role of structural DNA in border cell-mediated defense of the legume root tip

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Root tips are highly resistant to infection by pathogens that can readily invade other parts of the root. Even when concentrated inoculum is added directly to

this newly-generated 1–2 mm apical tissue, nearly 100% of root tips escape invasion. Populations of detached root border cells, a complex polysaccharide 'slime' layer, and a group of >100 extracellular proteins, the 'root cap secretome,' each contribute to this highly effective form of cellular resistance. Among the secretome are DNA-binding proteins also found within the extracellular matrix of vertebrate neutrophils. These specialized white blood cells recently have been shown to use extracellular DNA (exDNA) in pathogen defense. By feeding pea root tips with ^{32}P-dCTP or ^{35}S-methionine and harvesting root cap slime during a 1-h period when no cell death occurs, we demonstrated that living cells secrete exDNA as well as proteins. When the complex of proteins or the exDNA is degraded enzymatically, root tip resistance to infection is lost and 100% become necrotic. These results suggest that exDNA is a previously unrecognized component of plant defense. This 'polysaccharide' was implicated as a player in the human immune response >50 years ago but with the demonstration of an informational role for DNA, research into its other function(s) ceased. It will be of interest in future studies to determine whether exDNA-related defense in plants is unique to protection of the root meristem, or functions in other cellular resistance responses.

Uncoupling the cell-to-cell movement and RNA silencing suppressor functions of *Saguaro cactus virus* capsid protein

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The capsid protein (CP) of *Saguaro cactus carmovirus* (SCV) plays multiple roles during viral infection. In addition to encapsidation of viral RNA, SCV CP is required for viral cell-to-cell movement, long distance transport, and suppression of RNA silencing. A series of NAAIRS-scanning mutants were generated to dissect functional domains and to uncouple multiple functions of SCV CP. Silencing suppressor activities of the mutant CPs were assayed by co-agroinfiltration with a GFP-expressing construct. The ability of SCV mutants to move intercellularly was inferred from the amount of viral RNA accumulated in *Chenopodium capitatum* and *Nicotiana benthamiana*. Wild type *N. benthamiana* does not support intercellular movement of SCV and no SCV RNA can be detected by RT-PCR. However, SCV movement can be complemented in transgenic *N. benthamiana* expressing *Red clover necrotic mosaic virus* MP (MP+), resulting in a readily detectable level of SCV RNA. Our results showed that the intercellular movement and the RNA silencing suppressor functions could be uncoupled and that these functions required both shared and unique domains of SCV CP. In two scanning mutants that displayed normal intercellular movement in MP+ *N. benthamiana* but little or no suppressor activity, one moved readily in *C. capitatum* but the other failed to do so. An additional NAAIRS mutant retained a normal level of suppressor activity but its ability to move intercellularly was severely compromised.

Is there a balance in disease severity development within the SDS-*Heterodera glycines* complex?

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Fusarium virguliforme, cause of sudden death syndrome (SDS) of soybean and *Heterodera glycines* co-occur in many Midwest fields of the USA, and can cause a synergistic disease complex. How persistent is this complex in different soil microbial backgrounds in soybean monoculture? In a microplot trial, two levels of each of four factors, including soil fumigation, watering, *H. glycines*, and *Fusarium virguliforme* infestation were applied. Dynamics of the nematode population densities, root necrosis, and disease severity, as area under the disease progress curve (AUDPC), were monitored for 3 years after the initial treatments. Severity of root necrosis increased in fumigated plots, whereas it declined in the non-fumigated soils. Population densities of *H. glycines*, possibly residual after the initial fumigation, increased in fumigated, non-inoculated plots to levels similar to those in non-treated or fumigated and artificially re-infested plots. In the first year, addition of *F. virguliforme* increased root necrosis in plots with *H. glycines*. On average of the 3 years, plots that were amended with *F. virguliforme* had higher AUDPC values than the non-amended plots when *H. glycines* was present. Supplemental watering increased AUDPC overall, but did not change the balance between *H. glycines* and severity of foliar SDS symptoms. In this trial, the two pathogens did not decrease the pathogenic success of each other, suggesting that the disease complex was persistent.

Application of complementation tests in identifying pathogenicity determinants of the chickpea pathogen *Ascochyta rabiei*

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The necrotrophic pathogen *Ascochyta rabiei* causes chickpea Ascochyta blight. Very little is known about its pathogenicity mechanisms. The objective of this research was to identify pathogenicity determinants of *A. rabiei* using complementation tests. The hygromycin-resistant mutant ArW519 was non-pathogenic on chickpea by virtue of a single T-DNAinsertion. Genomic DNA regions corresponding to the T-DNA insertion site was isolated from a phage library of a wild type strain AR628. Six genomic fragments were moved separately into a T-DNA vector with geneticin resistance and used to re-transform the mutant ArW519. The pathogenicity of eight double transformants (resistant to both antibiotics) recovered from independent T-DNA integration events was compared to that of the parent mutant ArW519 and of the wild-type strain AR628 on chickpea using a minidome bioassay. One of the six clones tested was able to functionally restore pathogenicity to the ArW519 mutant. Each of the two antibiotic cassettes in the double transformants was verified to be intact. The complementing genomic fragment contains about 3000 bp of DNA upstream of the T-DNA insertion site and about 1000 bp of DNA downstream, and it carries DNA sequences that are similar to retrotransposon Molly from *Stagonospora nodorum* and the AvrLM1 avirulence gene from *Leptosphaeria maculans*.

Tuber symptoms are induced in potato by a range of Potato virus Y strains

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PVY isolates collected from a national survey were used to inoculate N. A. cultivars in order to characterize tuber symptoms. Plants in a screened greenhouse were ELISA tested for freedom from PVY, PVX, PVA, PVS, and PLRV before inoculation. Ten cultivars were inoculated with one of nine PVY$^{N\text{-}Wi}$, four PVY^{NTN}, or two PVY^{O} isolates using a spray gun. The plants were ELISA-tested for PVY at three weeks post-inoculation and if negative, tested again at later dates. At harvest, tubers were scored for symptoms of potato tuber necrotic ringspot disease (PTNRD) and again after storage at room temperature. Symptoms included external skin cracking and ringspots, and internal symptoms. Russet Norkotah and Premier Russet exhibited no PTNRD symptoms with any of the isolates tested. Yukon Gold, Yukon Gem, and Highland Russet, were all very susceptible to the development of PTNRD when infected by various isolates, including PVY$^{N\text{-}Wi}$ and one of the PVY^{O} isolates. PVY^{O} does not typically produce PTNRD. Also, asymptomatic PVY-infected tubers were shown to produce symptomatic tubers (in the second generation) from the seed-borne virus plants. While general assignment of susceptibility to PTNRD can be made from this bioassay, it is apparent that tuber symptoms are dependant upon cultivar and PVY isolate. Bioassay information is necessary to evaluate potential quality impacts due to the spread of PVY necrotic strains within the U.S. potato crop.

Investigating the roles of siderophores in the *Pseudomonas syringae* pv. *syringae* B728a lifecycle

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Iron is essential for the biosynthesis of syringomycin and syringopeptin, the pore-forming cyclic lipopeptides that serve as major virulence factors for *Pseudomonas syringae* pv. *syringae* B728a (*P.s.s.* B728a). Bioinformatic analysis has revealed that two iron-scavenging siderophore molecules, pyoverdine (Pvd) and achromobactin, are encoded in the *P.s.s.* B728a genome. Previous research has shown that Pvd provides a selective advantage to fluorescent pseudomonads in the phyllosphere and rhizosphere due to its high affinity binding of available iron. However, biosynthesis and uptake of Pvd are not essential for pathogenicity or virulence of *P.s.s.* B728a. The second siderophore cluster encoded in the *P.s.s.* B728a genome consists of 14 genes with high sequence similarity to the *Dickeya chrysanthemi* cluster responsible for synthesizing the citrate siderophore, achromobactin. We hypothesize that these two siderophores are utilized by the bacteria in separate phases of the *P.s.s.* B728a lifecycle, with Pvd contributing to epiphytic survival and achromobactin playing a role in plant pathogenicity. Deletion mutations of the ECF sigma factors regulating Pvd and achromobactin biosynthesis were developed to evaluate these hypotheses. Understanding the role of siderophores in the *P.s.s.* B728a lifecycle is a preliminary step in elucidating the complex role of iron in plant disease.

Effects of soil temperature on *Sclerotinia homoeocarpa* growth, survival, and pathogenicity

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Dollar spot, a disease caused by the fungus *Sclerotinia homoeocarpa*, is a widespread, important disease of most turfgrass species worldwide. Dollar spot on turf was described almost a century ago by F.T. Bennett. However, the basic biology and epidemiology of the pathosystem is still unclear. Two isolates of *S. homoeocarpa* were grown on native silt loam and Waupaca sand with and without creeping bentgrass (*Agrostis stolonifera*) debris and incubated at several temperatures (14, 20, and 26°C). Radial growth of mycelia was recorded at 24, 48, 72, and 96 hours to assess effect of soil temperature on survival and pathogenicity. *S. homoeocarpa* grows most rapidly at 26°C on native silt loam with bentgrass debris (36% faster than Waupaca sand with debris, 76% faster than bare native silt loam, and 86% faster than bare Waupaca sand). Growth is significantly slower at 14 on both soils. Growth on bare silt loam and sand is evident, but is extremely slow and variable. These data suggest that *S. homoeocarpa* is able to survive in the absence of plant debris on soil, and that higher temperatures (20–26°C) are conducive to growth and pathogenicity.

Resistance to crown and foot rot in wheat cultivars grown in Idaho

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In the higher elevation areas of the Intermountain West, common foot and crown rot limits the yield of wheat and barley under dryland production conditions. In 2006, a screening nursery was established in Aberdeen, ID, to test currently grown cultivars and advanced breeding lines for resistance to *Fusarium*. Inoculum was produced from isolates of *F. culmorum* previously obtained from infected plants in South eastern Idaho. Four replications of thirty six wheat lines were planted in a split-plot design, with the mainplot being variety and sub-plots being inoculation treatment (inoculated versus non-inoculated). Results showed that there was no effect of inoculation on seedling stand, number of whiteheads, yield or test weight. There were significant variety differences for seedling stand, number of whiteheads, yield, and test weight ($Pr>F < 0.0001$). The spring wheat varieties Otis, Pettit, Iona, Challis and IDO 377s yielded the highest under high disease pressure and water stress, while the lowest yielding varieties included Matt (durum), Puseas, Alzada (durum), Klasic, and Kronos (durum). Screening for foot rot resistance is critical in areas where water stress, especially at reproductive growth stages, increases crop susceptibility to foot and crown rot fungi.

Population structure of *Phytophthora capsici* in a yellow squash field in Michigan

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Oospores present in soil initiate primary infection of *P. capsici*. Once disease is established, the pathogen can spread through asexually produced propagules. The aim of this study was to describe the spatial distribution of oospores in a field and map the population structure over time and space during the evolution of an epidemic. On 25 June 2008 yellow squash seedlings were planted in a *P. capsici*-infested field at the Michigan State University Muck Soils Research Farm and scouted weekly for *P. capsici* infection. Symptomatic plants were sampled to verify *P. capsici* infection and disease incidence was monitored from 25 June to 3 September. Disease symptoms were first observed on 16 July and incidence reached 58% and 68% by 13 August and 3 September, respectively. A total of 277 isolates of *P. capsici*, recovered from diseased root, crown, stem, leaf, and fruit tissues, were tested for sensitivity to mefenoxam and the mating type determined. Isolates were also genotyped by sequencing their β-tubulin and elongation factor 1-α genes. When plants yielded more than two isolates, 56% had more than one phenotype present (different mating type and/or mefenoxam resistance level). Data indicate that plants may be infected with more than one genotype of *P. capsici*, suggesting that, at least on yellow squash, mating has the potential to occur fairly frequently within a growing season.

Pathogenic development of *Phytophthora capsici* on cucumber fruits

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Phytophthora capsici severely limits vegetable production in many areas of the U.S. Successful infection is usually initiated by motile, biflagellate zoospores that are released from sporangia under wet conditions. Studies were undertaken to document pathogen development from zoospores on pickling cucumber fruits using scanning electron microscopy, time-lapse photography, and quantitative measurements of lesion development. Zoospore aggregation, encystment, and attachment were apparent as early as 10 min post inoculation (pi) at room temperature (RT, 23°C). Germination was detected within 2 h pi at RT and limited appressorial formation was also noted. By 6 h pi at RT, cytoplasmic migration from zoospore cysts to developing hyphae was noted. Water-soaked lesions were formed within 48 h pi at 20–30°C and hyphal growth was observed at 30°C. At 72 h pi, sporulation was visible at 20–30°C. At 72 h pi at RT, individual sporangia could form in ~1.5 h, and sporangia formation continued throughout the duration of the experiment (120 h pi). Delayed and limited lesion development and sporulation (at 120 h pi) were observed at 15°C. This study found that infection takes place shortly after zoospore contact with the host. Once sporulation commences, new sporangia may be generated in hours, allowing for rapid and extensive polycyclic disease development. The results of this study suggest that chilling fruit at temperatures ≤15°C may delay lesion development.

The complete nucleotide sequence and genome organization of *Tomato infectious chlorosis virus*: A distinct crinivirus most closely related to LIYV

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Tomato infectious chlorosis virus (TICV) affects tomato production in many temperate to subtropical parts of the world where production is impacted by the presence of the greenhouse whitefly (*Trialeurodes vaporariorum*). Symptoms include interveinal yellowing, and leaves become thickened and crispy, breaking easily when bent. Yield is affected through decreased fruit size and number, as well as decreased plant longevity. The complete nucleotide sequence of TICV was determined and compared with other members of the genus Crinivirus. RNA 1 is 8271 nucleotides long with three open reading frames and encodes the replication module, consisting of the putative papain-like cysteine protease, methyltransferase, helicase and RNA-dependent RNA polymerase. RNA 2 is 7913 nucleotides long and encodes eight proteins including a HSP70 homolog, a 60 kDa protein, a major coat protein and a larger minor coat protein (75 kDa) involved in genome protection, movement, and other functions yet to be identified. The genome organization is similar to that of other members of the genus. Similarity between TICV and other criniviruses varies throughout the genome, but TICV is related more closely to *Lettuce infectious yellows virus* than to any other crinivirus. With the addition of TICV, phylogenetic analysis indicates that the genus, Crinivirus, could appropriately be separated into three distinct groups.

Study of *Cucurbit yellow stunting disorder virus* in southern California reveals an expanded host range including non-cucurbit weed and crop species

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Cucurbit yellow stunting disorder virus (CYSDV) was identified in southern California in the fall of 2006, and has severely affected cucurbit production in the southwestern US. Survival of CYSDV through the largely cucurbit-free winter months suggested the presence of weed or alternate crop hosts, although previous studies indicated a host range restricted to the *Cucurbitaceae*. As part of a study of CYSDV epidemiology, potential weed and crop reservoir hosts were collected from California's Imperial Valley over a 26 month period. Samples were tested for the presence of CYSDV by RT-PCR using CYSDV-specific primers. Surveys revealed a low incidence of CYSDV in spring melons, whereas fall crops had high incidences of infection (up to 100%). Many non-cucurbits collected from fields and nearby areas were symptomless and virus free; however, CYSDV was detected in alfalfa (*Medicago sativa*), lettuce (*Lactuca sativa*), and snap bean (*Phaseolus vulgaris*), as well as several prevalent weed species. Symptoms were observed in CYSDV-infected snap bean, alkali mallow (*Sida hederacea*) and ground cherry (*Physalis wrightii*), whereas other infected hosts were symptomless. Whitefly (*Bemisia tabaci*) transmission tests established that lettuce, snap bean, alkali mallow, Wright's ground cherry and buffalo gourd (*Cucurbita foetidissima*) can serve as reservoir hosts for CYSDV. These results greatly

expand the known host range of CYSDV, and have implications for development of disease management approaches.

Occurrence and impact of Goss's bacterial wilt and leaf blight on corn in Indiana

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Goss's bacterial wilt, caused by *Clavibacter michiganensis* subsp. *nebraskensis,* was confirmed in August of 2008 at the Purdue Plant and Pest Diagnostic Laboratory on field corn and popcorn samples submitted from northwest Indiana. Confirmation of the causal agent was obtained through DNA sequence analysis. Koch's postulates were completed to verify the diagnosis, making this is the first documented evidence of Goss's wilt in Indiana. This confirmation is of regulatory importance due to potential export restrictions on Indiana seed corn and popcorn for select countries. Approximately 6,879 hectares of hybrid dent corn and popcorn across two counties exhibited tan to gray lesions with dark freckling and bacterial exudate present on symptomatic leaves. The systemic wilt phase of the disease was also observed in several fields of popcorn. It is suspected that hailstorms early in the season, along with heavy rains and windstorms contributed to establishment and dispersal of Goss's wilt in the affected area. Some infected fields experienced substantial yield loss, and although partially resistant hybrids are available in other areas of the Midwest, the susceptibility level of most varieties of popcorn, sweet corn, and hybrid corn to Goss's wilt in Indiana is currently unknown.

Response of cotton to foliar applied fungicides

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The recent labeling of strobilurin fungicides, such as pyraclostrobin and azoxystrobin in cotton (*Gossypium hirsutum* L.), has generated interest in evaluating these compounds. Field trials were conducted from 2004 to 2008 in Georgia and Texas to evaluate the use of foliar applied fungicides in cotton. Fungicides were applied at first bloom. Disease development was monitored throughout the growing season. Hyper spectral imaging was used to determine differences in plant reflectance in two trials in Texas during the 2008 growing season. Plots were harvested at maturity and lint yields were calculated. Overall, disease pressure was low and did not warrant ratings. NDVI, NIR, and visible light values were similar among treatments. Lint yields did not differ among treatments for any of the trials. Additional studies are required to document fungicide efficacy towards foliar diseases of cotton.

Characterization of wheat streak mosaic severity trends across wheat fields

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Wheat streak mosaic virus, vectored by the wheat curl mite *Aceria tosichella* Keifer, is a major limiting factor in wheat production in the southern Great Plains. Infection usually begins at the edges of fields as the mites are blown in from nearby volunteer wheat fields or other grass vegetation. Thus, the disease is often severe around the edges of the fields and declines in intensity away from the edges. Wheat streak mosaic severity was quantified in three fields with remote sensing using a handheld radiometer at 555 nm at multiple locations in two-to-four transects per field. Several models such as linear, exponential, power law and modified power law, and state space then were evaluated for describing the diseases gradients across the field using several statistical indices including the root mean square errors and the percent of variations the models captured (R^2- for observed versus predicted). Disease severity trends were similar within a field but varied among fields. The models varied in their abilities to describe the disease gradients and no one model was consistently superior across all transects and fields but the state space model provided a better description of the disease gradients in transects in which other models failed to provide satisfactory fits.

A new PCR method for detection of *Acidovorax citrulli* on seed

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Acidovorax citrulli (Schaad et al. 1978), formerly *Acidovorax avenae* subsp. *citrulli*, causal agent of bacterial fruit blotch, is a seed-borne pathogen of cucurbits. Testing of melon and watermelon seed for this pathogen is a standard practice in the seed industry to prevent infected seed from entering the market. PCR-based detection methods have the advantage of being much faster than commonly used grow-out methods; however they require reliable and specific primers and a robust means to extract the bacterial DNA from infested seed that minimizes PCR inhibitors. To develop primers with increased sensitivity, we screened the genome sequence of *Acidovorax citrulli* for repetitive elements and identified a sequence specific to this pathogen for further evaluation. Primers were developed and validated against a diverse collection of strains of *Acidovorax citrulli* and related species using SYBR Green real-time PCR. Combining the enhanced sensitivity of the resulting primer set with DNA extraction in 96-well format provided a sensitive and rapid detection method for *Acidovorax citrulli* on seed. Seed extracts constitute a complex microbial and biochemical environment. To ensure that DNA extractions functioned properly and inhibitors did not interfere with PCR reactions, we deployed a related strain in the genus *Acidovorax* as an internal control during the extraction and PCR steps. Data on the performance of this method are presented.

Morphological identification and pathogenicity of *Botryosphaeria* spp. causing stem blight on southern highbush blueberries in Florida

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Two *Botryosphaeria* species, *B. rhodina* and *B. ribis,* have been recently identified as the primary fungi associated with stem blight of southern highbush blueberries (SHB), and *B. dothidea* was infrequently isolated. Symptoms include reddening and drying of attached leaves, and necrotic stem lesions with internal discoloration. Pathogenicity and conidia morphology were used for pathogen characterization. Pathogenicity tests were conducted on the SHB cv. 'Misty' by inoculating fresh pruning wounds with mycelium plugs. Lesions were allowed to develop for 3 wks. The extent of fungal colonization varied by species, after 3 wks the average lesion length was 15.5 cm (±1.6), 10.6 cm (±1.6), and 2.6 cm (±0.7) for *B. ribis, B. rhodina,* and *B. dothidea*. Conidia were produced directly on PDA, and they also were produced by inoculating fresh pruning wounds of the SHB cv. 'Jewel' with mycelium plugs grown on PDA. Conidia were produced after 5 wks *in vitro* and after 10 days on inoculated stems. The average length and width of 60 conidia from two isolates of *B. dothidea* was 26.1 µm (±0.1) and 5.6 µm (±0.1). The average length and width of 60 conidia from five isolates of *B. ribis* and *B. rhodina* were 18.6 µm (±1.5) and 6.6 µm (±0.1) and 28 µm (±1.6) and 15 µm (±1.0). These findings suggest that at least three *Botryosphaeria* spp. can cause stem blight of SHB; however, disease severity and conidia morphology varied by species.

Identification and molecular characterization of a new phytoplasma associated with Sunshine Tree Stem Fasciation (STSF) disease in China

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Phytopathology 99:S143

Senna surattensis, commonly known as sunshine tree or golden senna, is an evergreen to semi-deciduous ornamental plant with prolonged and beautiful flowers. Originated in India, Southeast Asia, and Australia, sunshine tree is now widely distributed in tropical and subtropical regions of the world. Recently, sunshine trees exhibiting unusual stem fasciation symptoms were observed in Yunnan, China. The morphological abnormities of the affected plants included uncontrolled shoot proliferation and stem enlargement and flattening. Since the disease occurred in a region where other phytoplasma diseases are prevalent, a phytoplasma infection was first suspected. Nested PCR was performed using phytoplasma universal primers P1/P7 and P1A/16S-SR, and a phytoplasma-specific 1.5-kb amplicon representing a near-full length 16S rRNA gene was obtained from all DNA samples extracted from diseased plants; whereas no PCR products were obtained from nearby symptomless plants. Subsequent nucleotide sequence analysis of the cloned PCR fragment suggested that STSF disease is associated with an infection by a '*Candidatus* Phytoplasma australiense'-related strain. The association of STSF disease with phytoplasma infection was further supported by transmission electron microscopic observation of phytoplasma-like bodies in sieve elements of diseased plants. Virtual RFLP analysis of the 16S rDNA sequence suggested that the STSF phytoplasma is a new member of the phytoplasma subgroup 16SrXII-A.

Evidence that *Phoma sclerotioides*, causal agent of brown root rot of alfalfa, is composed of a species complex

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Phoma sclerotioides, causal agent of brown root rot of alfalfa, causes severe root and crown lesions on alfalfa and other perennial forage legumes in

regions with harsh winters. The internal transcribed spacer (ITS) 1, 5.8S, ITS2, and the complete intergenic spacer of the nuclear rDNA and intron-spanning regions coding for actin, alpha tubulin, beta tubulin, elongation factor 1-alpha, glyceraldehyde 3-phosphate dehydrogenase, histone, and mitochondrial NADH dehydrogenase subunit 5 were sequenced for 154 single-conidium *P. sclerotioides* isolates collected in the western, central, and eastern United States and Canada and two single-conidium *P. medicaginis* isolates. Phylogenetic analysis of the concatenated multilocus sequence data suggests that at least four subtypes of *P. sclerotioides* are present in North America. Gene-jackknifing analyses confirm that this conclusion was not driven by a single locus, and single-gene analyses indicate congruence of sequence data across loci with respect to this conclusion. The subtypes differ in mycelial pigmentation and in production of aerial mycelium on potato dextrose agar, and field experiments suggest that the relative virulence of the subtypes may differ by alfalfa cultivar. Phylogenetic analyses, morphological characteristics of representative isolates from each subtype, results from field trials, and results from inoculation experiments conducted in controlled environments will be presented.

Fungicide resistance management guidelines for cucurbit downy and powdery mildew control in the mid-Atlantic and Northeast regions of the U.S.

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In the mid-Atlantic and Northeast regions of the United States over 65,000 ha of fresh-market vegetable crops are grown annually with a value over 794 million dollars. Cucurbit downy mildew (*Pseudoperonospora cubensis*) and powdery mildew (*Podosphaera xanthii*) are two important diseases of cucurbit crops in both regions. In recent years, several new fungicide chemistries labeled for use in cucurbit production have been registered in the U.S. Many of these fungicides have a specific mode-of-action (MOA) that targets pathogen development at a single site. Fungicides with a single-site MOA are considered at high-risk for fungal resistance development and possess a much greater risk than fungicides with multiple MOAs (i.e. protectant fungicides). A fungicide resistance management table has been developed for cucurbit growers in the mid-Atlantic and Northeast regions of the U.S. to i) promote the importance and understanding of FRAC (Fungicide Resistance Action Committee) codes to manage fungicide resistance while controlling cucurbit powdery and downy mildew; ii) optimize appropriate use of specific fungicides that have a high-risk for resistance development; and iii) provide an IPM tool to allow cucurbit growers to develop season-long cucurbit downy and powdery mildew control programs with an emphasis towards fungicide resistance management.

Detection of *Turnip yellows virus* in eight cruciferous crops in Mainland China

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Turnip yellows Polerovirus (TuYV) causes a yellowing disease of crucifer crops worldwide. To determine the occurrence of TuYV in Mainland China, 150 samples from eight different species of cruciferous species, including cabbage (Brassica oleracea var. capitata), chinese cabbage (Brassica pekinensis), cauliflower (Brassica oleracea var. botrytis), flowering chinese cabbage (Brassica chinensis), leaf mustard (Brassica juncea), oilseed rape (Brassica napus), radish (Brassica rapa), white glabrous mustard (Brassica alboglabra), were collected from 10 provinces. Total RNA from diseased leaf tissue was extracted and tested by RT-PCR using a universal primer pair to poleroviruses, and a specific primer set to the previously published sequence of TuYV from France. The results revealed that 48 of 150 samples were positive in all the eight cruciferous crops from the ten provinces. Furthermore, eight RT-PCR products of the expected size (1384 bp) from different cruciferous species were cloned, sequenced and submitted to GenBank. Sequence analyses revealed that the newly determined sequences had the highest nucleotide sequence identity (94.4–99.5%) with each other, and shared 92.9%–94.5% identity with the reference TuYV isolate, indicating that the isolates from different cruciferous species are identical to the previously reported TuYV.

Effect of environment on the abundance and activity of the nematophagous fungus *Hirsutella minnesotensis* in soil

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Environmental factors greatly affect the occurrence and activity of soil microorganisms. Not enough is known, however, about how environment affects the abundance and activity of many beneficial microorganisms after they are released into soil. *Hirsutella minnesotensis* is an important fungal parasite of second-stage juveniles (J2) of the soybean cyst nematode and has shown great potential in nematode control. The objective of this study was to measure the effects of environmental factors on the abundance and activity of *H. minnesotensis* in soil. Fungal mycelium was mixed with dry soil (1% wetwt/drywt) and placed into 50-mL plastic tubes. Soil temperature ranged from 5°C to 30°C; soil water content ranged from 6% to 22%; and the soil was supplemented with 0% to 70% of fine soil particles (silt and clay) or sand. After 24 days, fungal abundance was quantified by real-time PCR and its activity was inferred from the numbers of J2 parasitized. The amount of *H. minnesotensis* DNA g^{-1} soil and the percentage of J2 parasitized by *H. minnesotensis* were higher with lower soil temperature and water content, and higher fine particle content, demonstrating that *H. minnesotensis* has greater potential to multiply and control pest nematodes in cooler, drier, and heavier soils.

Analysis of *ZmPR10.1* promoter reveals regulatory regions for stress responses and strong expression in transgenic *Arabidopsis*

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Pathogenesis-related protein 10 (PR10) is one of the seventeen PR protein families. Recent studies identified two PR10 genes from *Zea mays* and showed that *ZmPR10.1* mainly expressed in root tissue and was induced by a variety of stresses. For a better understanding of the regulation mechanism of *ZmPR10.1*, a 1,591-bp promoter fragment of *ZmPR10.1* and three 5′ deletion series were fused to a β-glucuronidase (GUS) reporter gene and transformed into *Arabidopsis*. Comparison of the expression levels between *ZmPR10.1* in maize and *gusA* in transgenic *Arabidopsis* demonstrated that the 1,591-bp promoter fragment suffices for transcription. When driven by the -1,591, -969 and -419 promoter, the GUS activity in root tissue was over 11 folds higher than that in aerial tissues. However, deletion of a region between -419 and -340 drastically increased the GUS activities in both root and aerial tissues, which was higher than what obtained with a CaMV35S-GUS construct. In addition, promoter analysis of deletion series also identified additional important regions responsible for abiotic stresses, including abscisic acid, salicylic acid, methyl jasmonate, Cu^{2+}, H_2O_2, salinity, dehydration, darkness and wounding. The data indicated a complicated transcriptional regulation of *ZmPR10.1* expression.

Complete nucleotide sequence and taxonomy of *Sugarcane streak mosaic virus*, member of a novel genus in the family *Potyviridae*

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Sugarcane streak mosaic virus (SCSMV) was first identified as a member of the family *Potyviridae* in a sugarcane accession imported to the United States from Pakistan. Molecular characterization of its 3'-partial sequence indicates the virus is different from other members in the family, but its taxonomic position has not been decided due to lack of complete genomic sequence data. In this report, host range and complete nucleotide sequence of the SCSMV Pakistan isolate (SCSMV-PK) are described. The virus was mechanically inoculated onto 17 plant species, and the results showed that it infected sugarcane, sorghum, maize, millet and crabgrass. The complete genomic sequence of SCSMV-PK was determined to be 9782 nt in length, excluding the 3' poly(A) tail, and it comprised a large open reading frame encoding a polyprotein of 3130 amino acid residues. This genomic organization is very similar to the genera Potyvirus, Ipomovirus, Rymovirus and Tritimovirus in the family *Potyviridae*. However, sequence analyses indicated that SCSMV only shared identities of 41.1–45.6% at the genomic sequence level and 26.4–

31.5% at the polyprotein sequence level with members of these genera. Phylogenetic analyses of the SCSMV genomic sequence and deduced amino acid sequence of coat protein also revealed that SCSMV is different from other viruses in the family at the genus level. Therefore, SCSMV should represent a new genus, Susmovirus.

Evaluation of seed treatments to prevent bacterial canker in greenhouse tomatoes

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Bacterial canker is an important disease affecting the greenhouse tomato industry. The pathogen *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) is seed transmitted, therefore, seed must be the first point of focus in developing an integrated program to manage this disease. Seed sanitation treatments alone or combined with applications of the biological control agents *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated for efficacy in killing *Cmm* on/in seed and reducing seed to seedling transmission of *Cmm*, and effects on seedling vigor. The seed sanitation treatments included five disinfectants (HCl, NaClO, H_2O_2, KleenGrow and Virkon), two heat treatments (hot water and dry heat), two antibiotics (kasugamycin and streptomycin) and one plant oil (thymol). Among the sanitation treatments, the most effective in killing *Cmm* both on and in seed in laboratory tests were soaking seed with 1) 0.6% NaClO at 50°C for 15 minutes, 2) 6M HCl for 30 minutes, 3) 0.56% KleenGrow for 30 minutes, and 4) 2% Virkon for 20 minutes. Disease incidence and *Cmm* populations on seedlings subjected to these treatments were consistent with laboratory results. None of these disinfectants affected seedling vigor in laboratory tests and greenhouse grow-outs. Thymol was phytotoxic to tomato seed and seedlings, reducing emergence rate and increasing the numbers of abnormal seedlings. Neither biological control agent affected bacterial canker incidence or seedling vigor.

Detection of *Tobacco rattle virus* in stubby root nematodes by conventional and real-time RT-PCR

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Tobacco rattle virus (TRV) can infect potatoes and cause tuber necrosis. This virus has been detected from field potatoes in the Fraser Valley region in British Columbia, Canada in recent year. TRV is transmitted to healthy plants by viruliferous nematodes in the field and the non-feeding nematodes can retain the virus for many months in soil. Several nematode species of *Paratrichodorous* and *Tricodorous* have been confirmed to be the natural vectors of TRV. To control the diseases caused by TRV, it is critical to use virus- and vector-free soil for planting. This report describes the detection of TRV by RT-PCR and real-time RT-PCR in single and multiple stubby root nematodes (*P. allius*) isolated from soil samples. The total RNA was extracted from nematodes and TRV RNA was amplified in RT-PCR and real-time RT-PCR using primers targeting TRV 16K protein gene. Reliable detection of TRV RNA was achieved from the total RNA extract of single, three, five and ten nematodes and both PCR procedures produced the same results. Amplicons of both RT-PCR procedures were verified by restriction digestion of *Alu*I and melting temperature analysis, respectively. The ability of the stubby root nematodes to transmit TRV in soil was tested by growing healthy bait plants (*Nicotiana tobacum*, cv. Samsun) in the soil infested with the viruliferous nematodes. The infection of the bait plants was then confirmed by symptom development and the detection of TRV RNA in the upper leaves by both RT-PCR methods.

Exploring bacterial diversity in irrigation runoff water containment basins

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Containment basins (CBs) are an important part of the agricultural infrastructure to secure an adequate supply of irrigation water and reduce nonpoint runoff of nutrients. CBs can potentially accumulate and redistribute plant pathogens. Recent studies indicate that the populations of *Phytophthora* species declined with increasing distance from the entry point of runoff into the CB, but its underlying mechanism is not known. The objectives of this research were to develop reliable and effective strategies and methods to characterize microbial diversity and identify beneficial bacterial species that may have contributed to the pathogen decline in the CBs. We have developed a 16S ribosomal DNA based-DGGE (denaturant gradient gel electrophoresis) procedure to characterize bacterial diversity in two CBs at two ornamental plant nurseries. Bacterial diversity, as measured by the Shannon index, was determined according to DGGE profiles. Bands in the DGGE gel were excised for further sequence analysis. Bacterial population data from the DGGE analyses were compared to those generated using the culture-based method. Our initial results demonstrate that there is a great diversity of bacteria including a variety of beneficial species in these CBs. Some isolates identified belong to species that are potentially antagonistic to plant pathogens. Others belong to groups of nitrogen-fixing bacteria, and plant growth-promoting rhizobacteria.

A root-knot nematode secretory protein interacts with plant cell membrane proteins

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Proteins produced in specialized esophageal gland cells are secreted from the stylet of the root-knot nematode (RKN), *Meloidogyne* spp., that induce profound changes in recipient plant root cell gene expression and morphology to form multinucleate giant-cells to sustain nematode feeding. The 8D05 RKN parasitism gene encodes a novel 330 amino acid secretory protein that stimulates plant growth when expressed in transgenic *Arabidopsis thaliana*. Plant host-derived RNA interference targeted to 8D05 significantly reduced root galling induced by RKN, suggesting a functional role of secreted 8D05 protein in successful infection of host roots by the nematode. A positive interaction of the 8D05 protein with members of the tomato major intrinsic protein (MIP) superfamily was detected in yeast two-hybrid screens and confirmed in subsequent co-transformation experiments. MIPs are a family of membrane channels, including aquaporins, that facilitate the bidirectional transport of water and small uncharged solutes, consistent with required activity of giant-cells. Upregulation of plant MIPs in nematode feeding cells has been previously reported, providing a potential in vivo target for interaction with secreted RKN 8D05 protein to modulate water and solute transport across the giant-cell membrane.

Biology and sources of inoculum of *Geotrichum candidum* causing sour rot of peaches and nectarines in California

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Post-harvest decay is a major problem that complicates storage and shipment of stone fruits with serious economic consequences. *Geotrichum candidum* causes sour rot of fresh-market peaches and nectarines. We investigated the sources of inoculum of *G. candidum* in the field and packinghouses as well as insect transmission of the pathogen. Also, the role of culled fruits as inoculum sources and as potential sources of fungicide-insensitive strains was investigated. Soil, leaves, and fruits were collected from orchard sites in Fresno, Tulare, and Kings counties over a 3-year period. Soil samples contained the highest population of *G. candidum*, with as much as 3.3×10^3 CFU/g of soil. The pathogen was more frequently isolated from soil than from leaf and fruit surfaces. *G. candidum* also was detected in various stages of the processing lines in packinghouses, with the highest frequency on and immediately after brushes used for cleaning the fruit. Isolates from different sources and substrates were tested for their pathogenicity on nectarines and all were found to be pathogenic. Isolates from cull fruits treated with propiconazole and fludioxonil were assessed for their insensitivity to propiconazole by comparison to a baseline, sensitive population. The EC_{50} of insensitive isolates was approximately six-fold higher than the baseline population. These results indicate that cull fruit disposal in an orchard provides a potential source of fungicide-insensitive strains.

Seed-borne and systemic populations of *Agrobacterium tumefaciens* as sources of inoculum for crown gall development on Paradox walnut rootstock

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Paradox (*Juglans hindsii* × *J. regia*) is the dominant rootstock used by California walnut growers and is extremely susceptible to crown gall caused by *Agrobacterium tumefaciens*. The inoculum source for crown gall was traditionally thought to come from infested soil; however, recent observations and preliminary data from an infested nursery suggest systemic or seed-borne

inoculum sources. To investigate the prevalence of seed-borne populations, Paradox seeds were collected 1) directly from mother trees, 2) from the orchard floor soon after shaking the trees for seed harvest, and 3) from the orchard floor four weeks after shaking. *A. tumefaciens* was not isolated from seeds harvested directly from trees or harvested after a short period of contact with the orchard floor, but avirulent *A. tumefaciens* was detected in seeds contacting the orchard floor for four weeks. To investigate the impact of seed-borne populations on disease development, germinating seeds were soaked in a marked strain of *A. tumefaciens* or planted in soil inoculated with *A. tumefaciens*. All trees exposed to *A. tumefaciens* developed galls extensively on roots and/or crowns within three months. *A. tumefaciens* was detected systemically in both shoot and root tissue. Populations inside the shoots were discontinuous and a native avirulent *A. tumefaciens* population was also found. The implication and importance of early walnut seed contamination by *A. tumefaciens* for crown gall management will be discussed.

Molecular identification of cyst nematode species from wheat and barley fields in the Pacific Northwest

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Cyst nematodes (*Heterodera* spp.) are important plant-parasitic nematodes in the Pacific Northwest (PNW). Soil samples were collected from wheat and barley fields in Oregon, Washington and Idaho to investigate the population density and distribution of cyst nematode species. The *Heterodera* species occurring at each location were determined using PCR and restriction fragment-length polymorphism (RFLP) with up to six restriction enzymes. Grid soil sampling in the field where *H. filipjevi* was first reported in Oregon revealed that *H. filipjevi* was present at most of the infested grid sites but mixtures of *H. avenae* and *H. filipjevi* also occurred. Cyst nematodes were unevenly distributed across the field and a high population of *H. filipjevi* was present at some grid sites. All samples tested from nearby fields revealed only *H. avenae*. Very high populations of *H. avenae* were found in fields sampled near Palouse, WA and St. Anthony, ID. Cysts from soil under a barley crop in an irrigated field near Paul, ID were determined to be *H. schachtii*. Intraspecific variation was not observed within *H. filipjevi* populations or within PNW *H. avenae* populations but was observed between *H. avenae* populations from the PNW and France based on the ITS region of rDNA sequences. Accurate identification of cyst nematode species and awareness of high population density in fields is essential for designing effective control measures.

Identify genes important for conidiogenesis in *Magnaporthe oryzae*

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Appropriate localization of mRNA in many developing organisms is important for both cell polarity and morphogenesis. Three-celled pyriform conidia play a central role in the infection cycle of *Magnaporthe oryzae*, the pathogen causing the most devastating fungal diseases of cultivated rice worldwide. In this study, we hypothesize that RNA trafficking is important for conidium development. Genes that are conserved in eukaryotes for RNA trafficking were manually annotated. Sixty-three genes containing RNA recognition motif (RRM) were identified. Some of these RRM genes are unique to filamentous fungi. One of them is important for conidiation and hyphal growth in *Neurospora crassa.* Functional characterization of selected RRM genes in *M. oryzae* will be presented. In addition, we have two spore morphology mutants that produced predominantly two-celled and one-celled conidia, respectively. RNA transcripts present in these defective conidia will be identified by microarray analysis and compared with those of the wild-type conidia. We expect that genes important for normal conidiogenesis will be affected in these conidia that are defective in polarity (one-celled conidia) or cytokinesis (two-celled conidia).

Biological control of *Ralstonia* wilt in tomato

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Bacterial wilt of tomato caused by *Ralstonia solanacearum* is a destructive soil-borne disease and a serious threat for agricultural production in Southern China. A screening strategy was developed to assess the potential biocontrol agents (BCA) of this disease from tomato-associated bacteria. We isolated 592 bacterial strains from different microenvironments, and obtained 139 ones according to their antagonistic activity against *R. solanacearum* and three phytopathogenic fungi (*Phytophthora capsici, Pythium ultium, Fusarium oxysporum*), their production of hydrolytic enzymes and secondary metabolites. Based on the results of in vitro tests above described, an assessment scheme was developed and 23 isolates were considered as the best candidates. Tomato as host plant was included in the greenhouse experiments in which the biocontrol efficacies, plant growth promotion and colonization capacity of these bacterial isolates were confirmed. These 23 antagonists belong to genus of *Bacillus*, *Serratia*, *Pantoea*, *Enterobacter*, *Erwinia*, *Citrobacter* and *Pseudomonas* based on the 16S rRNA gene sequences. A good positive correlation coefficient (r = 0.837) between biocontrol efficiency and assessment points verified the feasibility of the assessment scheme. And all 23 antagonists showed the ability to improve the plant growth in the greenhouse. Besides, *Pantoea ananatis* 1FY7 was the best strain to control *Ralstonia* wilt of tomato. Thanks to National Natural Science Foundation of China (30800714).

Biological control of take-all disease of wheat by *Pseudomonas fluorensce*

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In Hebei and Jiangsu province which have different environment, 553 bacterial strains were isolated from rhizosphere in the different growing stages of wheat. Totally 105 stains of were found to be antagonistic to *Gaeumannomy cesgraminis*. Among these antagonists, the bacterial strain GN4 and JB19 showed most effective inhibition against mycelial growth of *Gaeumannomy cesgraminis*. These bacterial strains were identified as *Pseudomonas fluorensce.* The bacterium also suppressed mycelial growth *in vivo* of other plant fungal pathogens such as *Fusarium graminearum, Bipolaris sorokiniana, Sclerotinia sclerotiorum*. Greenhouse experiment indicated that the biocontrol efficacy of these two strains were 55.07% and 60.31% respectively towards take all disease caused by *G. cesgraminis* on the average. This experiment was repeated 3 times, and each treatment was done with 60 wheat plants. In the field experiment, these strains achieved biocontrol efficacy of 51.25% and 55.46% respectively in 2008 in Jiangsu province. These results indicated that *P. fluorensce* GN4 and JB19 have a good application potential in the future. Thanks to Chinese 863 High-Tech Program (2006AA10Z431).

Mutation in *tctD* reduces virulence of *Xanthomonas oryzae* pv. *oryzae* KACC10859

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The RpfC and RpfG were well characterized as a two component regulatory system that regulates virulence and pathogenicity factors by a cell-cell communication in *Xanthomonas campestris* pv. *campestris*. Mutations in *rpfC* and *rpfG* homologs in *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) reduced virulence, which suggests that the function of the genes is conserved in *Xoo*. Microarray analysis of the *rpfC*$^-$ mutant was carried out to screen the RpfC regulons in *Xoo*. One of the significantly down regulated genes in the *rpfC*$^-$ mutant was *tctD* that is known as a two-component regulator involved in tricarboxylate transport in *Salmonella typhimurium*. Down regulation of the *tctD* in the *rpfC*$^-$ mutant was confirmed by RT-PCR. Marker-exchange mutation in *tctD* reduced the lesion length to 30% of wild type's. Growth of the mutant was not different to wild type in rich medium, nutrient broth. Cellulase, motility and EPS were reduced in the mutant, whereas xylanase was not different to wild type significantly. The phenotype change in the mutant was restored in the complementation strain. These results suggest that *tctD* is involved in virulence expression of *Xanthomonas oryzae* pv. *oryzae* KACC10859.

A novel nuclear protein Com1 is required for normal conidium morphology and full virulence in *Magnaporthe oryzae*

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Magnaporthe oryzae, the causal agent of rice blast, produces pyriform conidia that are the primary inoculum and the main source for disease dissemination

in the field. To address importance of the fungal conidial morphology in plant infection and disease dissemination, we isolated three insertional mutants that formed slender conidia. Sequences analysis showed that the three mutants were disrupted in the same *COM1* gene. The Δ*com1* mutants and the *com1* disruption mutants had similar defects in conidium morphology, and were significantly reduced in virulence on rice and barley seedlings. Microscopic examination revealed that the Δ*com1* mutants were defective in appressorium turgor generation, penetration, and infectious growth. Conidia of Δ*com1* mutants were also slow in sedimentation and were less in lipid body-like structure. *COM1* encodes a nuclei localized protein unique to filamentous ascomycetes and was expressed constitutively in *M. oryzae*. The Com1 protein had the putative helix-loop-helix structures and three predicted nuclear localization signal sequences. Deletion of the second NLS sequence, PPVKRPRE, eliminated the nuclear localization of the fusion proteins. Our data indicated that the *COM1* gene may encode a transcription regulator that regulates conidial development and invasive growth in *M. oryzae*.

NDR1/HIN1-like genes in _Glycine max_ with potential roles in defense against the Soybean Cyst Nematode, _Heterodera glycines_

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NDR1/HIN1-like (*NHL*) genes constitute a large gene family in plants that encode putative membrane associated proteins with potential functions in plant responses to pathogens downstream of initial signal recognition. Certain members of this gene family in *Arabidopsis* have been shown to be induced in response to avirulent bacteria, expression is suppressed by virulent bacteria, and overexpression correlates with increased resistance. However, the molecular function of NHL proteins and how they may be contributing to plant defense against pathogens remains unknown. Several soybean (*Glycine max*) genes showing sequence homology with *Arabidopsis NHL* genes were identified to be differentially expressed in microarray studies comparing the gene expression profiles of laser-microdissected feeding cells (syncytia) induced by the soybean cyst nematode (SCN; *Heterodera glycines*) from resistant and susceptible soybean lines. *NHL promoter-GUS* fusions confirmed nematode-inducible expression of these genes specifically within developing syncytia. Quantitative real-time PCR determined that the timing and level of expression of these *NHL* genes in response to SCN infection is faster and higher in the resistant line. Moreover, *NHL* transcript levels are suppressed by a virulent SCN population. We are currently assessing soybean lines overexpressing *NHL* genes for increased resistance to SCN.

Members of soil bacterial communities sensitive to tillage and crop rotation

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Microorganisms play a major role in soil fertility and agricultural practices are known to exert influences on the community diversity of soil microorganisms. By using high-throughput sequencing approaches, we examined microbial populations in four cultivation and crop rotation treatments from a long-term field experiment in Kansas. From the two years of sampling, a total of 20,180 DNA 16s rDNA sequences were generated and a total of 2337 operational taxonomic units (OTUs) were assembled using a 97% similarity cutoff. The most abundant taxonomic group was the phylum Acidobacteria. Many of the Acidobacteria Group 1 OTUs showed a significant preference for continuous wheat over a wheat-soybean rotation and half the Acidobacteria Group 2 OTUs showed a preference for no-till over conventional tillage. Seventy percent of the contigs of Acidobacteria group 3 showed no treatment preference, but 22% showed a preference for the continuous wheat rotation. Unlike the other Acidobacteria groups, 75% of Group 4 contigs showed a preference for the wheat-soybean rotation. OTUs in the genus *Gemmatimonas* showed a range of treatment preference similar to Acidobacteria group 1. Estimated differences in the abundance of different taxa were validated by quantitative real-time PCR. Differences in specific taxa are easy to detect by high-throughput sequencing approaches, which identified bacterial taxa that were sensitive to tillage and rotation effects.

Culture- and non-culture based methods to detect _Lysobacter enzymogenes_ in soil

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Strains of *Lysobacter enzymogenes* (*LE*), a bacterial species with biocontrol activity, have been detected by 16S rDNA in soil in many parts of the world. In most cases, however, their occurrence could not be confirmed by isolation, presumably because the species occurred in lower numbers than faster-growing bacterial species. In this study, we developed DNA-based detection (DBD) and enrichment culturing method (ECM) for *Lysobacter* spp. and *LE* specifically. In DBD, a region of 16S rDNA conserved among *Lysobacter* spp. (L4-F: GAGCCGACGTCGGATTAGCTAGTT) was used as the forward primer in PCR amplification. When L4-F and universal bacterial primer 1525R were used to amplify DNA from bacterial species, an 1100-bp product was found in *Lysobacter* spp. exclusively. ECM involved culturing soils for 3 days in modified chitin broth. Bacterial strains in the enrichment culture were isolated on yeast-cell agar and then identified by 16S rDNA analysis. A strain of *LE* added to soils was detected as low as 10^2 and 10^4 CFU/g soil by PCR amplification and enrichment culturing, respectively. In a survey of 56 soil samples, *Lysobacter* was detected by PCR in 19 samples, of which 5 yielded 16 strains of *Lysobacter* spp. via enrichment culture. All 16 isolated strains were identified to be *LE,* with the exception of a strain of *L. antibioticus*. Although neither method alone is fully effective at detecting *LE,* they are complementary when used together and may provide new information on the spatial distribution of the species in soil.

Genetic diversity of _Citrus tristeza virus_ isolates collected recently in California

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Surveys conducted over the past several years show a dramatic increase in CTV incidence in several locations in Central California. Our objective was to assess genetic diversity of CTV populations that are currently being spread and determine their phylogenetic relationship with representative isolates from collections established over the past 40 years at the Citrus Clonal Protection Program, Riverside, CA and CCTEA, Tulare, CA. Over 385 field isolates were assessed by RT-PCR, MMM analysis, SSCP and sequencing of the CP and other relevant genes, and qRT-PCR with strain-specific probes. More than 90% of these isolates had a T30-like genotype and 37 field isolates contained a non-standard (NS) CTV genotype. From 2000, field surveys have not detected severe CTV genotypes in either CTV eradicative or non-eradicative citrus districts in Central California. The NS isolates reacted with the CTV monoclonal antibody, MCA13 but were mild in biological characterization tests. Sequence homology in the CP gene of the NS isolates was found with *Poncirus trifoliata* resistant-breaking CTV strains. Tests are underway to determine if these isolates replicate in *P. trifoliata* or its hybrids. Comparative analyses with collection isolates showed that NS genotype isolates have been occasionally intercepted in California since 1993. Knowledge of CTV population structure and dynamics are being used to design more effective strategies to mitigate diseases caused by severe strains of CTV.

Epichloë endophytes from cool season grass germplasm

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Epichloë endophytes are agriculturally important fungal symbionts that form mutualistic symbioses with cool season grasses. They are known to produce a range of bio-protective alkaloids, peramine, lolines, ergot alkaloids and lolitrems, with anti-insect and anti-mammalian properties that help protect their grass host. Cool season grass germplasm has been obtained from the USDA National Plant Germplasm System and collections made throughout USA, Mexico, Greece and Argentina. This material was screened to identify endophytes that enhance plant performance without having detrimental effects on livestock health. Plants or seed stocks are screened for the presence of epichloae utilizing a high throughput PCR system that is specific to these endophytes. Isolates of interest are cultured from the plant material and maintained as lab stocks for further analysis. Molecular screens are conducted by PCR to characterize the presence or absence of known alkaloid biosynthesis genes, such as those for ergot alkaloids and lolitrems. Tissue from endophyte infected plants is analyzed to confirm alkaloid profiles. Correlation between natural genetic variants and alkaloid profiles can be used to identify candidate genes involved in the production of specific compounds and pathway intermediates. Promising endophyte isolates can be inoculated into plants to determine host range and ultimately the bio-protective and mammalian toxicity potential of an endophyte.

Epidemiology of soybean rust (*Phakopsora pachyrhizi*) in soybean (*Glycine max*) sentinel plots in Florida

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The overwintering of soybean rust (SBR) in the Southeastern United States has been variable due to weather conditions which may influence disease incidence and severity in the major soybean producing regions of the Midwest, making it important to understand the epidemiology of the pathogen in Florida. This study examined the incidence and severity of SBR in relation to prevailing weather data, growth stage, and maturity group (MGIII, MGV, MGVII) in 15 m square soybean plots across the Panhandle of Florida from 2005 through 2008. Of the three majority groups, the MGIII soybean became infected first the least often. Plots became first infected at growth stage R4 (full pod) or later. On average, plots became infected 40 days earlier in 2008 than 2005. Precipitation was the principle factor affecting disease progress, where disease increased rapidly after rain events and was suppressed during dry periods. The area under the disease progress curves (AUDPC) for incidence and severity was the lowest in 2007, most likely due to dry conditions. In 2008, there was a significant increase in disease incidence and severity as reflected in the AUDPC. This can be attributed in part to the occurrence of Tropical Storm Fay, which deposited up to 290 mm of water in the plot locations during the third week of August. Results from this study may lead to a better understanding of the impact of weather on the epidemiology of this pathogen.

Stigmasterol and cholesterol down-regulate the expression of elicitin genes in *Phytophthora sojae*

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Elicitins are extracellular proteins unique to oomycetes of the genera *Phytophthora* and a few *Pythium* species. Class-I elicitins are presumed to function as sterol carrier proteins in these organisms, but the relationship between sterol availability and elicitin secretion has never been explored. We examined the expression of class-I elicitin genes when stigmasterol and cholesterol were supplemented into the growth medium of the soilborne pathogen of soybean *Phytophthora sojae*. The growth of *P. sojae* was stimulated by nanomolar concentrations of stigmasterol and cholesterol, which also resulted in the down-regulation of its elicitin genes over time when expression profiles were monitored using real time Reverse Transcription Polymerase Chain Reaction (RT-PCR). The down-regulation of elicitin genes in response to the two sterols also coincided with a reduction in the amount of elicitins detected in spent filtrates. Our study is the first to show the influence of sterols on the expression of elicitin genes in *Phytophthora*, which is important with respect to the ecology of elicitin secretion as sterol carrier proteins in the environment.

Purification, characterisation and immunolocalization of extracellular β-1,3-glucanase secreted by take-all pathogen in infected wheat roots

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The properties of β-1, 3-glucanase secreted by *Gaeumannomyces graminis* var. *tritici* (*Ggt*), the causal agent of take all disease, and role in the pathogenesis were studied. An extracellular enzyme Glu*Ggt* was purified from filtrate of *Ggt*. The pI of active protein determined by IEF-PAGE was 6.3. The Glu*Ggt* yielded two subunits with molecular masses of 66.2 kDa and 56.0 kDa, respectively, in SDS-PAGE. The special antibody against Glu*Ggt* was prepared and allowed localization of these β-1, 3-glucanases *in situ* in inoculated wheat roots by immunogold labeling. Hyphal cell walls and septa as well as membraneous structures showed regular labeling, while few gold particles were detected over the cytoplasm and other organelles such as mitochondria and vacuoles by TEM observation. In host tissues, cell walls in contact with the hyphae usually showed a few gold particles, whereas host cytoplasm and cell walls distant from the hyphae were free of labeling. Furthermore, over lignitubers in the infected host cells labeling was detected. No gold particles were found over sections of non-inoculated wheat roots. The results indicate that β-1, 3-glucanase secreted by *Ggt* may be involved in pathogenesis of the take-all fungus through degradation of callose in postinfectionally formed cell wall appositions, such as lignitubers.

Dynamics of rice blast resistance genes in the *Pik* cluster and molecular dissection of the *Pik-p* gene

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The *Pik* cluster located on rice chromosome 11, which consists of *Pik*, *Pik-s*, *Pik-h*, *Pik-m*, and *Pik-p* alleles, is a major resistance gene resource for rice breeding programs in China. Dynamics of resistance of these alleles was characterized with a total of 612 isolates of *Magnaporthe oryzae* collected from various regions in China in combination with 16 main resistance genes being used in the breeding programs. Each allele is an independently and dominantly acting gene in the *Pik* cluster, and conditions differential reactions against many isolates. For molecular dissecting the *Pik-p* gene, a genetic and physical map of *Pik-p* locus was constructed using genomic position-ready markers. Then, the *Pik-p* gene was isolated through an approach called map-based cloning, *in silico*. The function of the *Pik-p* gene was dissected using both forward and reverse genetic approaches such as transformation, RNAi, and gene interaction. The detailed results will be presented in the meeting.

Assessment of longleaf pine on high-risk and low-risk loblolly pine decline sites at Fort Benning, Georgia

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Southern pine decline negatively affects forest productivity in the southeastern United States. Loblolly pine is the species most affected, though longleaf pine decline has also been reported less frequently. Site risk factors and biotic agents, including *Leptographium* spp., associated with loblolly pine decline have been explored yielding the Loblolly Pine Decline Risk Model (LPDRM). To test the applicability of the LPDRM to longleaf pine decline, plots were installed in predicted high and low risk longleaf pine stands at Fort Benning, GA. Tree health was assessed by crown rating, resin sampling, and growth measurements. *Leptographium* spp. were monitored by isolation from pitfall-trapped beetle vectors and by direct isolation from excavated roots. In this study, longleaf pine stands did not exhibit decline symptoms on predicted high risk sites. Trees in all age and decline classes were infected with *Leptographium* spp., mainly *L. procerum* and *L. terebrantis*. Infected trees did not exhibit crown symptoms or differences in resin mass and effects on growth were limited. Beetle capture numbers and levels of fungal infestation were similar on high and low risk plots. While longleaf pine may be susceptible to decline, these results suggest that with proper management longleaf pine may be a reasonable alternative species to plant on high risk loblolly pine decline sites.

Comparison of Brassicaceae seed meals for *Meloidogyne incognita* and *Pratylenchus penetrans* control

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There is growing interest in utilizing Brassicaceae seed meals in pest management systems. To achieve consistent and reliable pest suppression with these materials we must have a deeper understanding of their relative toxicities and the application rates necessary for acceptable control. Towards this end, we evaluated four mustard seed meals, *Brassica juncea*, *Sinapis alba*, *B. napus* 'Sun' (low glucosinolate-containing seed meal) and *B. napus* 'Dwarf Essex' (high glucosinolate-containing meal) at rates ranging from 0 to 10% dry w/w against *Meloidogyne incognita* second-stage juveniles (J2) and *Pratylenchus penetrans* mixed stages. At rates greater than 5% all of the meals resulted in almost 100% nematode mortality compared to an untreated control. The meals differed in their toxicity against the plant-parasitic nematodes. At a 0.5% rate the relative order of meal potency was *B. juncea* > *B. napus* 'Dwarf Essex' = *S. alba* > *B. napus* 'Sun'. At application rates lower than 5% the toxicity of these seed meals to plant-parasitic nematodes was related to glucosinolate type and concentration.

Field effects of biological control products and potassium silicate on *Sclerotinia sclerotiorum* in soybean

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Field trials were conducted in PLP farm and Clarksville in Michigan to evaluate the efficacy of Actinovate (*Streptomyces lydicus*), Sil-MATRIX

(potassium silicate), Contans (*Coniothyrium minitans*), and PlantShield (*Trichoderma harzianum*). Fungicide Endura was used as a control. A randomized complete block design (RCBD) with 4 replications in PLP and split (pre-treated with Contans in the previous fall or non-treated) RCBD with 3 replications in Clarksville were applied at recommended rates. *S. sclerotiorum* sclerotia were spread in the soil surface and slightly disked in the top soil. The products described above were applied in soil a month before soybean was sowed. A sprinkler irrigation system was used to increase the soil moisture in PLP. Sclerotia were retrieved from soil at planting and harvesting, and plant yield, and sclerotia number per volume of beans (SNB) were measured. Due to a low disease incidence, SNB was used as disease measurement. Contans had the greatest effect on reducing SNB and sclerotia in soil by 91.7% and 96.9% respectively. Actinovate, Sil-MATRIX, PlantShield, and Endura reduced disease (SNB) by 85.9, 84.9, 80.5, and 69.9%, and sclerotia in soil by 88.2, 96.8, 77.9, 61,4, respectively. PlantShield also increased the soybean yield by 11%. Pre-treatment with Contans gave better efficacy on sclerotial reduction and disease control. Therefore, Contans had the best efficacy on controlling *S. sclerotiorum*.

Genotype shift in a *Venturia inaequalis* population during an Apple scab epidemic in Pennsylvania

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Apple scab, caused by the heterothallic Ascomycete *Venturia inaequalis* continues to be one of the most serious and widespread problems of apple orchards in temperate regions, despite the intensive fungicide-based management of this disease. Spring primary inoculum is composed of ascospores and the disease undergoes six to eight asexual secondary cycles (conidial inoculum) throughout the growing season. Knowledge about population biology of the pathogen is crucial to design new and efficient control strategies. Our overall goal is to understand how populations of *V. inaequalis* change during an epidemic and what component of fitness is the main driving force of genotype changes. The growing season of 2008 presented ideal climatic conditions for development of Apple scab epidemics in PA. We sampled *V. inaequalis* from leaf and fruit lesions at three times during 2008, representing the beginning (May), middle (July) and end (September) of a scab epidemic. Four hundred isolates were obtained from scab lesions of untreated trees of cvs. Golden Delicious and Rome Beauty in an orchard located in Biglerville (PA). Isolates were genotyped using fourteen polymorphic microsatellite markers. Our hypothesis is that there is a significant reduction of genotype diversity as the population is overtaken by few, very fit individuals during the epidemic development. We will discuss the potential impact of *V. inaequalis* population dynamics in Apple scab management.

Functional characterization of *SREA* in *Cochliobolus heterostrophus*

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Phytopathology 99:S149

A GATA-type transcription factor encoding gene, *SREA*, was characterized in *Cochliobolus heterostrophus*, a fungal pathogen of maize. SREA had been illustrated to play a central role in iron uptake and siderophore biosynthesis in other model systems including *Aspergillus nidulans* and *Ustilago maydis*, in which it represses the siderophore biosynthesis under sufficient iron supply. In this study we found that the *sreA* deletion mutants had decreased growth rate and conidium germination rate, and increased tolerance to oxidative stress compared to the wide type (WT). However, the virulence of the mutant was not significantly different from the WT. To identify members of SREA regulon in *C. heterostrophus*, 28 candidate genes were selected for gene expression analyses, based on the knowledge of the iron uptake, regulation, and related pathways. qRT-PCR results indicated that in *C. heterostrophus*, two genes in the siderophore biosynthesis pathway (*NPS6 and SIDA2*) were derepressed in the *sreA* mutant under iron sufficient conditions. Genes seem to be regulated by SREA in other forms include an antioxidative enzymes catalase gene (*CAT1*), a superoxide dismutase gene (*SOD1*) and an ATP binding cassette gene (*ABC6*).

Application of acibenzolar-S-methyl and silicic acid for suppressing Phytophthora blight of squash under greenhouse conditions

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Phytopathology 99:S149

Phytophthora blight, caused by the oomycete *Phytophthora capsici* Leonian, is a devastating disease to cucurbit production in the US and worldwide. Control of this disease remains an intractable problem. Highly resistant varieties with ideal horticultural traits are currently not available. The long-term survival of *P. capsici* oospores in the soil limits the effectiveness of crop rotation. No fungicides are highly effective against *P. capsici*. More importantly, it has been reported that *P. capsici* has developed resistance to certain fungicides applied for Phytophthora blight control. Greenhouse studies have been conducted to evaluate the potential of the use of acibenzolar-S-methyl (ASM; Actigard 50WG) and silicon nutrient (silicic acid) for control of Phytophthora blight on squash. Applied as soil drenches or foliar sprays, Actigard at 20 and 30 mg/l significantly ($P < 0.05$) reduced disease severity compared to the nontreated control. Actigard at 30 mg/l provided the greatest protection in squash against *P. capsici*, and no disease symptoms developed on treated plants whereas the nontreated inoculated plants collapsed due to the infection of *P. capsici*. Silicic acid applied as a soil drench at 0.15 mM significantly suppressed disease severity of *P. capsici*. The results suggest that ASM and silicic acid are effective against *P. capsici* in squash and may be incorporated in integrated management strategies for control of Phytophthora blight of squash.

Evaluation of plant growth-promoting rhizobacteria for their effect on Phytophthora blight of squash in the greenhouse

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Phytopathology 99:S149

Phytophthora blight caused by *Phytophthora capsici* is a serious threat to vegetable production in the US and worldwide. It is imperative that practical and cost-effective alternatives to chemicals be developed in order to sustain production of vulnerable vegetables in Florida. Greenhouse studies were conducted to evaluate the potential of the use of plant growth-promoting rhizobacteria (PGPR) for control of Phytophthora blight on squash. PGPR strains were applied as soil drenches 1 and 2 weeks after planting (WAP) in soilless potting mix, and squash seedlings were inoculated with *P. capsici* 3 WAP. PGPR strains SE34, SE49, SE76 and IN937a applied at 10^7 CFU/ml significantly ($P < 0.05$) reduced disease severity of Phytophthora blight of squash compared to the nontreated control. Interestingly, treatment with certain other PGPR strains in combination significantly reduced the disease severity of *P. capsici* while each individual strains alone did not. No significant effect on Phytophthora blight disease was observed by seed treatment with any PGPR strains tested. Data from this research indicated that PGPR are effective against *P. capsici* in squash, and improved disease control can be achieved by multiplexing PGPR strains. The effect needs to be confirmed under field conditions to determine if the strategy may be incorporated in integrated management strategies for management of Phytophthora blight of squash.

Efficiency and stability of *Foot and mouth disease virus* vp1 epitope expression from a Chinese isolate of *Tobacco necrosis virus A*

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Phytopathology 99:S149

In the current study, an infectious cDNA clone of *Tobacco necrosis virus A* (TNV-A^C) was used for fusion of different epitopes of *Foot and mouth disease virus* (FMDV) serotype O VP1 downstream of the TNV-A^C coat protein. *Chenopodium amaranticolor* plants infected with *in vitro* transcripts of the recombinant viruses developed symptoms similar to those caused by wild type TNV-A^C. Western blotting with antiserum against TNV-A^C particles revealed that there combinant viruses were highly expressed. Large amounts of chimaeric virus (~330 µg/g fresh weight leaf tissue) were purified from systemically infected *Nicotiana benthamiana* leaves, whereas much lower yields (~ 30 µg/g) were recovered from other plants. In addition, large numbers of virions were observed in infected leaves by electronic microscopy. Western blotting with rabbit antiserum against FMDV VP1 or serum from infected cattle revealed highly expressed FMDV epitopes. Additional immunoblotting and DNA sequence analyses showed that most of the chimaeras contained unmodified foreign peptides even after six successive passages in *C. amaranticolor*. The results also suggest that the amino acid sequence and its length has a substantial influence on viral morphogenesis and systemic infections. Immunoelectron microscopy was used to identify FMDV VP1 epitopes on the surface of virus particles, and the abilities of the purified viruses to induce humoral and cell-mediated immune responses in BALB/c mice were also determined.

Detection and distribution of mating-type of *Setosphaeria turcica* causing northern corn leaf blight in China

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Northern corn leaf blight, caused by heterothallic ascomycetous fungus *Setosphaeria turcica*, can cause the yield losses of corn production severely. *MAT1-1* encodes for a protein with an α-box motif and *MAT1-2* encodes for a protein of the high mobility group (HMG). A pair of specific primers was designed by comparing the sequences of α-box from 16 fungal species of Pleosporales to amplify *MAT1-1* and another pair of specific primers was designed based on the sequence of HMG (Accession No. E15510) to amplify *MAT1-2*. The mating-types of 115 isolates collected from Beijing and 11 provinces of China were detected using the two pairs of primers, respectively and examined by pairing culture on Sach medium at the same time. The results from PCR method and the traditional detection method were consistent with each other, showing that 49 isolates were *MAT1-1* and 66 isolates were *MAT1-2*. It is indicated that there were two mating-types and the ratio of *MAT1-1* and *MAT1-2* was approximately 1:1 in *S. turcica* from China, suggesting that sexual reproduction of the pathogen could occur if the environmental conditions are suitable for perithecia formation in nature, despite the perfect stage has not been found in the field.

Effects of a crude toxin from cultures of *Fusarium oxysporum* f. sp. *conglutinans* on the germination of cabbage seed

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Fusarium wilt of cabbage, caused by soilborne fungus *Fusarium oxysporum* f. sp. *conglutinans*, was first reported in Yanqing County, China, in the summer of 2001. The effect of a crude toxin (CT), extracted from a culture filtrate of *F. oxysporum* strain GLW3, on cabbage seed germination was investigated in the laboratory. Three concentrations (100%, 50% and 10%) of the crude toxin were incorporated into Czapek and Richard media. Sterile distilled water and media without the toxin served as controls. Cabbage seed from two local cultivars, *Beinongzaosheng* (susceptible to wilt) and *Zhenqi* (resistant to wilt), were used. Seed were placed on the media amended with the different levels of CT for 24 hours. The seed was then removed and germinated on two layers of filter paper in Petridishes maintained at 18–20°C for 3 days. CT both in Czapek and Richard media suppressed the germination of cabbage seeds. Increasing concentrations of CT were increasingly inhibitory to seed germination. Germination of cabbage seed in Czapek medium with 50% CT was more suppressive (86.9–92.6%) than in Richard medium (50.8–69.6%).

Antagonistic activities and properties of metabolites from *Bacillus* sp. CE

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Phytopathology 99:S150

The strain *Bacillus* sp. CE, isolated from the rhizosphere of peach tree in Beijing, China, showed obvious antagonism against *Monilinia fructicola*. The antifungal component of the metabolites from CE was roughly purified by precipitating with ammonium sulfate of 60%, and it could endure the treatment of a high temperature of 100°C for 20 min, and maintain the antifungal activity after treatment of proteinase K, trypsinase and ultraviolet radiation. The antagonistic substance of CE can inhibit the mycelium growth of *M. fructicola* and the diameter of inhibition zone was 33 mm. When tested in the solution with the antagonistic substance produced by CE electrical conductivity of hyphae increased and more vacuoles were observed in mycelium cells under the electronic microscope which indicated that the antagonistic substance damaged the membrane permselectivity and internal structure of hyphae. The conidia treated with the antagonistic substance were also abnormal in morphology and cannot germinate. Moreover, the antagonistic substance inhibited the infection to peach fruit and delayed the lesion expansion of *M. fructicola in vitro*. The metabolites from CE also showed strong antifungal activity against other 8 tested fungi, such as *Valas mali*, *Sclerotinia sclerotiorum*, *Alternaria brassicae*, *Fusarium graminearum*, and *Verticillium dahliae* et al. Thanks to Beijing Municipal Education Commission (KM200710020003).

The behaviour of *Marssonina coronaria* in early stage of infection on apple leaves

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Phytopathology 99:S150

Marssonina coronaria, the causal agent of *Marssonina* leaf blotch of apple, leads to severe premature leaf fall since early summer which weakened the trees and reduced the yield and quality of apple fruit. The infection behaviour of *M. coronaria* on apple leaves was examined by using fluorescence and electron microscopy. Conidia used as inoculum were washed from either diseased leaves or culture of the pathogen on potato carrot dextrose agar at 25°C for 14 d. Excised apple leaves were inoculated by dropping 20 µl of spore suspension (concentration approximately 1×10^5/ml) on upper and lower surface separately, and placed in a chamber at 25°C to keep satisfied moisture. The germinated conidia were observed 6 h after inoculation. The most two-celled conidia produced one germtube from one cell of the conidia. But two germtubes formed from each cell of the conidia were observed too. Occasionally, the conidia germtube were found with branch or with septum. Penetration occurred on both side of the leaves 12 h after inoculation. Most conidia formed appressorium and penetrated apple leaves between adjacent epidermal cells. Invading through stomata was not observed. The infection and expansion process of *M. coronaria* in leaf tissues, and the host cell reactions are unclear and need to be clarified in further study.

Resistance to thiabendazole and sensitivity to fludioxonil and pyrimethanil in *Botrytis cinerea* populations from apple and pear in Washington State

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Phytopathology 99:S150

Gray mold caused by *Botrytis cinerea* is a common postharvest disease of pome fruits. Thiabendazole (TBZ) was the most commonly used postharvest fungicide prior to the registration of fludioxonil (FLU) and pyrimethanil (PYR) in 2004. In this study, 83 and 40 isolates of *B. cinerea* that had not been exposed to FLU and PYR were obtained from apple and pear, respectively, screened for resistance to TBZ, and tested for sensitivity to FLU and PYR. Three isolates from apple were highly resistant to TBZ, while all remaining isolates were sensitive to TBZ. EC_{50} values ranged from 0.003 to 0.038 (mean = 0.005) µg/ml for FLU and from 0.013 to 0.173 (mean = 0.056) µg/ml for PYR. One apple isolate exhibited reduced sensitivity to FLU with EC_{50} of 0.038 µg/ml, which was significantly higher than those of remaining isolates tested and was considered resistant to FLU. After 20 successive generations on PDA and four generations on apple fruit, the FLU-resistant isolate retained the same level of resistance to FLU as the original generation. However, it was less pathogenic and virulent on apple fruit and produced fewer conidia in vivo at 0°C than FLU-sensitive isolates. On apple fruit at 0°C, the FLU-resistant isolate was completely controlled by TBZ and PYR but only partially controlled by FLU. The results indicate that the vast majority of isolates in the baseline population of *B. cinerea* from pome fruits in the region were sensitive to the two new postharvest fungicides.

Multiple personalities of *Streptomyces* spp. from the rhizosphere of apple cultivated in brassica seed meal amended soils

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Phytopathology 99:S150

Brassicaceae seed meal soil amendments provide control of Rhizoctonia root rot of apple, in part, through the proliferation of indigenous *Streptomyces* spp. Studies were conducted in an attempt to assess the relative role of antibiosis and nitric oxide (NO) production in the capacity of *Streptomyces* strains to control *Rhizoctonia solani* AG-5. Among the four dozen isolates tested, there existed no clear association between the capacity to suppress in vitro growth or produce NO and the ability to suppress apple root infection by *R. solani* AG-5. Among those providing suppression of Rhizoctonia root rot, the NO-producing population provided greater disease control than the non-producing population, however several exceptions were observed. Isolates with affinity to *S. atratus*, *S. avidinii*, and *S. cirratus* consistently suppressed apple root infection by *R. solani* AG-5. Surprisingly, root infection by *R. solani* was significantly elevated in the presence of *S. vinaceaus.* When co-inoculated with certain isolates having affinity to *S. herbaricolor*, *R. solani* root infection was significantly elevated resulting in elicitation of novel leaf symptoms that were not observed on plants in soils infested with the pathogen alone. When examined individually, these same *S. herbaricolor* isolates induced leaf necrosis while *S. anulatus* inhibited root development of apple seedlings initiated from seed.

A bi-directional promoter from rice drives high level expression of report genes in Monocotyledon and Dicotyledonous plants

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Phytopathology 99:S150

A bi-directional promoter is desired when multiple genes are introduced to develop genetically modified plants with a novel trait. To isolate such a

promoter, we performed negative subtractive screening *Magnaporthe oryzae*-induced rice genes and identified two rice genes, *OsSCI2* and *OsSCI3* that were linked to each other head by head in the genome. Thus, the intergenic region between *OsSCI2* and *OsSCI3* was supposed to be a bi-directional promoter and was designed as *pSCI*. Constructs containing the *pSCI* fused with reporter genes were introduced into rice and *Arabidopsis thaliana*, respectively. Histochemical staining, GUS enzyme activity assay and northern blot Analyses showed that *pSCI* activated expression of reporter genes in transgenic plants of both species. In rice, reporter genes were constitutively expressed in roots but were induced to express in leaves by infection of *M. oryzae*; while in *A. thaliana*, reporter genes were constitutively expressed in both roots and leaves. Further analysis revealed that a short deletion in one end of *pSCI* significantly reduced expression level of reporter genes fused at the other end. These results demonstrated that *pSCI* is a bi-directional promoter, which will be useful for simultaneously engineering expression of two genes to generate disease-resistant plants.

Flower crinkle of *Phalaenopsis* orchids — A new disease caused by an old virus

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A new disease with symptom showing flower crinkle on *Phalaenopsis* orchids bearing white flowers has been observed in Taiwan, China and Japan for three years. This disease decreases the flower longevity and was considered as a physiological disorder. The objectives of this study were to identify and characterize the real causal agent of this new *Phalaenopsis* disease. Five plants of Phal. Sogo Yukidian "V3" (Phal. Yukimai × Phal. Taisuco Kochdian) bearing flower crinkle symptom were collected and tested by enzyme-linked immunosorbent assay with antisera against 18 viruses. The leaves and flowers from one diseased plant reacted positively only to the antiserum against *Odontoglossum ringspot virus* (ORSV), while those from the other four plants reacted positively to the antisera againt ORSV and *Cymbidium mosaic potexvirus*. To identify the causal agent of flower crinkle disease, viruses were isolated from the diseased V3 orchid plants and back inoculated on to healthy *Phalaenopsis* plants. By three individual tests and molecular diagnosis of the inoculated plants, ORSV was found to be the causal agent for this new disease. To our knowledge, this is the first report of ORSV causing flower crinkle on *Phalaenopsis* orchids.

Influence of acibenzolar-S-methyl, nonpathogenic *Fusarium oxysporum* 'CS20' and hybrid common vetch winter cover crop on Fusarium wilt of watermelon

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Applying abiotic or biotic agents that induce systemic disease resistance in watermelon may be an effective approach to manage Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (FON). The objectives of this study were to i) determine effective rates of the resistance inducers acibenzolar-S-methyl (Actigard) and nonpathogenic *F. oxysporum* isolate CS20 in the greenhouse and ii) evaluate their effectiveness when used with 'Cahaba White' hybrid common vetch in the field in Maryland and South Carolina. In the greenhouse, Actigard at 0, 20, 100 or 500 ppm (equivalent to 0, 8, 40 and 200 g/ha) was sprayed on seedlings and CS20 drenched into soil to achieve 0, 10^3, 10^4 or 10^5 microconidia/g. Actigard and CS20 at all rates reduced infection by FON. Actigard at 500 ppm was phytotoxic. Vetch and rye were planted as winter cover crops and incorporated into soil in 2007 and 2008 in both states. CS20 was applied to seedlings prior to transplanting and Actigard was sprayed on watermelons four times after transplanting. In Maryland, Actigard reduced wilt incidence in 2007. CS20 and its combination with Actigard reduced wilt incidence in both years and increased fruit yield in 2008. Vetch decreased wilt incidence and increased yield compared to rye. In South Carolina, Actigard increased yield in 2007 but wilt incidence did not differ among cover crops and inducers. Vetch increased soil populations of *Bacillus* spp. in Maryland but not in South Carolina.

Genetic diversity of citrus huanglongbing bacterium '*Candidatus* Liberibacter asiaticus'

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Candidatus Liberibacter asiaticus (Las) is one of the most important species associated with citrus Huanglongbing (HLB) worldwide, and is the only species detected in Florida to date. However, different types of symptoms and different bacterial titers were observed in HLB-affected citrus and periwinkles both in the field and greenhouse. It is interesting to note that the different HLB phenotypes on periwinkles can be maintained by graft transmission. In this study, eight of HLB-infected citrus, periwinkle plants with distinct symptoms were used to construct different library based on rRNA operon, outer membrane protein (OMP) gene and rplKJAL-rpoB loci. Single nucleotide polymorphisms (SNPs), were found in all the libraries, raging 1.7 to 12%. But there is no significant deletion and insertion in these genetic loci. These results indicated that Las bacteria present as a mixed population in a single ecological niche, which could be important for the disease complex. Our recently completion of Las genome sequencing allowed us to decipher further on the genetic diversity of Las bacterium using other variable loci.

Multilocus sequence analysis of *Monilinia fructigena* from China

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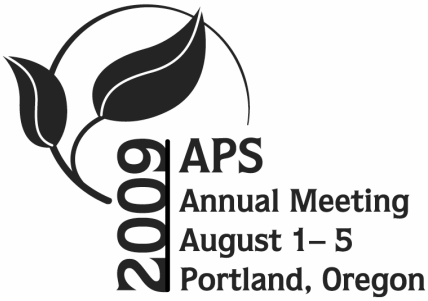
Brown rot is an important disease on stone and pome fruits in China. As sequence difference was found in the species specific primer based on the internal transcribed spacer region of rDNA in a previous study, multilocus sequence analysis was used to investigate the diversity among the population of *Monilinia fructigena* from China. Isolates collected from apple, pear and peach with symptomatic brown rot in several provinces in China, which were identified as *M. fructigena* by morphology characters, and *M. fructigena* from Europe and *Monilia polystroma* from Japan were used in this study. Isolate of *Botrytis cinerea* was used as an outgroup. Three protein coding genes (β-tubulin gene, *bt*; elongation factor 1-α gene, *ef-1α*; laccase 2 gene, *lcc2*) and ribosomal DNA in the internal transcribed spacer region were sequenced. Sequence alignments were conducted and phylogenetic trees were reconstructed by maximum likelihood with software DNAMAN 5.2.2. Phylogenetic trees from these three genes are similar and the isolates of *M. fructigena* are divided into three groups based on the sequences of these genes. The first group contains only the European isolates. The second group contains isolates from China and the isolates of *Monilia polystroma* from Japan. The third group contains isolates solely from different provinces in China. Our preliminary results suggest that isolates of *M. fructigena* from China is different from those European isolates, and some of them are close to *Monilia polystroma* from Japan.



2009 APS Annual Meeting
Abstracts of Special Session Presentations

Biology of Plant Pathogens

Coordinated Regulation of Fungal Development and Secondary Metabolism During Pathogenesis

Aspects of habitat important to *Fusarium verticillioides* during pathogenesis of maize kernels

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Fusarium verticillioides has at least two discernible interactions with its host. The most obvious is the destruction of host tissues: causing root rot, stalk rot, and kernel/ear rot. In a second type of interaction, the pathogen grows as an endophyte without visible signs or symptoms. Evidence suggests that *F. verticillioides* responds to its microenvironment during colonization of the maize kernels. The responses are triggered by plant metabolites within kernel, often resulting in a modification of the kernel environment by the fungus. The most noted change is pH. Fungal growth on germ tissues leads to alkalinization of the tissue, in contrast to endosperm tissues, which becomes acidic during colonization. Alkalinization also occurs when the fungus infects blister stage kernels and high-amylose genotypes of maize. Production of fumonisin B1 (FB1) is also part of the fungal response to its host environment. In mature kernels, the starch-rich tissue of the endosperm supports higher levels of FB1 than germ tissue. The uptake and subsequent utilization of sugars derived from starch in endosperm may drive kernel acidification, which is conducive for FB1 biosynthesis. Recently, we have identified several genes that appear to have roles in the perception of and/or the response to inducers in the kernel. We are investigating the potential mechanisms responsible for regulating FB1 production during the destructive or endophytic states of the pathogen.

Regulation of morphogenesis, secondary metabolism, and pathogenicity by the VeA system in *Aspergillus* and *Fusarium* species

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Phytopathology 99:S152

A number of biological processes are intimately connected to an organism's developmental program. This is true of processes such as production of toxins and other secondary metabolites in pathogenic fungi. In many cases the signaling pathways involved in these cellular processes are very complex and often the cross-talk between them is not well understood. Here we describe the role of the VeA regulator in morphogenesis, secondary metabolism and pathogenesis in two agriculturally important fungal genera: *Aspergillus* and *Fusarium*. We showed that the *veA* gene positively regulates sterigmatocystin production in *Aspergillus nidulans* and aflatoxin production in *A. parasiticus* and *A. flavus*, species often found infecting oil seed crops worldwide. In addition, *veA* is required for the production of resistant structures called sclerotia in these fungi. Furthermore, our recent studies revealed that virulence of *A. flavus* on peanut, corn and cotton was reduced in the absence of the *veA* gene product. Whether *veA* homologs have a role in regulating secondary metabolism and pathogenicity in other fungal genera was unknown. In our studies, we examined the role of the *veA* homolog, *FvVE1*, on the production of two mycotoxin families, fumonisins and fusarins, in the important maize pathogen *Fusarium verticillioides*. We found that *FvVE1* deletion completely suppressed fumonisin and fusarin production on natural substrates, such as corn and rice. Moreover, deletion of *FvVE1* resulted in strains unable to cause disease symptoms on corn, and unable to produce fumonisins in plant tissue. Leaf lesion incidence and severity correlated with fumonisin presence and disruption of sphingolipid metabolism.

Bioprotective secondary metabolites from fungal endophytes of cool season grasses

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The epichloae (*Epichloë* and *Neotyphodium* species) are important fungal symbionts that form mutualistic associations with cool season grasses. They are capable of producing a range of bioprotective alkaloids, such as peramine, lolines, ergot alkaloids and lolitrems, with anti-insect and anti-mammalian properties that help protect their grass host. The genes required for the synthesis of these four classes of alkaloids have been cloned and characterized from a number of endophytes found in agriculturally important grasses. As seen with other fungal secondary metabolite biosynthesis genes, the genes for three of the four alkaloids are present as co-regulated gene clusters. The lolitrem (*ltm*) biosynthesis genes from *Neotyphodium lolii*, an endophyte of perennial ryegrass (*Lolium perenne*), are found in a complex gene cluster interspersed with AT rich repetitive regions that are preferentially and highly expressed in planta. Metabolic profiles using HPLC and LC-MS/MS, and characterization of the indole-diterpene biosynthetic pathway using gene knockouts and naturally occurring isolates with variation at the *LTM* locus has shown that lolitrem B is produced by a complex biosynthetic grid rather than linear pathway.

Elicitors to toxins: Plant interactions with *Trichoderma virens*

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As a ubiquitous soil inhabitant capable of developing symbiotic associations with plant roots, *Trichoderma virens* can provide significant protection against pathogens through induced systemic resistance (ISR). The activation of this response in maize and cotton roots colonized by *T. virens* is mediated through the elicitor SM1, a small secreted, cysteine-rich protein and a member of the cerato-platanin family (CP). We have established the role of glycosylation in elicitation properties and this property appears distinct for some members of the CP family. Crystallization of SM1 has further provided a model for comparison to the phytotoxic members of the CP family. A bioinformatic survey of the *T. virens* genome has revealed the presence of more than 200

proteins with putative elicitation properties; many of these are root-induced. In order to establish the role of non-ribosomal peptide synthetases (NRPSs) in ISR, we have studied the expression of over 30 putative genes encoding NRPSs, and estimated that approximately half are expressed during interactions between maize roots and the fungus. A reverse genetic approach has demonstrated the involvement of several NPRSs in siderophore, peptaibol, and gliotoxin production. One peptaibol initiates ISR whereas other biosynthesized compounds affect developmental stages. However, the majority of the metabolites encoded by these NRPSs remain to be identified.

Light and pathogenesis among *Cercospora*: Evidence for coordinated responses to photoperiod?
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The fungal genus *Cercospora* is a diverse and destructive group of foliar fungal pathogens. During infection, many species of *Cercospora* display an extraordinary ability to orient hyphal growth in the direction of the nearest stomate, whereupon they form appressoria to initiate entry. After penetrating leaves, many *Cercospora* species produce the phytotoxin cercosporin, a photosensitizing perylenequinone. Light strongly induces cercosporin biosynthesis, thus underscoring a relationship between photobiology and virulence in this group of pathogens. We recently discovered that the maize foliar pathogen *C. zeae-maydis* requires light in order to find stomata and produce appressoria, and that blue light induces cercosporin biosynthesis. These findings led us to characterize *CRP1* in *C. zeae-maydis*, a member of the *white collar-1* family of blue-light photoreceptors that regulate circadian rhythms in filamentous fungi. Disruption of *CRP1* almost completely abolished appressorium formation, resulting in a concomitant loss of virulence. Intriguingly, the basal regulation of stomatal aperture is circadian, governed by an endogenous molecular clock that is entrained by daily cycles of day and night. We hypothesize that coordinated, light-dependent signal transduction pathways dictate the outcome of interactions between *C. zeae-maydis* and maize. As the first gene known to regulate non-thigmotropic stomatal infection in fungi, *CRP1* provides a key foothold to unravel the molecular dialogue underlying light-dependent pathogenesis.

'New' Nuances in Virus-Vector Biology

Elucidating the functional role of Crinivirus capsid proteins in mediating semi-persistent virus transmission by whitefly vectors
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Phytopathology 99:S153

Semi-persistently transmitted viruses include the aphid-transmitted *Cauliflower mosaic caulimovirus* (CaMV) and members of the whitefly-transmitted genus *Crinivirus*. Evidence suggests that semi-persistently transmitted viruses are borne in the vector foregut, although virion retention in the aphid stylet has also been reported for CaMV. Studies of *Lettuce infectious yellows crinivirus* (LIYV) and *Lettuce chlorosis crinivirus* (LCV) showed that whitefly, *Bemisia tabaci* biotype A, pre-fed with high concentrations of LIYV virions followed by decreasing concentrations of LCV virions drastically reduced or, in most cases, abolished the transmission of LCV, suggesting that vector acquisition of LIYV interfered with some aspects of LCV-vector interaction. One objective of our study was to use immunofluorescent localization to determine if acquired LIYV virions are retained in the vector foregut, and if this corresponds with virus transmission. Our results revealed that fluorescence was seen in the foregut of viruliferous vectors, and virus transmission occurred when the signal was observed in >20% of the whiteflies. This is only the first step; many issues remain unexplored. Are virions retained in the foregut of non-vector whiteflies? What is/are the viral protein(s) that mediate(s) foregut retention? Do LIYV and LCV share a similar retention site? These and other developments will be discussed.

-Omics for exploring whitefly-begomovirus interactions
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Whiteflies are pests and vectors of plant viruses. The most damaging viruses are members of the genus *Begomovirus* (*Geminiviridae*), which are transmitted by the *Bemisia tabaci* complex (Gennadius) in a persistent and circulative manner. Such tight specificity is suggestive of co-evolved protein-protein interactions. We are focused on elucidating both conserved and specific molecular and cellular mechanisms that underlying circulative types of virus-vector transmission. Using a proteomics approach, we identified a suite of whitefly proteins (putatively) involved in 'stress' and 'defense' pathways, and in macromolecular transport. A large-scale 5' expressed sequence tag (EST) project was undertaken in which non-normalized (Leshkowitz et al. 2006) and normalized (Sarapalli et al., in press) whitefly (B biotype) cDNA libraries were sequenced. Non-subtracted EST consensi were re-annotated, and normalized ESTs were annotated for the first time against available insect sequences (12+ genomes) in public databases. BLASTN hits were predominantly to genes of *A. pisum* and *M. persicae*, a result that supports the close taxonomic relatedness of whiteflies and aphids, e.g. homopterans in the Sternorrhyncha. The translated ESTs were assigned Gene Ontology functions (www.geneontology.org), and candidates were selected for validation. Determining the genome sequence for *B. tabaci*, a subtropical, hompteran hallmark, is now imperative.

Vector and virus proteins contributing to the regulation of Yellow dwarf virus (*Luteoviridae*) transmission by aphids
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Phytopathology 99:S153

The circulative transmission pathway of luteoviruses through their aphid vectors requires that the virus be actively transported across both gut and salivary tissues as well as survive in the hostile environments of the gut and the hemocoel. This journey is orchestrated by two virus proteins and an unknown number of aphid proteins. The transmission of two related viruses that cause yellow dwarf disease of cereals by *Schizaphis graminum* is controlled by two distinct, but overlapping sets of aphid genes. Aphid genes regulating the transmission of both viruses segregate independently as do genes regulating the transport of virus through gut and salivary tissues. A proteomic analysis of vector and nonvector genotypes has identified numerous aphid and endosymbiont proteins that are involved in cell surface binding, endocytosis, macromolecular transport and trafficking, and immune defense responses. These proteins are correlated with vector and nonvector genotypes or specific tissue types and ultrastructural studies suggest sites of protein function influencing virus transmission. Several of these proteins have been shown to bind to virus particles. Several proteins have also been shown to be allelic in nature, differing between vector and nonvector genotypes. This is the first system to allow the identification of specific aphid proteins involved in the genetic regulation of virus transmission.

Exploiting vector specificity to inhibit tospovirus transmission
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Arthropods can be significant pests of humans, animals and plants directly and in their role as vectors of viral pathogens. More than 70% of viruses infecting plants and 40% of viruses infecting mammals are transmitted by arthropod vectors. We are addressing this problem using Tomato spotted wilt virus (TSWV), the prototypic member of the genus Tospovirus within the family Bunyaviridae. TSWV is a prominent plant pathogen with a worldwide distribution and a large host range. TSWV is transmitted by at least seven species of thrips (Thysanoptera: Thripidae) in a persistent, replicative manner. Vector specificity (which virus can be transmitted) and efficiency (usually measured as per-cent transmission) are determined by (i) the acquisition of virus from an infected host; (ii) passage of virus through the insect midgut; (iii) entry into the salivary glands; (iv) delivery of virus in a viable form into another host. Each of these steps is governed by specific molecular interactions between the virus and the vector and every step represents possible barriers to successful transmission. We have shown that a truncated version of a viral surface protein specifically attaches to thrips mid-gut cells and significantly interferes with transmission of TSWV. Our objective is to use this information to develop a practical, bio-rational means to specifically target peptides derived from viral proteins to vectors of TSWV that will inhibit virus transmission.

Molecular and cellular interactions between rhabdoviruses and their insect hosts
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Plant rhabdoviruses are transmitted in a persistent propagative manner by their arthropod vectors. These viruses must cross several tissue layers in the arthropod for successful transmission to occur. Studies have demonstrated that the

insect gut serves as an important barrier for rhabdovirus transmission, and in other insect-microbe interactions, it has been shown that a substantial number of genes are differentially regulated upon pathogen invasion of midguts, including immune-response genes. In recent years, significant advancements have been made towards identifying the essential viral components of virus-vector interactions, but the molecules that determine vector competence remain largely uncharacterized. Our goal is to identify proteins that facilitate virus acquisition and/or innate immune responses during infection of the insect midgut. We hypothesize that *Maize mosaic virus* (MMV) infection of the planthopper vector, *Peregrinus maidis*, alters the insect midgut transcriptome. To that end, we sequenced the *P. maidis* midgut transcriptome and identified 200 ESTs that are predicted to play a role in innate immune response. Two possible virus receptor transcripts were identified in our EST collection based on sequence similarity to receptors for animal-infecting rhabdoviruses. These sequence resources provide us with the necessary tools to identify insect proteins that play a role in virus transmission.

Quorum Sensing and Biofilm Formation in Plant-Associated Bacteria

Quorum sensing in the plant pathogenic bacteria: The *Pantoea stewartii* paradigm
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Phytopathology 99:S154

In *Pantoea stewartii* subsp. *stewartii* the major stewartan exo- and capsular polysaccharide (EPS/CPS) virulence factor is governed by the EsaI/EsaR cell-cell communication system together with the environmental sensing Rcs phosphorelay. The integration of these regulatory networks ensures the cell density-dependent synthesis of stewartan, but only under the appropriate environmental conditions. The strict regulation of stewartan synthesis is absolutely essential for *P. stewartii* to cause Stewart's vascular wilt in maize. In fact, the premature or constitutive synthesis of stewartan diminishes the virulence potential of the pathogen significantly. One of the reasons for this is that stewartan production interferes with microbial adhesion to surfaces, including maize tissue, thereby compromising the organism's ability to aggregate and form biofilms. *P. stewartii* is motile and exists in the xylem as cell wall-adherent biofilms. We also resolved a related, long-standing question showing that stewartan CPS synthesis coincides with stewartan EPS synthesis supporting our hypothesis that various surface glycoplymers provide different functions during the *P. stewartii* life-cycle. We will correlate our findings to the disease biology of the pathogen.

Polar attachment, a unipolar polysaccharide adhesin and cellular asymmetry determinants of *Agrobacterium tumefaciens*
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Phytopathology 99:S154

Agrobacterium tumefaciens is well known for its ability to genetically modify plants through transfer of a segment of DNA (T-DNA) into the plant nucleus. T-DNA genes direct rapid cell proliferation and production of opine nutrients that are utilized by the infecting bacteria. Despite extensive studies of the T-DNA transfer, there is minimal understanding regarding productive attachment to plant surfaces. *A. tumefaciens* attaches and forms complex biofilms on a variety of surfaces. We have identified a cell surface adhesin that associates with the pole of the cell which contacts surfaces, including plant tissues and abiotic structures. The adhesin can be visualized with fluorescently-tagged lectins on single cell poles, suggesting that it is at least partially comprised of polysaccharide, and hence we describe it as the unipolar polysaccharide (UPP). The *A. tumefaciens* UPP bears facile similarity to the holdfast adhesin of *Caulobacter crescentus*. UPP elaboration requires the *upp* gene cluster, which encodes putative polysaccharide biosynthetic functions, and is conserved and syntenous among several rhizobia. Elaboration of the UPP is required for *A. tumefaciens* polar attachment to abiotic surfaces and binding to *Arabidopsis* root tissues. Co-localization of the UPP with flagellar basal bodies reveals that this adhesin is on the opposite end of the cell from the flagellar tuft in *A. tumefaciens*. Several presumptive polar development genes of *A. tumefaciens* are required for or influence proper cell septation, flagellar placement and motility, biofilm formation, pellicle generation and UPP localization.

The role of quorum sensing and phenazine antibiotics in biofilm formation by *Pseudomonas chlororaphis 30-84*
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Phytopathology 99:S154

Pseudomonas chlororaphis 30-84 is a rhizosphere-colonizing bacterium that is able to control take-all disease of wheat caused by the fungal pathogen *Gaeumannomyces graminis* var. *tritici* (Ggt). *P. chlororaphis* produces primarily two different phenazine (PZ) compounds, phenazine-1-carboxylic acid (PCA) and 2-hydroxy-PCA (2OHPCA). Production of PZs by strain 30-84 is the primary mechanism of pathogen inhibition and contributes to the persistence of strain 30-84 in the rhizosphere. PZ production is regulated by a complex sensory pathway that includes the PhzR/I quorum-sensing system (QS). Using flow cells, we demonstrated that QS and PZ are involved in biofilm formation. Derivatives of *P. chlororaphis* were constructed that produced only PCA or more efficiently converted PCA into 2OHPCA. These derivatives produced similar levels of PZs and PZ gene expression was controlled by QS. We found that the PZ-altered derivatives of *P. chlororaphi*s differed from the wild type in initial attachment, biofilm architecture, and dispersal. The PCA-only derivative produced thicker, denser biofilms than the wild type, whereas the 2OHPCA enhanced strain adhered more rapidly than either the wild type strain or the PCA-only derivative. The PZ-altered derivatives also differed in their ability to inhibit Ggt. Preliminary microarray data suggested that PZs themselves serve as signaling mechanisms. Our findings demonstrate that PZ play multiple roles in the ecology of *P. chlororaphis.*

Plant factors and other bacterial residents modulate iron levels on leaves thereby influencing quorum sensing controlled epiphytic fitness and virulence in *Pseudomonas syringae*
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The majority of cells of *Pseudomonas syringae* are found in relatively large (>100 cells) cell aggregates on the surface of healthy leaves, facilitating quorum sensing (QS) via its production of 3-oxo-hexanoyl homoserine lactone (AHL). QS enhances epiphytic fitness but suppresses swarming motility of *P. syringae*. The increased numbers of lesions incited by QS mutants on bean compared to wild-type strains was associated with increased rates of invasion of leaves, apparently due to incessant movement of cells on plants. As many as 8% of the bacterial residents of healthy leaves produced an AHL to which *P. syringae* responded and reduced the numbers and size of lesions incited when co-inoculated onto plants with *P. syringae*. QS in *P. syringae* is directly related to levels of ferric iron and about 9% of bacteria on leaves produced iron sequestering compounds that inhibited QS and increased lesions size and numbers when co-inoculated with *P. syringae*. Invasion of leaves by *P. syringae* and disease incidence is suppressed by topical applications of iron and stimulated by tannins that bind ferric ions. Thus considerable cross-talk appears to occur between *P. syringae* and other residents on leaves which may alter the likelihood of disease from an existing *P. syringae* population. Pathogen confusion by bacteria that alter signal perception by plant pathogens and modulating iron availability on plants may be important new strategies of disease control.

DSF signalling and biofilm formation in *Xanthomonas campestris*
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Phytopathology 99:S154

Xanthomonas campestris pathovar *campestris* (Xcc) is the causal agent of black rot of crucifers. The ability of Xcc to incite disease depends on cell-cell signaling involving the diffusible factor DSF. The synthesis of DSF depends upon RpfF and perception and signal transduction involves the RpfC/RpfG two-component system. Mutations in *rpfC* lead to DSF over-production. In certain liquid media, *rpfF*, *rpfG* and *rpfC* mutants grow as large multicellular aggregates rather than the dispersed planktonic form of the wild type. Addition of DSF causes dispersal of aggregates formed by *rpfF* strains but not *rpfG* or *rpfC* strains. Strains carrying additional mutations in *gumB*, which is required for biosynthesis of xanthan, are unable to form aggregates indicating that this polysaccharide is a component of the bacterial matrix. In minimal medium without shaking a different picture emerges. Wildtype Xcc forms microcolonies that develop into a structured biofilm, whereas *rpfF* and *rpfC* mutants form only unstructured arrangements of bacteria. A *gumB* mutant is unable to develop the typical wildtype biofilm. Mixed cultures of *gumB* and *rpfF* mutants form a typical biofilm; in contrast, the *rpfC* mutant prevents the formation of the structured biofilm by the wildtype. These findings suggest that DSF signaling is finely balanced during biofilm formation.

Schroth Faces of the Future Symposium in Bacteriology

***Erwinia amylovora* IQ and gene regulatory network (GRN)**
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Phytopathology 99:S155

Microbes are in constant contact with their environment, and the ability of a bacterium to monitor the ever-changing environmental conditions is a prerequisite for its survival. Two-component signal transduction systems (TCSTs) are the most elaborate sensory systems in bacteria to monitor dramatic fluctuations in their environment and to elicit adaptive responses through changes in gene expression. It has been proposed that TCSTs can be used to measure the ability of an organism to adapt to diverse conditions as the bacterial intelligence quotient (IQ). Recent advances in genome sequencing have provided a unique platform to identify and decode bacterial IQs. In this study, we used a systems approach to identify and characterize TCSTs in *Erwinia amylovora*, a serious pathogen of apples and pears. Bacterial genetics, comparative genomics and bioinformatics approaches were employed. Our results revealed that TCSTs in *E. amylovora* play major roles in virulence and in regulating critical virulence gene expression, suggesting the presence of regulatory networks governing expression of major virulence genes; and multiple TCSTs may form complex, highly connected circuits and signaling networks in this important pathogen. Deciphering gene regulatory networks using functional genomics such as microarray and computational models will be our next goal. Here, I will highlight our findings including discovery of new global regulators and our future directions of this project.

***Xylella fastidiosa* transmission by vectors – from molecules to models**
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Phytopathology 99:S155

Xylella fastidiosa is a xylem-limited vector-borne bacterium that causes disease in several crops of economic importance, including grape, almond, citrus and coffee. As with other vector-borne plant pathogens, developing practices that may reduce the impact of *X. fastidiosa* diseases requires interdisciplinary approaches, ranging from molecular- to field-based studies, integrating pathogen, vector, host plants and the environment. We will argue that research approaches used decades ago are still valuable methodological tools and can be used to address new questions. Likewise, technological advances permit us to revisit and challenge paradigms, and explore novel research venues. Using *X. fastidiosa* transmission as a model system, we will discuss recent research from the molecular characterization of pathogen-vector interactions to the role of host plant, pathogen strain and vector species in determining transmission rates, and how these factors may affect disease epidemiology. We hope to provide evidence that interdisciplinary research is more than the sum of its parts, and that the future of bacteriology and development of disease management practices lies in the integration of classic and novel, molecular and ecological approaches.

Individual-based ecology of plant-associated bacteria
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Phytopathology 99:S155

Ecology is the study of an organism's interactivities with its biotic and abiotic environment. Little is known about how individual bacteria perceive and respond to their local surroundings, or how far their sphere of perception and influence reaches beyond their own micrometer dimensions. To address this question and determine to what degree individual variation in perception contributes to the structure and activity of bacterial communities, our lab employs bioreporters for high-resolution habitat characterization. We focus on the phyllosphere, or leaf surface, as a model microbial habitat, and are using novel GFP-based bioreporter strains to quantify the experience, behavior and reproductive success of individual bacteria in relation to their whereabouts on the leaf surface. The overall objective of this work is to come to a better appreciation of the world from a bacterial perspective, using an individual-based, experimental ecology approach. More specifically, we aim for an enhanced understanding of the establishment, (inter)activities, and fate of plant-associated pathogens and nonpathogens at the microscale, so as to improve our ability at the macroscale to predict and manage plant disease and health in natural and/or agricultural systems.

Biology of *Enterobacter cloacae* and its association with onions
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The bacterium, *Enterobacter cloacae*, is the causal agent of Enterobacter rot of onion bulbs in storage. Evaluation of naturally infected bulbs from onion fields and storages in the Pacific Northwest identified that the incidence of *E. cloacae* was greater than previously thought. *E. cloacae* was found to be present in ~50% of the infected onion bulbs assayed and coinfecting onion bulbs with another bacterial plant pathogen such as *Burkholderia cepacia*, *Burkholderia gladioli*, *Pantoea ananatis* or *Pectobacterium* spp. ~25% of the time. This indicates that *E. cloacae* could be causing more onion bulb rot than previously thought. Evaluation of curing temperature and duration determined that *E. cloacae* is favored by higher temperatures (>35°C) and a longer exposure time at the higher temperatures significantly impacts disease progression of *E. cloacae* in onion bulbs. Artificial inoculation of onion plants with *E. cloacae* does not result in a phenotypic response. This supports the hypothesis that the pathogen is present as a latent infection when onion bulbs are placed in storage. Investigations into movement of *E. cloacae* in onion leaves demonstrate that the pathogen can move as quickly as 1 cm or more per week through the phloem and xylem. This indicates that the timing of the infection of the plant and the pathogen entering the bulbs as a latent infection could be quite small. Finally, the roll of exopolysaccharide in the biology of *E. cloacae* will be discussed.

Disease of Plants

APS – ISF Collaboration to Implement a System to Standardize Naming of Plant Pathogen Races and Strains

Overview of proposed system
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Phytopathology 99:S155

Inconsistency in protocols used to name plant pathogen races and strains can undermine the value of disease resistance claims made for specific cultivars, particularly in specialty crops such as vegetables. A broader understanding of the impact of naming pathogen races and strains on the vegetable industry is needed. For example, inconsistencies in naming of races, pathotypes and or strains of the downy mildew pathogens of spinach and lettuce, as well as the Fusarium wilt pathogens of tomato, melon and watermelon, cause continuing confusion for growers, the vegetable seed industry and academia. There is no internationally recognized authority on nomenclature for new races and strains. Readily available sets of differential host cultivars and reference cultures of pathogen races and strains are needed to help standardize the nomenclatural system and provide clarity for claims of disease resistance. Members of APS, the American Seed Trade Association and the International Seed Federation are collaborating to implement a network of private and public research laboratories and seed companies in the U.S. for the maintenance, storage, multiplication and distribution of reference pathogen cultures and seed of differential host cultivars, to facilitate standardizing the naming of plant pathogen races and strains. The proposed system should complement existing systems in Europe (i.e., Naktuinbouw in the Netherlands and GEVES in France) and comply with U.S. regulatory requirements. The complexity and challenges of implementing this system, demand for such a system, and proposed protocols will be discussed in the symposium. Feedback from the APS community is critical to development of a system of reference pathogen cultures and differential host sets for naming of pathogen races and strains with guidelines that are accepted globally by the scientific community.

Current European systems
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Phytopathology 99:S155

Disease resistance is a primary goal in vegetable breeding. In Europe, governmental scientific institutes traditionally have been responsible for the maintenance, nomenclature and distribution of pathogen reference cultures. Due to changing priorities in financing these institutes, the pathogen collections became lower priority, resulting in poorer quality control. Affected seed companies in France and the Netherlands joined with Plant Breeders Rights testing authorities at GEVES (Group for Control and Testing of Varieties and Seeds) and Naktuinbouw, respectively. Different strategies were developed. 1)

In the French system, GEVES, INRA and seed companies created a network, MATREF (Reference Materials) based on the mutualization of differentials, controls and pathogen strains. Seed companies produce seeds of host differential cultivar sets that are stored at GEVES. Seed health tests are performed by seed companies or GEVES-SNES on the regenerated seeds. Pathogen reference cultures are maintained at GEVES and distributed to participants of the MATREF network. A duplication of pathogen strains is ensured by the members of MATREF. The differential seeds and pathogen reference cultures are distributed freely to members of MATREF and sold to non-member participants. 2) In the Netherlands, the focus is on maintenance of pathogen reference cultures. Seed companies maintain pathogen reference cultures that are sent to Naktuinbouw for verification of identity and pathogenicity, and then distributed to seed company member participants or sold to non-member participants. In each of these programs, clear identification and nomenclature of pathogen races/strains is critical. The definition and maintenance of host differential sets is also crucial for accurate pathogen race or strain identification. The International Seed Federation (ISF) Resistance Code Working Group has taken the lead in developing this at a global level. Public scientists, private industry, and regulatory authorities are essential to these programs to ensure quality sources of pathogen reference cultures and host differential sets to improve efficiency and quality control in developing resistant plant cultivars.

Proposed U.S. Permitting Strategy for pathogen race and strain distribution

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The USDA Animal and Plant Health Inspection Service (APHIS) regulates plant pathogens and other organisms that are of limited distribution or do not occur in the U.S. For an individual to import and maintain cultures of regulated pathogens, APHIS issues permits with safeguarding requirements based on the potential risk of the organisms in question to agriculture and the environment. For organisms classified as "widely prevalent", APHIS issues permits that have standardized and comparatively minimal permit conditions. At the request of the APS-ISF ad hoc committee on naming plant pathogen races/strains, three sets of pepper differentials with associated reference pathogen races or strains classified as "widely prevalent" were presented to APHIS to begin developing a pilot permitting system. This system will enable identification and/or calibration of pathogen races and strains using reference pathogen cultures and differential host cultivar sets. Under this system, laboratory facilities will be certified by APHIS for maintenance of reference pathogen races or strains, and electronic permits will be issued for distribution of pathogen cultures for resistance testing. Seeds of the host differential sets will be stored, catalogued in the GRIN database, and distributed by the USDA Agricultural Research Service with associated reference cultures of pathogen races or strains. This presentation will provide detailed information on this template permit system and demonstrate how the system will function.

Naming of Spinach downy mildew races, a case study

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Spinach downy mildew, caused by *Peronospora farinosa* f. sp. *spinaciae*, is an economically important disease of spinach worldwide. In recent years, there has been a dramatic expansion in both spinach production and consumption. With this increase in production, there has been an increase in the number and frequency of new races of the spinach downy mildew pathogen. There are now 11 described races of the pathogen, with three races (races 1–3) having been identified between 1824 and 1990 (166 years) and eight races (races 4–11) identified between 1990 and 2008 (18 years). Because of the globalization of spinach production and the need for independent verification of reportedly resistant spinach cultivars, standardized race identification, and clear communication on race development, an international committee known as the International Working Group on Peronospora (IWGP) was organized. The committee, administered by Plantum in Gouda, the Netherlands, is composed of public and private sector spinach research personnel. The committee provides an important and objective balance to the demands of scientific discovery, company proprietary concerns, and industry, breeder, and grower imperatives. The continued effectiveness of the committee will depend on periodic review of the committee objectives, operating procedures, and communication methods with various international stakeholders. Specific examples will be discussed illustrating the practical needs in dealing with the development and nomenclature of new races of the downy mildew pathogen on spinach. The committee continues to evolve, and the benefits to industry and the challenges the committee faces will be discussed.

Current Status of Citrus Huanglongbing Research and Control

Citrus HLB, its pathogens and vectors

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Huanglongbing (HLB) has been known for over 100 years, probably originating in Asia. The disease was named after the symptoms associated with the declining trees; in China it was commonly called yellow shoot disease, likubin decline in Taiwan, citrus dieback in India, citrus leaf mottle yellows in the Philippines, citrus vein-phloem degeneration in Indonesia, and blotch mottle or greening in South Africa. Even to the present, Koch's postulates have not been fulfilled for HLB. Based on diagnosis of conserved regions of prokaryotic DNA, usually the 16S rRNA gene, three species of *Candidatus* Liberibacter have been associated with HLB: asiaticus, africanus, and americanus. In Brazil and China, Phytoplasmas have been associated with HLB symptoms in trees where *Ca*. Liberibacter sp. tested negative. In African, HLB is vectored by *Trioza erytreae*, African citrus psyllid, while in Asia and the Americas, the Asian citrus psyllid, *Diaphorina citri*, is the vector. Control of HLB currently depends on psyllid control, planting healthy plants in block plantings, and timely removal of symptomatic trees. Growers having to adapt to these practices to stay in production complain about the high costs. Generally, the economic benefits of programs which result in avoiding large economic losses are hard to document. Available literature will be reviewed which document the economics of living with HLB.

Historical and current status of HLB in China

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Citrus Huanglongbing (HLB, yellow shoot disease) was first observed in Chaoshan area of Guangdong Province, China over 100 years ago. As inferred by the name, typical symptoms of HLB are leaf/shoot chlorosis. HLB was first systematically studied in Chaoshan during the 1930–40's, when HLB was epidemic there. The disease was found to be spread probably by unknown biological vector(s). The infectious nature of HLB was further demonstrated and confirmed in the 1950's. Since then, HLB management has focused on pathogen elimination and vector control. Efforts to identify HLB pathogen(s) has, however, met with great challenges. Suspected causal agents include *Fusarium*, water damage, nutrient deficiency, nematode, virus, mycoplasma-like organism, *Rickettsia*-like organism and bacteria-like organisms. Currently, "*Candidatus* Liberibacter asiaticus" is confirmed to be associated with HLB. A strain of "*Ca*. phytoplasma asteri" has recently been identified. However, Koch's postulates have not been fulfilled. Citrus psyllid (*Diaphorina citri*) was proved to transmit HLB. No HLB-resistant citrus cultivar has so far been identified. Presently, HLB remains as a critical threat to the booming citrus industry in China. HLB control includes: 1) Strict implementation of quarantine procedures; 2) Production and utilization of disease-free seedlings; 3) Control of insect vector(s); 4) Prompt elimination of affected trees; and 5) Optimization of orchard management.

Current HLB research in Brazil

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Huanglongbing (HLB) is a destructive and fast spreading citrus disease associated with *Candidatus* Liberibacter americanus (Lam) and *Ca.* L. asiaticus (Las), both transmitted by *Diaphorina citri*. Lam and Las also infect orange jasmine trees. A phytoplasma of group 16SrIX has also been detected in citrus trees showing typical HLB symptoms, but free of Liberibacter. HLB was first found in 2 locations in São Paulo State in March 2004, and has been reported in 241 municipalities in São Paulo, Paraná and Minas Gerais States as of March, 2009. Lam and Las differ in ability to grow *in planta* and in sensitivity to high temperature, which helps to explain their uneven spatial and temporal progress. Since pruning is not effective in removing all infected tissues, disease management relies on symptomatic tree elimination and

repeated insecticide applications. Success has been dependent on the intensity of inoculum pressure in adjacent areas. To improve HLB management, current research focuses on determining: (i) the role of asymptomatic citrus and orange jasmine as sources of inoculum, (ii) feeding and dispersion patterns of *D. citri*, (iii) the efficacy of systemic insecticides in preventing Liberibacter transmission, (iv) the best frequency and timing of orchard inspection and insecticide application, (v) the relative importance of these practices in HLB management, and (vi) the temperature limits for Lam and Las multiplication in citrus and in the insect vector.

Research on HLB in South Africa

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Citrus Huanglongbing (HLB = citrus greening) was first observed on citrus in South Africa during the 1920s. It was shown later to be transmitted by the psyllid, *Trioza erytreae*, and to be caused by the phloem-limited bacterium *Candidatus* Liberibacter africanus. The disease is currently managed by a certified disease-free budwood program, nursery location, total psyllid control in nurseries and timed control in orchards, and infected tree removal. Current research is directed towards host range studies of indigenous and wild rutaceae, surveys for variants of *Ca.* L. africanus and the presence of other species, and resistance to HLB through embryo recovery from chimeric mutants. *Vepris lanceolata* and *Clausena anisata* have been identified as indigenous hosts of the bacterium, while *Calodendrum capense* has been shown to be widely infected by a sub-species, *Ca*. L. africanus ssp. capensis. No other species have been detected thus far in any plants. Two clones of Valencia orange seedlings derived from asymptomatic fruit chimeras are undergoing field testing for resistance/tolerance.

Huanglongbing in India

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The earliest evidence of the presence of huanglongbing (also known as citrus greening) in India probably comes from the detailed descriptions of symptoms associated with Asian citrus psyllids (ACP, *Diaphorina citri*) in Punjab state (Husain and Nath, 1927). The symptoms were suspected to be caused by a toxin secreted by the psyllid at that time. In India, the symptoms associated with ACP were known as "Indian citrus dieback". Fraser (1966) associated the dieback symptoms with the South African citrus greening. She found widespread incidence of this disease throughout India. At the same time, Capoor's laboratory in Poona established, for the first time, that *D. citri* is the vector of the disease. In the 1970s, a phytoplasma was reported to be associated with citrus greening, but was soon thought to be an error because of later research on association of a bacterium with the disease. In India, the disease appeared to have spread slowly because of several cultural and environmental factors. The effect of disease on the industry in several citrus growing regions is discussed.

HLB diagnosis

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Initial diagnosis of huanglongbing is based on symptoms of phloem dysfunction, which can be caused by diseases and disorders other than huanglongbing. Diagnosis of huanglongbing must be confirmed by PCR testing. Both standard format and real-time PCR tests are available, most based on the 16S RNA gene or the rRNA operon. PCR products are sequenced for final diagnostic confirmation. Several species of *Ca*. Liberibacter have been identified from citrus, and these can be differentiated on the basis of these tests. A difficult challenge in PCR-based diagnosis is the collection of the samples from the tree. Symptomatic mature leaves are preferred, although even in trees with established infections, the distribution and titer of the pathogen can be erratic. For this reason multiple leaf samples should be collected, and if huanglongbing is suspected, negative tests should be repeated. Although hundreds of real-time PCR tests can be completed in a day, extraction of DNA in quantity and quality from midribs or petioles for PCR testing is very laborious and determines the rate at which samples can be tested. Judgment is required in the review of PCR test data. A 'positive' result with a high Ct value may be valid or may result from laboratory contamination. Negative controls and internal controls for DNA quality are both essential. As with any test, lab-to-lab variation exists. Ultimately the results must be interpreted with the sample source and test purpose in mind.

Epidemiology of HLB in U.S.

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Phytopathology 99:S157

Results from studies on the increase in HLB incidence and spread in China and Reunion Island indicate a rate of disease increase leading to a multi-year epidemic requiring 7 to 10 years for infection to approach an asymptote of 100%. In contrast, more recent studies in Brazil, Vietnam, and Florida suggest a much more rapid rate of disease increase and spread. An HLB epidemic was examined in a plantation of over 4,800 ha in South Florida where no new citrus had been introduced for 10 y and thus spread was entirely dependent on psyllid transmission. The level of psyllid infestation was unprecedented compared to previously recorded psyllid infestations. The psyllid vector was relatively newly introduced to Florida and thus lacks the biological and environmental constraints found in its native range. Consequently the HLB epidemic in Florida is undoubtedly one of the worst on record. Stochastic Markov-Chain Monte Carlo models indicated a prevalence of secondary spread with occasional primary spread from outside the plots. Interpretations of the stochastic models combined with survival analyses show spread over multiple scales from local to regional are occuring simultaneously and continually in Florida. Edge effects analyses indicate a prevalence of infections that accumulate at the transition of plantings and areas devoid of citrus such as the plantation perimeter, internal roads, canals, ponds, etc. This edge effect diminishes rapidly toward the interior of the planting and is generally well described by an inverse power function.

Isolation, cultivation, and Koch's postulates of the HLB bacterium

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Huanglongbing (HLB) is a serious bacterial disease of citrus, world-wide. One of the three suspected pathogens of HLB, *Candidatus* Liberibacter asiaticus, emerged in 2005 in Florida. Recent findings of the psyllid (*Diaphorina citri*) vector of HLB in Southern California pose a potential threat of introducing the pathogen into California. The suspected causal agents *Ca.* L. asiaticus, *Ca.* L. americanus, and *Ca.* L. africanus are phloem-limited bacteria and have been only recently grown in pure culture (Sechler et al., 2009, Phytopathology, In Press). Isolations from infected tissue were made by streaking extracts of sterilized leaf petioles onto Liber A agar. Plates were sealed and incubated for 3–4 d at 28°C, until colonies were visible with a binocular microscope. The colonies were 0.1 mm or less in diameter and irregular shaped after 7 d. Cells were 0.3 to 0.4 × 0.5 to 0.8 µm with numerous fimbriae. Inoculation of citrus seedlings and trees with cultured bacteria resulted in typical symptoms of HLB. The symptomatic tissue and typical isolated bacteria were positive by a *Ca.* L. asiaticus-specific real-time PCR assay.

Genome sequencing of "*Ca.* Liberibacter asiaticus"

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Phytopathology 99:S157

Citrus huanglongbing is the most destructive disease of citrus worldwide. It is spread by citrus psyllids (*Diaphorina citri* and *Trioza erytreae*) and is associated with a low-titer, phloem-limited infection by any of three uncultured species of alpha-Proteobacteria: '*Candidatus* Liberibacter asiaticus'; (Las), '*Ca*. L. americanus'; and '*Ca.* L. africanus'. A complete circular Las genome has been obtained by metagenomics using the DNA extracted from a single Las-infected psyllid. The genome of 1,227,204 bps has an average 36.5% GC content. Annotation revealed a high number of genes involved in both cell motility (4.5%) and active transport in general (92 genes), which may contribute to its virulence. Las appears to have a limited ability for aerobic respiration and is likely auxotrophic for at least 5 amino acids, all of which contribute to its fastidious nature. Consistent with its intracellular nature, Las lacks Type III and Type IV secretion systems as well as typical free living or plant-colonizing extracellular degradative enzymes. Las appears to have all Type I secretion system genes needed for both multidrug efflux and repeats in toxin effector secretion. Multiprotein phylogenetic analysis confirmed *Ca.* L. asiaticus as an early-branching and highly-divergent member of *Rhizobiaceae*. This is the first genome sequence of an uncultured alpha-Proteobacteria that is both an intracellular plant pathogen and insect symbiont/parasite.

Perplexing Potato Problems

Potato early dying
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Phytopathology 99:S158

Potato early dying (PED) is a chronic disease endemic to many potato production regions. Chlorosis and premature senesce of foliage with reduced tuber yield characterize PED. The primary pathogen of PED and Verticillium wilt is the soilborne fungus *Verticillium dahliae*. The PED disease is distinguished from Verticillium wilt by a more rapid and extensive progression of symptoms in the field, and the coinfection of plants by *Pratylenchus penetrans* or various fungi. It is generally agreed that detection of *V. dahliae* is diagnostic for PED, but criteria for soil inoculum thresholds, symptoms, or epidemiology vary as do conclusions about the significance and role of other pathogens in PED. Controlled inoculations of *V. dahliae* and *P. penetrans* cause PED, but potato fields are typically infested with other nematode species and soil fungi such as *Colletotrichum coccodes*. A perplexing problem for scientists and producers is to construct models that reflect combinatoric interactions manifested as PED in potato fields. Root infection by fungi and nematodes is a continuous process with physiological impacts on the crop that may take weeks to manifest. No single sampling scheme or assay is adequate to represent fungal and nematode population biology. The complexity of PED and the push towards control measures more specific than soil fumigation justify a multidisciplinary approach towards this most perplexing potato disease.

Impact of nematodes on potato quality
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Phytopathology 99:S158

While nematodes that reduce plant growth can cause significant yield reduction, nematodes that impact crop quality can result in complete loss of crop value. Root-knot nematodes infect tubers and develop to adult females. These infection sites produce brown spots that are considered a quality defect. Only a few spots can degrade a tuber to a cull and if a field contains 5–15% culls the crop may be devalued or rejected. Some species produce galls on the tuber surface which can also cause tubers to be graded as culls. The most damaging species is *Meloidogyne chitwoodi* (Columbia root-knot nematode) which can cause crop rejection at densities of 1/250 g soil or less. Tobacco rattle virus (TRV) is vectored to potato by stubby-root nematodes, SRN (*Paratrichodorus* spp. and *Trichodorus* spp.). TRV causes corky ring spot disease (CRS) which produces necrotic arcs, rings and/or diffuse spots in tubers. If more than 5% of the tubers in a field have excessive CRS, that crop may be rejected. SRN as low as 3/250 g soil have resulted in rejected crops. Fumigation with 1,3-dicloropropene (1,3-D) is generally effective for both nematodes. Metam sodium (MS) applied through irrigation (chemigation) is not effective but shanked-in MS may be adequate in fields with low disease pressure. Best control is with 1,3-D followed by MS. Oxamyl can be effective but timing of applications is critical. Crop rotation and green manure crops can suppress root-knot nematode but are less effective for SRN.

Important soilborne fungal diseases of potato
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Phytopathology 99:S158

Soilborne fungi caused a number of important disease problems in potatoes. Three that are arguably the most important in North America are: *Pythium ultimum* (leak), *Rhizoctonia solani* (Rhizoctonia canker and black scurf) and *Helminthosporium solani* (silver scurf). The former disease causes a tuber rot in the field that can further develop in storage. Losses in the field are usually minor but losses due to secondary problems in storage can be severe. This disease is controlled by a combination of cultural (i.e. watering, harvest conditions, and/or storage temperatures) and chemical (Mefenoxam) methods. Recent reports of resistance to Mefenoxam may result in reduced control. *Rhizoctonia solani* inoculum can originate in the soil or be seedborne, and infect stems, roots and stolons (canker diseases) or infect the surface of tubers (black scurf). Yield loss can result or in the case of black scurf, the formation of sclerotia on tubers may prevent fresh marketing. Infection is controlled by cultural (crop rotation, planting conditions, soil moisture) and chemical (seed and in-furrow treatments) methods. Silver scurf infects tubers in the field and causes a surface discoloration but no rot. However field and secondary infection in storage can result in total crop losses. Control of silver scurf requires the use of both cultural (seed selection, quick harvest) and chemical (seed and pre and post storage treatment) methods and even then adequate control may not be achieved.

Detection and control of infestation foci of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*)
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Phytopathology 99:S158

A computer software program 'SAMPLE' was developed to evaluate sampling methods for the detection of infestation foci of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*). In "SAMPLE" the distribution patterns of potato cyst nematodes at different scales are integrated: • The models describing the expected medium scale infestation foci; • Bivariate models for the variation of the parameters of the focus; • The Negative Binomial Distribution giving the probability of finding a certain number of cysts in a core, given the core size; • The growth of the infestation foci as a function of time. The methods to be evaluated are a.o.: the new EU method and some deviations, the Dutch AMI-50 and AMI-100 method, some Australian and New Zealand sampling methods. In the software package "NemaDecide", the detection algorithms of SAMPLE are included, providing the possibility to evaluate the effect of control measures on the detection of a focus, for instance: (partial) resistant cultivars, soil fumigation, crop rotation, non fumigants, trap crops and 30 years growth of non-hosts. NemaDecide contains models and parameters of: • Population dynamics of the nematodes under host and non-host crops; • Plant growth and yield as a function of nematode density; • Dose/effect of control measures on the model parameters. Further NemaDecide has a cultivars list with all relevant quantitative information and is connected with financial data and sampling data. SAMPLE and NemaDecide are used for governments to develop detection methods for legislation, quarantine and export protection, for farmers and agro business to give recommendations for optimum control measures leading to maximum returns and for bio-tech companies to evaluate resistant crops.

Epidemiology/Ecology/Environmental Biology

9th I. E. Melhus Graduate Student Symposium: Integrating Pre- and Post-Harvest Views of Yield and Quality Loss

Aflatoxins in Kenyan maize: Etiology holds clues to recurrent human aflatoxin poisonings
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Phytopathology 99:S158

Aflatoxins are secondary metabolites produced by members of the fungal genus *Aspergillus*. Immunosuppressive and carcinogenic activities of these toxins negatively impact human health especially in emerging and developing countries. Severity of contamination is influenced by both fungal community structure and the environment to which the crop is exposed both prior to and after harvest. Consumption of maize contaminated with lethal levels of aflatoxins has resulted in repeated epidemics of human death in the Eastern Province of Kenya. Analysis of fungal community structures revealed that the S strain morphotype of *Aspergillus flavus*, previously unknown in Africa, was the causal agent of aflatoxin contamination events associated with lethal aflatoxicosis from 2004 through 2006. The S strain dominated fungal communities in contaminated maize and occurred at lower frequencies or not at all in adjacent provinces. Reduced intraspecific competition resulting from the unusual fungal community structure may have contributed to the extreme aflatoxin. Selectively increasing native atoxigenic fungal competitors could break the dominance of the S strain in Kenya and result in reduced aflatoxin levels in maize. Identification of specific causal agent(s), a traditional early step in development of plant disease management, has been underemphasized for aflatoxin contamination events. Improved etiologic knowledge may result in new opportunities to limit aflatoxin contamination.

Distinct roles of VeA and LaeA in *Aspergillus flavus*
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Phytopathology 99:S159

Aspergillus flavus, a mycotoxigenic filamentous fungus, colonizes several important agricultural crops, such as maize and peanuts. Two proteins, VeA and LaeA known to form a nuclear complex in *A. nidulans*, have been found to positively regulate developmental processes in several *Aspergillus* species. In *A. flavus* both proteins are required for aflatoxin and sclerotial formation. Here an examination of near-isogenic *A. flavus* mutants differing in copy number of *veA* and *laeA* alleles (0, 1 or 2+ each) revealed critical roles for VeA and LaeA in *A. flavus* development and seed colonization. Both null mutants were unable to metabolize host cell lipid reserves and were inhibited in growth by oleic acid, a primary component of peanut seed. Copy number of LaeA but not VeA appeared critical for a density dependent sclerotial-to-conidial shift as the MC*laeA* strain produced relatively constant sclerotial numbers with increasing population size rather than the decrease in sclerotia seen in both wildtype and MC*veA* strains. The MC*veA/laeA* strain yielded an intermediate phenotype. This study revealed unique roles of VeA and LaeA in seed pathogenesis and fungal biology, distinct from their cooperative regulatory functions in aflatoxin and sclerotial development.

Development of biological control strategies for management of pre- and postharvest diseases of apple in Pennsylvania
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Disease prevention is an essential for producing quality fruit. Fungicides are widely used for controlling pre- and postharvest diseases of apples and development of pathogen resistance has resulted in fewer fungicides available for disease control. The objective of this research is the development of biological control agents for foliar and fruit diseases that can be implemented into existing conventional, reduced risk and potentially organic apple production systems to reduce the use of fungicides. Bacteria were collected from abandoned, low input, organic and conventionally managed orchards in PA and screened for the ability to produce chitinase enzymes, endospores and endophytically colonize apple leaves and fruit. Following preliminary screening, field experiments were conducted to determine the effectiveness of selected bacterial isolates to suppress preharvest and postharvest diseases on apple. Isolates applied at full bloom along with successive later season sprays reduced apple scab (*Venturia inaequalis*) severity on 'Rome Beauty' and 'Golden Delicious' leaves. Several isolates also reduced severity of scab on 'Rome Beauty' fruit up to 40% compared to untreated controls. Evaluation of application timing indicated a synergistic effect of combining pre-harvest and postharvest applications on the reduction of bitter rot (*Colletotrichum acutatum*) disease severity. Successful results will improve the use of biocontrol for management of apple diseases.

Trichothecene dynamics and *Fusarium graminearum* infection patterns in wheat heads
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Phytopathology 99:S159

The primary causal agent of Fusarium Head Blight of wheat in North America is *Fusarium graminearum*. Shortly after infection, the fungus produces trichothecene mycotoxins, including deoxynivalenol (DON), which contaminate floral tissue and grain. However, the relationship between toxin production and fungal growth is not fully understood. The objective of this research was to study the effects of temperature on fungal biomass, estimated by ergosterol, and DON accumulation in wheat heads. Two spring wheat cultivars were used in this study: Alsen (moderately resistant) and Wheaton (susceptible). A central spikelet was inoculated during mid-anthesis. Plants were incubated at 15 or 22°C. Spikelets, each containing two florets, were harvested daily until 12 days post-inoculation. One floret was placed on Nash agar to determine *F. graminearum* incidence. DON and ergosterol were extracted from the remaining floret and analyzed simultaneously by gas chromatography. This method was also designed to detect deoxynivalenol-3-glucoside, a conjugated mycotoxin. Fungal colonization of spikelets beyond the inoculated point and DON translocation to spikelets not colonized by the pathogen were both observed by three days post-inoculation. During early stages of infection, DON production appeared to be stimulated by 15°C, whereas fungal growth was slightly inhibited by the cooler temperature. Toxin accumulation was observed more frequently in Wheaton florets, but Alsen florets contained higher DON concentrations. This suggests that DON production is a cultivar specific response. Results indicate DON production is a mechanism the fungus uses to adapt to stressful environmental conditions, such as temperature and resistant host.

Pre-harvest moisture impacts wheat quality through Fusarium head blight (FHB) development and deoxynivalenol (DON) accumulation
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Field experiments (split-split-plot) were conducted to examine the effect of moisture on the production and accumulation of deoxynivalenol (DON) in *Fusarium*-infected wheat. Main plot treatments were length of mist-irrigation after inoculation (14, 21, 28 and 35 days after inoculation [DAI]); sub-plot were wheat cultivars: Alsen (moderately resistant), 2375 (moderately susceptible) and Wheaton (susceptible); and sub-sub-plots were *Fusarium graminearum* isolates (5) differing in aggressiveness and DON production capacity. Plots were inoculated at anthesis with *F. graminearum* (1×10^5 conidia ml^{-1}). Heads were harvested (10/plot) at 0, 7, 11, 14, 21, 28 and 41 DAI for DON analysis. Disease severity was assessed at 21 DAI (20 heads/plot) and the percentage of visually scabby kernels (VSK) and DON was determined on samples of grain harvested at maturity. Across all isolates and mist-irrigation treatments, the susceptible cultivar Wheaton had significantly higher FHB severity, VSK and DON. VSK was significantly lower in all treatments receiving the least amount of mist-irrigation (14 DAI). DON was significantly lower in the longest mist-irrigation treatment (35 DAI) compared to all other treatments. The reduction of DON observed with increased moisture duration was also evident in the sampled heads. The reduction of DON was larger in Wheaton than the other cultivars. This suggests that DON may be reduced by late season moisture, either from mist-irrigation or rainfall, despite increased damage to grain. Leaching of DON from plant tissues may explain the observed reductions in DON.

Resistance in winter wheat to Fusarium head blight
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Phytopathology 99:S159

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is an important disease of wheat because of mycotoxins in grain. Developing cultivars with adequate resistance has been difficult because resistance is controlled by several genes with small effects on five components of resistance that are difficult to quantify. The objective of this research was to improve methods for quantifying the five components and to fully characterize the resistance in 15 diverse lines. Quantifying resistance to initial infection was improved by standardizing inoculum and environmental conditions, and a new method was developed for quantifying resistance to spread within a spike in the same experiment. Injecting florets with deoxynivalenol (DON), the principal mycotoxin, demonstrated that *FHB1*, the most-studied resistance gene, has a unique mode of action and that measuring relative yield loss after DON injection may be useful for identifying lines with tolerance to FHB in the field. Late-season epidemics are being investigated as a means of separating resistances to kernel infection and DON accumulation from interactions with other components, and a real-time PCR technique has been developed to quantify kernel colonization. Some of the methods developed in this research are already being used to evaluate breeding lines for resistance. Results of this research should be useful for phenotyping lines in mapping studies to identify markers for genes controlling resistance components.

Forensic Plant Pathology: Science in the Courtroom

Critical issues in determining if disease outbreaks were deliberate attacks on U.S. agriculture
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Phytopathology 99:S159

Most plant diseases are naturally occurring, and producers, Extension agents, crop consultants and plant disease diagnosticians are appropriately focused on identifying a pathogen to the extent necessary to effectively manage the disease, and then moving quickly to limit the damage. New concerns about the possibility of a rare intentional plant pathogen introduction, however, have resulted in a need to discriminate between natural and human-assisted plant diseases. Because law enforcement personnel would become involved only in

cases involving criminal activity, guidelines are needed for rapid assessment of the likelihood that illegal activity has occurred. Features of a disease event that could arouse concern about the nature of its introduction include current and recent weather, pathogen identity and geographical range, crop species and cropping history, soil conditions, disease pattern, presence or absence of vector insects (if involved), physical evidence, and other factors. We have created a decision tool consisting of a series of key questions and/or assessments, each of which is assigned a numerical point value that is weighted to reflect its significance. The tool helps non-plant pathologists to focus on the most relevant criteria, and the final point values add a quantitative aspect that can be used to rank the probability of intent or to compare two disease events.

Engaging plant pathologists to meet law enforcement needs
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Because of the availability of pathogenic microorganisms and the relatively low cost of preparing and disseminating bioweapons, there is a continuing threat of biocrime and bioterrorism. Thus, enhanced capabilities are needed that enable the full and robust forensic exploitation and interpretation of microbial evidence from acts of bioterrorism or biocrimes. To respond to the need, greater resources and efforts are being applied to the burgeoning field of microbial forensics. Microbial forensics is a discipline dedicated to the characterization, analysis and interpretation of evidence for attributional purposes from a bioterrorism act, biocrime, hoax or inadvertent agent release. The goal of attribution is the identification of those involved in the perpetration of the event, which is necessary for criminal prosecution, or for actions that may be taken as a result of national policy decisions. The forensic information sought in bioterrorism and biocrime cases evolves over the course of the investigation, but begins as soon as an attack or potential attack is realized. Farmers and agricultural health officials will likely be the first ones involved with initial analyses using traditional diagnostic approaches. First responder data are invaluable and will be used in a microbial forensic investigation. A major component of a microbial forensic case is trace-back to a cause and source. In this sense, a search for commonalities and clustering of the primary sources of infection to be employed in microbial forensics is no different from that of the standard epidemiologic methods. A microbial forensic investigation encompasses many aspects that are beyond those pertaining to health management. Detailed characterization assays are required for clues regarding the origin of a pathogen or vector. All pertinent evidence can be exploited, i.e., materials directly and indirectly associated with the biological weapon, processes used to prepare and disseminate the weapon, and evidence found at the crime scene. These include both traditional forensic evidence and biological, physical, and chemical characteristics of the microorganism and associated materials related to weapon preparation, purification, stabilization and/or dissemination.

Role of imagery, spatial pattern analyses, and sampling in plant pathogen forensics
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One of the most critical decisions needed early-on following the detection of a new, threatening plant pathogen is whether or not to treat the new threat as a biocrime (deliberate, human-assisted introduction) or as a natural or accidental introduction. This decision could be made (in near-real time) if a 10 × 10 km high-resolution satellite or aerial image of the affected agricultural area could be obtained and analyzed for the presence of pathogen-specific signatures/patterns. Such imagery could provide forensic data concerning the spatial patterns of a new biothreat within and among agricultural fields. Within-field focal gradients, their spatial patterns, and temporal and spatial expansion rates can provide diagnostic and quantitative forensics data. By determining GPS coordinates of initial disease foci, forensics personnel will know where to search for key forensic evidence to support or refute that the new threat was as a result of a biocrime. Imagery also provides time-insensitive, quantitative data that can be analyzed and re-analyzed by experts to obtain scientifically-valid geospatial evidence. Knowledge concerning the GPS location of initial fields, and disease foci within fields, ensures the collection of sufficient, geospatially-referenced pathogen isolates to detect population genetics anomalies atypical of natural events. A protocol for image acquisition, image processing, spatial analyses, and gradient analyses involving the wheat-leaf rust pathosytem will be presented.

New molecular tools for microbial forensics investigations
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The National Bioforensic Analysis Center (NBFAC) was established as the lead federal agency to conduct and coordinate the scientific analyses of evidentiary samples from biocrime and bioterror investigations in support of the lead federal investigative agency. The NBFAC since its inception in 2004 has been a leader in the develop of techniques to identify and characterize biological threat agents in evidentiary samples associated with biocrime and bioterror investigations. The NBFAC identifies and characterized biological threat agents using overlapping complementary techniques such as culture, molecular analyses, antigenic analyses and electron microscopy. With the advent of new techniques such as inexpensive rapid sequencing, microarrays and binformatic analyses, bioforensic analytical capabilities are becoming more comprehensive and capable of more rapidly identifiying and characterizing new emerging agents and genetically engineered agents. The various commonly used bioforensic approaches for identifying and characterizing biological threat agents and new approaches for the future will be discussed.

Forensics in the trenches: Learning through exercises
C. Thomas
UC Davis
Phytopathology 99:S160

The cross roads of criminal forensics and plant pathology meet many challenges in the field due to differences in traditional primary objectives and paradigms. This presentation will discuss similarities and differences in the approaches taken by these two different communities, using real-world and exercise cases. Seamless, coordinated standard operating procedures for detection, chain-of-custody, chain-of-communications, response and recovery in the event of a suspect criminal activity involving plant health are essential to a successful investigation. Lessons learned from exercise efforts, including a recent field training event in Oklahoma, and actual events will be presented. Focus will be on a likely progression of engagement that could involve a plant health professional, such as yourself, should the need arise. Potential roles and responsibilities of the university - including extension, industry, and government at the local, state and national levels will be discussed.

Globe Trotting Plant Pathogens and Factors Making a Difference in Management Outcomes

Globalization and new waves of immigration of plant pathogens
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Immigration of plant pathogens across national borders and geographic boundaries through trade has been present throughout much of human history. Recent movements through internationalization of trade by merging the world into one market unhampered by state boundaries/borders has greatly facilitated free trade, but also effectively enhanced long distance dissemination of plant pathogens. To combat this unwanted consequence of free trade, countries have paid increasing attention to collecting information on the emergence or re-emergence of plant diseases around the world. Pest risk assessment, phytosanitary clearance negotiation and plant quarantine are major tools used by trading partners in the prevention of entry of invasive pathogens. Plant materials, agricultural and forestry products and soils infested with exotic fungi, bacteria, viruses or nematodes are intercepted at the border and regularly denied entry. Within the country, elaborate surveillance systems are used to monitor diseases of economic, quarantine and national security significance. The development of new, sophisticated diagnostic tools and methods provides rapid and accurate diagnosis of pathogens and further aids the gathering of new information on diseases which might have been misdiagnosed or unknown previously. The new disease information, in turn, has a significant impact on mitigation; phytosanitary protocol negotiations and trade restrictions among countries.

Invasive bacterial pathogens with vectors: Management success and failure
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Phytopathology 99:S160

Many bacterial diseases are intractable due to the lack of effective bactericides, durable host resistance or other management tactics. We present two

examples of economically important invasive bacterial pathogens, citrus huanglongbing (HLB) and banana xanthomonas wilt (BXW), dependent on vectors for spread. HLB, vectored by two psyllid species, was first found in the Western hemisphere in 2004 and is now in Brazil, Florida, Cuba, and Louisiana. Management of the disease depends on psyllid control, planting healthy plants, and timely removal of symptomatic trees. Management is hampered by the lack of diagnostic methods for early detection of the associated *Candidatus* Liberibacter species in plants. Quantitative PCR (qPCR) for the detection of the bacterium in the vectors in Florida demonstrated the usefulness of this approach for early detection of HLB. We report the use of this approach for monitoring the effectiveness of different management systems in Brazil. BXW, caused by *Xanthomonas campestris* pv. *musacearum*, was first reported in Uganda in 2001. BXW has spread rapidly in East and Central Africa. The bacterium is spread by infected planting materials, contaminated tools and insects that visit infected fruit and flowers. A robust PCR assay based on the *hrpB* operon of the *hrp* gene cluster of *X. campestris* pv. *musacearum* has been developed. This assay was tested in African laboratories for detection of the pathogen and to determine the distribution of the bacterium in pseudostems in banana mats, resulting in refinement of sanitation recommendations.

Advance of the fungi in a world without borders
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Phytopathology 99:S161

Invasive fungal pathogens cause serious crop losses and environmental damage that can eliminate entire species and impose serious economic hardship on a society. Historical examples include Dutch elm disease and Chestnut blight, but current threats may be just as damaging. Reduced production efficiency from direct damage, increased costs of control, and imposed crop management changes often threaten sustainability of a society's necessary agricultural infrastructure and the health and well-being of populations far removed from the actual event. With regionalization of production, a disease in one area impacts the entire world community. Control of exotic fungal threats such as witches broom of cacao (*Crinipellis perniciosa*), laural wilt of red bay and avocado (*Rafiella* spp.), downey mildews of corn, sorghum, and sugarcane (*Peronosclerospora philippinensis/P. sacchari*), and late wilt of corn (*Harpophora maydis*); and reemerging diseases such as late blight of tomato and potato (*Phytophthora infestans*), take-all of cereals (*Geaumannomyces graminis*), and fusarium wilts (*Fusarium oxysporum*) require international understanding and cooperation in a global economy. Innovative approaches integrated into the crop production system are needed to meet fungal challenges to agriculture and the environment of the 21st century.

Capsids with wings
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Phytopathology 99:S161

Exotic, insect-vector borne plant viruses and viroids are among the most difficult pathogens to manage, owing to their propensity to hitchhike on plants and plant parts, mediated by human activities. In other instances vector-pathogen complexes invade new locales, deployed by natural weather phenomena. Many such vector-pathogen complexes exhibit invasive traits, are more fit than their endemic counterparts, and lack natural enemies in the invaded zone. Despite quarantine programs, the use of genetically similar (and susceptible) cultivars, together with expanded production in mild climatic zones have added fuel to the fire. Here, we will discuss very recent vector-pathogen invasions that have caused untold economic losses, including the whitefly-transmitted viruses: *Cassava brown streak virus* (*Ipomovirus; Potyviridae*), *Cucurbit yellow stunting disorder virus* (*Crinivirus; Closteroviridae*), *Cowpea mild mottle virus* (*Carlavirus*), severe African cassava mosaic disease complex, *Tomato yellow leaf curl virus*, and *Squash leaf curl virus* (*Begomovirus; Geminiviridae*). In addition the *Tomato chlorotic dwarf viroid* (*Pospiviroid; Pospiviroidae*) poses a serious threat to tomato production in controlled environment systems. These new and emergent pathogens and their insect vectors challenge the resilience of food and fiber production systems worldwide.

Stealth invaders: Lessons on nematode dissemination
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Phytopathology 99:S161

Plant parasitic nematodes present major economic constraints to agriculture, horticulture and forestry production. Many of these disease problems are the consequence of invasions by non-native nematode species. Nematodes have evolved many strategies that favor their dissemination. Active mobility of individual nematodes allows for only limited movement. Passive dispersal by physical forces or biological vectors may operate within a field or on a limited regional scale. However, human activities have been the main vehicles for nematode dissemination from local to intercontinental scales. The spread of exotic nematodes relies foremost on their ability to survive the transport from their origin to the new location. Establishing and maintaining their population requires suitable host plants and environmentally conducive conditions. Ecosystem requirements and the nematode's ability to adapt and to thrive under such conditions ultimately determine the success of the pathogen's introduction and its disease potential. Awareness of the means of nematode dissemination and their anticipated consequences allows risk assessment and provides information for potential regulatory action and nematode management strategies.

Lessons learned for successful management of invasive pathogens
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Phytopathology 99:S161

Some epidemics spread across continents, while others slow and come to a stop. Of the many strategies and tactics available for stopping epidemics of invasive pathogens, which have been most successful? The U.S. developed an extensive monitoring plan in response to the introduction of soybean rust, including deployment of sentinel plots to indicate the annual northern movement of the pathogen from overwintering sites to the south. Chrysanthemum white rust and Karnal bunt have been successfully restrained to date in the U.S. through quarantine programs and monitoring. The new stripe rust race UG99, with virulence to previously undefeated resistance genes, has been spreading, but it appears possible that efforts to restrict its movement will pay off before it reaches all vulnerable countries. Invasive diseases of palm caused by viruses and phytoplasmas offer less promising examples, where many epidemiological features still need more study to optimize management. We evaluate the strategies and tactics that have been employed for these and other pathogens of all taxonomic groups. As a function of the life history characteristics of hosts, pathogens, and vectors, we compare the utility of management efforts based on exclusion, eradication, containment, inspection, monitoring, and the use of pesticides and biological control.

Meta-Analysis for Evidence Synthesis in Plant Disease Epidemiology and Management

Introduction: What is meta-analysis and how is it used for evidence synthesis?
L. Madden
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Phytopathology 99:S161

Meta-analysis is the analysis of the results of multiple independent studies in order to synthesize the evidence from many possible sources in a formal probabilistic manner. In a simple sense, the outcome of each study becomes a single observation in the meta-analysis of all available studies. The discipline developed originally in the social sciences, based on earlier pioneering contributions by Fisher and Pearson, and has now been embraced within many scientific disciplines, especially in medical research. Since 1980, over 25,000 journal articles have been published on the topic; however, only a handful of articles have been published in plant pathology and related fields utilizing this methodology. After reviewing basic concepts and approaches, the advantage of meta-analysis will be presented in terms of the high statistical power that can be achieved for detecting significant effects of treatments or significant relationships between variables. Meta-analytical results can be biased, however, if the analysis is based on a nonrepresentative sample of study results. Therefore, novel approaches for characterizing the upper bound on the bias will be discussed, in order to show the robustness of the approach to possible violation of assumptions.

How should one measure the effect of a treatment (effect size) and obtain this information from published and unpublished studies?
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Phytopathology 99:S161

Like many other areas of statistics or data synthesis, meta-analysis begins with a question, and it is that question that determines what information is sought in published or unpublished studies or databases. The question may be related

to the effect of some treatment or the association among variables; hence, information is gathered that allows for a quantitative synthesis of the magnitude, direction, and significance of that effect or association in the form of a variable called an effect size. There are several different types of effect sizes for continuous and discrete data, including correlation coefficients, regression coefficients, mean differences, standardized mean differences, response ratios, and log odds ratios. These may be readily available in published literature or may be (easily) calculated from available raw data. However, in many cases, the effect size of interest may not be available in the desired form in all studies; it may be hidden in some other information or statistic that is provided in the literature or database. Some effect sizes are interrelated, and as such, it may be possible to estimate one effect size from another. Methods for estimating effect sizes from available data or statistics, the interconnection between effect sizes, their relationship with properties of the synthesized studies (moderator variables), and interpretation of results for different effect sizes will be discussed.

Effect of foliar fungicides used to control soybean rust
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Since the discovery of soybean rust (*Phakospora pachyrhizi*) in South America in 2001 and in the United States in 2004, there has been a tremendous increase in the number of foliar fungicide trials conducted in both regions. The goal for many of these trials was to improve management of soybean rust, however, in the United States, many of these trials were conducted under cropping conditions where soybean rust was not observed. Thus, it is important to improve our understanding of the conditions where the use of a foliar fungicide may be warranted. The focus for this talk will be the application of meta-analysis to address the use of foliar fungicides for soybean. In particular, a discussion of the current results from Brazil in comparison to results from the United States will be presented. Furthermore, in this talk special emphasis will be placed on how specific questions were formulated to screen foliar fungicide trials for inclusion in analyses and also a brief discussion of fixed and random effects as applied in meta-analysis.

What is the best treatment for biocontrol of fire blight?
H. Ngugi
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Phytopathology 99:S162

Fire blight caused by *Erwinia amylovora* remains an important disease limiting the productivity of apples. Several biocontrol products have been evaluated for fire blight control but their efficacy in the eastern U.S. has been inconsistent. A multi-treatment random effects meta-analysis was performed using data obtained from 47 trials carried out in the eastern U.S. from 1999 to 2007. Treatments included in the meta-analysis had been applied solely, or in a sequential combination of two products. A method for estimating the least significance difference (LSD) for studies with at least three significant mean separations was developed and used to derive the within-study variances for weighting the effect sizes. Based on this method, the estimated LSD was an excellent predictor ($r^2 = 0.994$, $P < 0.0001$, $n = 35$) of the observed LSD for 35 studies for which the raw data was available. Except for a treatment combining products based on *Pseudomonas fluorescens* and *Pantoea agglomerans*, all other treatments significantly reduced ($P < 0.01$) fire blight relative to the untreated control. The best control was noted for treatments combining the antibiotic streptomycin with a product based on *P. agglomerans* (56% disease reduction) or *Bacillus subtilis* (53% reduction). Disease suppression was 34, 25, and 24%, respectively, for products based on *B. subtilis*, *P. agglomerans*, and *P. fluorescens*, suggesting that the higher efficacy of the combination treatments was due to the antibiotic.

A Bayesian approach to meta-analysis
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Phytopathology 99:S162

This presentation reviews the use of Bayesian methods in meta-analysis of plant pathology studies. The use of meta-analysis has exploded over the last few years, as well as the use of Bayesian methods, facilitated by recent advances in computational technology. Whilst in most cases meta-analyses have been carried out using frequentist methods, there are a number of specific advantages conferred by the Bayesian approach. These include: full allowance for all parameter uncertainty in the models, the ability to include other pertinent information that would otherwise be excluded, and the ability to extend the models to accommodate more complex, but frequently occurring, scenarios. In the present paper the applications and advantages of Bayesian methods will be discussed and demonstrated with data assessing the evidence that application of a Systemic Acquired Resistance agent provides control against fire blight of apples, caused by *Erwinia amylovora*. Effect sizes computed with a frequentist approach will be compared with those obtained using a Bayesian approach. This evidence is available through synthesis of several experiments with meta-analysis methods.

Phytophthoras in Forests: New Paradigms for an Old Genus

Phytophthora in forests: New species, new threats, and new questions
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Phytopathology 99:S162

Phytophthora defines the science, the history, and the promise of plant pathology, from the potato famine to the new world of pathological genomics. Many *Phytophthora* species are known as pathogens of agricultural crops, but their origins lie in wild ecosystems. Speakers in this session look to forests for new insights into the evolutionary history of the genus. New ideas about population genetics and speciation are coming from forest populations of both indigenous and invasive species. Agricultural fields are too flat, and agronomic crops too genetically uniform to hold our attention for long in studies of epidemiology or pathogenesis. Crop losses in agriculture can be neatly measured by yield, but in the forest, the impacts of *Phytophthora* ripple through the entire ecosystem. New species are being described at an accelerating rate. Some are aggressive plant pathogens and threaten new diseases in new places in this era of global trade. Others appear to be benign, or even saprophytic in the wild. New pathogens are being reported in each of the major phylogenetic clades. ITS clade 6 encompasses much of the behavioral complexity in the genus. It includes the well-known *P. megasperma*, which is often not what it appears to be, and *P. gonapodyides*, ubiquitous despite its sterility. The full complexity of this clade is only beginning to be sorted out.

Progress in understanding Phytophthora evolutionary biology: 1983 revisited
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Phytopathology 99:S162

In the 1983 APS golden volume '*Phytophthora*: its Biology Taxonomy Ecology and Pathology' the author reviewed the evolutionary biology of the genus and, perhaps unwisely, presented a tentative *Phytophthora* phylogenetic tree based solely on morphological and behavioural characters. At the time only about 50 Phytophthora species were known and molecular variation was assessed mainly by protein and isozyme polymorphism. Today, in 2009, we have more than 100 known *Phytophthora* species and maybe another 100–500 species are still to be discovered. We also have the ability to construct molecular phylogenies and to probe the environment for *Phytophthora* biodiversity. Natural ecosystems are being sampled more frequently for endemic *Phytophthora* species than ever before. While at the same time the international plant trade is unwittingly distributing previously unknown *Phytophthora* taxa and promoting interspecific hybridisation. The significance of these developments for understanding *Phytophthora* evolutionary biology will be discussed. Some issues highlighted in 1983, including *Phytophthora* phylogeny, will be assessed from a current perspective.

Examining the population diversity of *Phytophthora* species in natural and agricultural ecosystems
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Phytopathology 99:S162

Their virulence and ability to spread rapidly throughout the world establish *Phytophthora* species as some of the most important groups of plant pathogens. Species in this genus are receiving increased attention as causal agents of emerging forest diseases and commercial plant trade has been implicated in their introduction and movement. In recent years, numerous types of molecular techniques have been used to investigate the intraspecific population diversity of *Phytophthora* spp. known to infect plants in both agricultural and natural ecosystems. In general, *P. ramorum* and *P. cinnamomi* are known to exist as a limited number of distinct clonal lineages of asexually reproducing populations, as these lineages are represented mostly by single

mating types. Recent research has indicated the A2 mating type as the oldest type in *P. cinnamomi*, while the A1 EU lineage may be older than the A2 NA lineages of *P. ramorum*. Often, their limited genetic and phenotypic diversity is presumed to be the result of founder events and used to support their exotic origin. While studies of some *Phytophthora* spp. have detected significantly higher genotypic diversity among nursery populations, isolates collected from forests have been mainly limited to terrestrial ecosystems. Researchers in numerous states and countries are currently investigating inter- and intraspecific diversity of *Phytophthora* spp. collected from watercourses in natural ecosystems and finding intriguing discoveries.

Phytophthora in forests: Feedbacks between pathogen and plant communities in forests
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Phytopathology 99:S163

Understanding the ecology of *Phytophthora* in forests requires integrating feedback among hosts, pathogens and their environment. Because generalist pathogens, such as *P. ramorum* or *P. cinnamomi*, infect many woody and herbaceous plant species, linkages between positive and negative feedback loops will be complex. Which plant species will be successfully recruited in the face of these pathogens and how successional patterns will develop are important questions for forest managers. In the sudden oak death system, pathogen-mediated competition between California bay laurel and tanoak appears to be a key component of plant community structuring. Transmission and pathogen impacts are asymmetric between the two hosts; bay laurel can support up to ten times greater sporulation and suffers no mortality compared to tanoak. Positive feedback may result in population increases for both bay laurel and *P. ramorum*. In contrast, as tree mortality increases, site associated environmental changes may create negative feedbacks on *Phytophthora* species. Opening of forest canopy gaps may result in unfavorable microclimatic changes for pathogen establishment, reproduction and survival. On sites with very high levels of pathogen caused tree mortality, accumulations of coarse woody debris (CWD) may be many times higher than in pathogen free areas. Contribution of CWD to wildfire severity may then lead to negative feedback on pathogen populations. The variety of *Phytophthora* species found in forests offer many opportunities to test hypotheses that will contribute to our understanding of disease ecology.

Landscape epidemiology of *Phytopthora ramorum*: Measuring, mapping, and modeling spread
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Phytopathology 99:S163

Landscape- to regional-scale models of plant epidemics are direly needed to predict large-scale impacts of disease and assess practicable options for control. While landscape heterogeneity is recognized as a major driver of disease dynamics, epidemiological models are rarely applied to realistic landscape conditions due to computational and data limitations. Here we describe a stochastic susceptible-infectious epidemic model, applied to temporally and spatially heterogeneous landscape parameters, to predict the spread of the invasive forest pathogen *Phytophthora ramorum* in California (1990–2030). Three epidemiological processes (production of inoculum, dispersal, and infection) are modeled on a weekly time step across a 250 m by 250 m lattice composed of variable susceptible and infected host units. We describe how field, lab, and geospatial data were combined to parameterize and map the key system variables affecting transmission of *P. ramorum*, including weather conditions, host infectiousness and availability, and a Markov Chain Monte Carlo estimated dispersal kernel. Replicated 1000 times to examine stochastic variability in epidemic outcomes, model predictions have a high degree of correspondence with 784 field plot observations that were collected across the pathogen's potential geographic range to validate model performance. Results show that most disease spread occurs via local dispersal (<250 m) but infrequent long-distance dispersal events can substantially accelerate epidemic spread in regions with large amounts of highly suitable habitat, such as the northern coastal forests. While the epidemic is currently well established, model predictions show that, under no control, epidemic spread will increase ten-fold by 2030 with most infection along the north coast to Oregon. In addition, results also show that wetter than normal weather conditions between 2009 and 2030 would double the rate of this spread. This research illustrates how stochastic epidemiological models can be applied to realistic geographies and be used gain a predictive understanding of plant disease dynamics in heterogeneous landscapes.

Pathogenicity of *Phytophthora ramorum*
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Phytophthora spp. infect an extremely diverse array of host plants resulting in a variety of diseases, ranging from root rots to foliar blights. In particular, *Phytophthora ramorum*, has the ability to infect more than 100 plant species. This talk will present a broad overview of the *Phytophthora* secretome and its potential contribution to host susceptibility and *P. ramorum* pathogenicity. Topics include the potential contribution of *Phytophthora* spp. toxins (e.g., NPP1) and effectors (e.g., elicitins) on disease development. Greater detail will focus on our own work exploring the contribution of elicitins to *P. ramorum* pathogenicity. In one study, we have examined elicitin production, virulence, and sporulation in 15 *P. ramorum* isolates belonging to the three clonal lineages (EU1, NA1 and NA2). The EU1 and NA2 isolates are generally more virulent, produce more sporangia, and produce more elicitin in vitro than NA1 isolates. Plants possess a number of defense pathways that also interact with elicitins, contributing directly and indirectly to *P. ramorum* pathogenicity. For example, elicitins may trigger a hypersensitive response in some hosts, which depending upon the timing and degree of this process may lead to complete resistance or physiological impairment (i.e., reduced photosynthesis and/or cell death) without limiting *Phytophthora* colonization. Finally, research on the potential contribution of plant tannins on elicitin activity, *P. ramorum* growth and sporulation will also be discussed.

Molecular/Cellular Plant Microbe Interactions

Application of Advanced Sequencing and Gene Expression Technologies for Characterization of Phytopathogens

Integrating molecular and computational methods to evaluate the *Pseudomonas syringae* transcriptome I and II
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Much information can be gathered from the genomic sequence of a bacterium. However, to more fully understand the coding potential of the genome, experimental identification of the transcribed fraction is required. In particular, strand-specific information is essential to thoroughly characterize transcriptional activity. Several methods exist for capturing the complete set of transcripts in a cell, using deep-sequencing technologies, however, most of these techniques have been limited to the study of eukaryotes and lack strand specific information. We will present combined computational and experimental approaches for precisely evaluating the transcriptome of the plant pathogen *Pseudomonas syringae* using RNA-Seq. The power of this approach is demonstrated by the fact that a single experiment has generated a number of important questions regarding gene expression in *P. syringae* for future investigations. The establishment of RNA-Seq for analyzing bacterial transcriptomes on a global scale significantly impacts bacterial genome annotation as well as the study of bacterial gene regulation. In Part I, we will describe the molecular methods used to prepare RNA samples and the development of a strand-specific protocol to sequence RNA using the Illumina Genome Analyzer. Next, the computational methods developed to analyze the vast amount of sequence data will be discussed. Then, we will show the application of transcriptome sequencing to the identification of polymorphisms and candidate transcriptional start sites. For Part II, we will describe a unique classification method developed to qualitatively assess transcriptional activity that combines RNA-Seq with proteomics data. Using this approach, we are able to identify transcriptional activity in areas of the genome inconsistent with the genome annotation and transcriptional activity in un-annotated areas of the genome, allowing for transcript discovery. Specific examples of areas in the genome that display unusual transcriptional activity will be highlighted.

Genomic perspectives on plant-associated enterobacteria
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The family Enterobacteriaceae includes plant-associated bacteria of the genera *Benneria*, *Dickeya*, *Erwinia*, *Pantoea* and *Pectobacterium*. We are using a

combination of Sanger, 454 and Illumina sequencing approaches to obtain complete and draft genome sequences from multiple representatives of each of these lineages. Comparisons of the sequences are assisted by reordering and alignment of genomes using the Mauve program and annotation using the ASAP database. Our analyses reveal core components conserved across lineages as well as features specific to particular species, genera and other groups of evolutionarily or phenotypically related pathogens. The sequences are used to design species-specific ORF or tiled microarrays for transcriptional profiling and chromatin immunoprecipitation experiments under varying growth conditions. These multi-omic datasets are integrated in the ASAP database and used to reconstruct the evolutionary history of genetic and regulatory networks among these bacteria.

GeoChip: A high throughput genomics technology for characterizing microbial functional community structure

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Microarray technology provides the opportunity to identify thousands of microbial genes or populations simultaneously. A comprehensive functional gene array (GeoChip) was developed to detect and monitor microbial communities important to various biogeochemical, ecological and environmental processes. Based on the second generation of GeoChip, a new generation, GeoChip 3.0, has been developed with several new features. First, GeoChip 3.0 contains ~25,000 probes and covers ~47,000 sequences for 292 gene families. Second, the homology of automatically retrieved sequences by key words is verified by HUMMER using seed sequences so that the sequence retrieving process is automated. Third, a universal standard has been implemented so that data normalization and comparison of different microbial communities can be conducted. Fourth, a genomic standard is used to quantitatively determine absolute gene abundance. In addition, GeoChip 3.0 includes phylogenic markers, such as *gyrB*. Finally, a software package has been developed to facilitate the management of such a complicated array, especially for data analysis and future update. GeoChips were successfully used to analyze microbial functional structure from a variety of environments such as wastewater treatment, microbial fuel cells from hydrogen production, hydrothermal vents, uranium-contaminated groundwater, the distribution of microbial functional communities across different oil fields, and grassland microbial communities in response to elevated CO2 and soil warming. New insights and implications in these systems were obtained. The results also indicated GeoChip is a novel, powerful high throughput, quantitative genomics technology for characterizing microbial functional community structure from a variety of natural habitats.

Comparative and functional genomics of oomycete infection

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Oomycete pathogens, including *Phytophthora* and *Pythium* species and many downy mildews, cause billions of dollars of damage to crops, forestry and ornamental plantings each year. Draft genome sequences have been developed so far for *Phytophthora sojae*, *P. ramorum*, *P. infestans*, *P. capsici*, *Hyaloperonospora arabidopsidis* and *Pythium ultimum*, and Illumina survey sequences of *P. phaseoli*, *P. mirabilis*, *P. andina* and *Albugo candida* have been produced. We have also compared the genome sequences of the four major genotypes of *P. sojae* using 454 pyrosequencing. Comparisons among these genome sequences have identified large numbers of rapidly evolving genes, including toxin and effector genes, that are likely involved in the interaction with host plants. Closer examination of the sequences has revealed highly conserved members of effector families that may play key roles in infection. We have also used transcriptional profiling, including both Affymetrix GeneChips and 454 cDNA sequence tags, to identify *P. sojae* and soybean genes that may play an active role in promoting or impeding infection, respectively. Transcriptional profiling revealed that very large numbers of host and pathogen mRNAs change in level during infection; in the case of soybean, more than 98% of the genes were transcriptionally reprogrammed. Combining information about the transcriptional program of *P. sojae* effector gene expression with high throughput functional screens for plant defense suppression has revealed a coordinated interplay among the effectors to maximize defense suppression.

Comparative genomics, sequence mining and transcript profiling of cyst nematodes during plant parasitism

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Genomic approaches have begun to unravel the responses of plants to nematode infection but information about pathogenesis-related gene expression changes in plant-parasitic nematodes themselves is still scarce. High-throughput sequencing of expressed sequence tags (EST) and microarray analyses are powerful approaches to study nematode infection of plants from the parasite's perspective. Concerted efforts enabled the generation of a life stage-specific EST set of close to 22,000 sequences, representing up to 6,860 unique genes, for the soybean cyst nematode *Heterodera glycines*. EST sequence mining led to the pre-selection of candidate genes for secreted effector proteins, including a putative histone deacetylase. Secreted effectors allow cyst nematodes to establish a feeding site within their hosts. Furthermore, this EST set has been incorporated into the Affymetrix Soybean Genome Array GeneChip. Using this microarray, life stage-specific transcript profiling of *H. glycines* revealed distinct shifts in transcript abundance during pathogenesis. Comparative genomics between *H. glycines* and the model nematode *Caenorhabditis elegans* showed that developmental arrest might be based on different mechanisms in these two species. Together, large scale transcript profiling of plant-parasitic nematodes and comparative genomics will aid in determining what defines the parasitic nature of certain nematodes and enable the development of novel control strategies to protect crops.

Evolutionary & Functional Genomics of Virus-Plant Interactions

Small RNA-directed silencing pathways in plants

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Plants possess several posttranscriptional silencing systems involving distinct small RNA biogenesis and effector components. We have explored biogenesis, effector and specificity mechanisms of miRNA, trans-acting siRNA (tasiRNA), antiviral siRNA, and other small RNA classes using *Arabidopsis thaliana*. Small RNA are formed through the activity of four DICER-LIKE (DCL) proteins, and sorted among 10 ARGONAUTE (AGO) proteins through AGO-specific 5′ nucleotide and size preferences. For example, the vast majority of miRNA possess a 5′U, which directs association with AGO1. RNA silencing in response to viruses involves a set of DCL, amplification and AGO factors that partially overlap those required for tasiRNA, which form through an RNA-dependent RNA polymerase6 (RDR6)-mediated mechanism after initial processing of primary transcripts by miRNA-guided cleavage. Analysis of the tasiRNA biogenesis pathway has been particularly informative about mechanisms that direct a target RNA through the RDR6 amplification pathway, and has revealed the importance of specific small RNA-AGO complexes in recruiting RDR6 for dsRNA production. Antiviral silencing, however, involves additional factors, which have been revealed through analysis of silencing suppressor-defective mutant viruses. These and other features of Arabidopsis posttranscriptional and antiviral silencing pathways will be discussed.

Mechanisms of plant resistance to viruses

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Resistance (R) proteins are components of a plant surveillance system that serves to recognize pathogen-encoded effector proteins from organisms including viruses, bacteria, fungi and even nematodes. The majority of R immune receptors contain nucleotide binding (NB) and leucine-rich repeat (LRR) domains which are both present in mammalian intracellular pattern recognition receptors (PRRs) required for animal innate immunity. However, despite the structural similarities with animal innate immunity molecules, plant immune receptors recognize specific pathogen effectors while mammalian receptors recognize non-specific MAMPs. Intriguing questions in

the last few years in the innate immunity field is how plant R proteins recognize the presence of pathogen-derived effector proteins and activate downstream signaling. To this end, we are using N immune receptor that confers resistance to tobacco mosaic virus (TMV) as a model system. I will discuss our recent findings on how N immune receptor recognizes TMV and activates defense signaling.

The diverse routes of plant virus evolution
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Each major lineage of cellular life forms supports the reproduction of diverse viruses. The repertoire of viruses infecting a given group of hosts depends on the internal dynamics of the virus world and on the unique cellular and tissue organization of the host. The plant virome is heavily dominated by positive-strand RNA viruses with smaller contributions from double-stranded RNA, negative-strand RNA, retroid, and single-stranded DNA viruses. Double-stranded DNA viruses that are dominant in prokaryotes and common in animals are conspicuously absent from plants, conceivably, owing to their inability to pass through plasmodesmata. Comparative-genomic analysis shows that the plant virome was shaped by a multitude of evolutionary processes of which perhaps the most prominent one is gene exchange among diverse viruses that is not limited to plant viruses but also involves viruses of fungi and bacterial plasmids. The radiation of the major superfamilies of positive-strand RNA viruses, most likely, occurred concomitantly with eukaryogenesis, and representative of all superfamilies were inherited by plants. In contrast, the small DNA viruses of plants (geminiviruses and circoviruses) were originally derived from bacterial plasmids. The subsequent evolution of plant viruses was defined primarily by intervirus gene exchange under common selective pressures as exemplified by the spread of homologous movement proteins among diverse viruses.

TMV MP gates plasmodesmata via ANK, a tobacco ankyrin-repeat protein which down-regulates callose deposits
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For decades, TMV MP has been known to increase plasmodesmal permeability and traffic between cells, but how it achieves these goals remains an enigma. We have identified a tobacco ankyrin-repeat protein, designated ANK, which, when transiently overexpressed in tobacco leaf epidermal cells, enhanced the TMV MP cell-to-cell movement. On the other hand, in transgenic plants with native ANK expression decreased by RNAi-based knockdown, the TMV MP movement was dramatically suppressed. ANK contains a conserved glycosyl hydroxylase catalytic motif at its C-terminus. Point mutation of the glutamic acid residue, known to be critical for the glycosyl hydroxylase activity, abolished the ability of ANK to assist TMV MP movement. Furthermore, coexpression of TMV MP with ANK resulted in 80% decrease in the amount of callose whereas coexpression with the inactive ANK mutant (E313A) had no such effect. Importantly, expression of ANK alone, without TMV MP, also did not alter callose deposits. Thus, we suggest that TMV MP utilizes ANK to decreases callose accumulation to enable plasmodesma gating and its own traffic from cell to cell. Another, as yet poorly researched aspect of viral infection is regulation of host gene expression. In the case of green algae-infecting PBCV1, it encodes a histone methyltransferase that epigenetically represses host genes. We will present our data characterizing plant chromatin-modifying r complexes potentially involved in such repression.

Yeast as a model host to explore plant virus - host interactions
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Plant RNA viruses, which are important and emerging pathogens, depend greatly on host proteins. Selected host proteins are subverted to facilitate virus replication and other steps in the viral infection cycle. To identify all host factors affecting the RNA replication and RNA recombination of tombusviruses, we performed genome-wide approaches based on yeast model host developed in our lab. The studies included systematic testing of 5,500 yeast genes via deletion or down-regulation. In addition, we conducted proteomics approaches based on mass spectrometry analysis of the highly purified viral replicase complex and yeast protein arrays carrying 4,100 proteins. The systems biology approaches led to the identification of ~200 host proteins. Follow up experiments demonstrated that, surprisingly, tombusvirus replication could switch from the peroxisomal membrane to ER in the absence of peroxisomes. We also show that GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and heat shock 70 proteins are components of the tombusvirus replicase, and they are re-distributed to the site of viral replication. Mechanistic studies revealed the role of GAPDH in viral RNA replication, whereas HSP70 was shown to affect the assembly of the viral replicase. The relevance of yeast studies has been confirmed using Nicotiana host. Based on the above and additional data, we propose novel roles for several host proteins in regulation of viral RNA replication and discuss novel antiviral strategies.

Virus-host arms race as a shaping force of virus evolution
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Although the virus-host interactions are inherently complex, recent advances in revealing the mechanisms of the innate and adaptive immunity, and virus counterdefensive strategies provide a glimpse of the emerging holistic picture. One apparent common theme is that every single feature of a virus that makes it distinct from a cell can be targeted by a cognate host defense. But one example of this is hypermutation of the retroviral single-strand DNA by APOBEC defense system. The second common theme is that, eventually, virus mirrors each of the host defenses with a counterdefense. For instance, HIV-1 has evolved Vif to suppress hypermutation. From an evolutionary standpoint, such arms race preconditions gradual growth of the viral genome complexity that, in turn, provides a bigger and easier target for the host defenses. Accordingly, the genomes of the larger viruses contain a larger proportion of genes that function in evasion or suppression of the host defenses. This evolutionary tendency can be illustrated by closteroviruses, the largest positive-strand RNA viruses of plants that contain multiple suppressors of RNA interference and other antiviral systems. The next challenge for the evolutionary virology is to unravel the chronological context of the arms race. Important clues to this problem were provided by recent studies of the origins and evolution of retroviruses and picorna-like viruses including plant potyviruses.

Mechanisms of Post-Transcriptional Control of Gene Functions in Plant-Microbe Interactions (AS, PTGS, Sumoylation and More…)

Alternative splicing in plant-microbe interactions
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Intron retention (IR) is the most abundant type of alternative splicing (AS) in plants. To investigate the prevalence of stress-induced AS in Arabidopsis, we performed a genome-wide IR analysis using the entire Arabidopsis dbEST EST/cDNA data set (~ 1.5 million sequences). We found that the frequency of IR versus intron skipping was 2.8 times higher in libraries derived from treated (biotic/abiotic agents) samples, suggesting IR involvement in plant stress adaptation. The search yielded 439 genes showing IR specifically upon treatment. Remarkably, about 70% of these genes were annotated as having non known function, process or cellular localization. We then investigated the genes' expression pattern in response to stimulus using GENEVESTIGATOR tools. About 10% of the stress-specific AS genes did not show any differential level of expression in response to biotic stressors. We interpret these results to indicate AS undergoes regulation upon stress exposure and that this response involves appearance of novel, treatment-induced splice variants. Furthermore, AS genes often change their splicing patterns but not their overall expression level. The current lack of knowledge on the role of AS in plant biology is most likely the result of prioritizing gene functional analyses mainly on the basis of overall mRNA abundance rather than on changes in sub-populations of transcripts produced at a locus of interest.

Regulation of plant disease resistance gene function by alternative splicing
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Alternative transcripts are being described for a growing number of plant resistance genes, and are most prominent among the large family of genes

encoding Toll/interleukin-1 receptor (TIR) - nucleotide binding site (NBS) - leucine-rich repeat (LRR) domain-containing resistance proteins. While the mechanism of alternative splicing in this gene family varies, these alternative transcripts usually encode putative truncated TIR-NBS proteins. A first indication that these alternative transcripts provide a crucial function came from the observation that intronless cDNAs of the tobacco N gene were non-functional. We study the Arabidopsis RPS4 gene and showed that intronless RPS4 transgenes, despite being expressed, failed to complement an rps4 mutant line. However, combining full-length and artificially truncated cDNAs that mimic the prevalent RPS4 transcripts provided resistance, demonstrating directly that a combination of RPS4 transcripts is required for function. RPS4 alternative transcript levels were temporally regulated after stimulus perception, suggesting that alternative splicing is fine-tuned to optimally regulate the plant innate immune response. We will present a model for the function of alternative transcripts in regulating plant resistance gene-mediated responses, and will discuss how the study of resistance genes can lead to insights into the important process of alternative splicing in plants.

A novel role for protein farnesylation in plant innate immunity
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Plant innate immunity involves tightly regulated signaling pathways leading from pathogen recognition by Resistance (R) proteins to the expression of immune responses. By using the autoimmune model snc1, which expresses a constitutively active R protein in *Arabidopsis thaliana*, we were able to dissect the signaling events downstream of R protein activation and to identify new players involved in plant defense. In the snc1 suppressor screen, using fast neutron, we identified modifier of snc1, 8 (mos8), which restores wild type susceptibility to virulent pathogens in snc1. MOS8 was identified through a map-based approach and is allelic to Enhanced Response to ABA 1 (ERA1), mutations in which affect seed germination, ABA responses, flower morphology and drought tolerance. ERA1 encodes the beta subunit of protein farnesyltransferase, a bipartite enzyme required for C-terminal prenylation of specific target proteins, which is important for correct protein localization and interactions. Mutations in ERA1 render plants more susceptible to virulent bacterial and oomycete pathogens and also affect several R protein mediated responses. ERA1 acts additively with the known resistance regulator Non-expressor of PR genes 1 (NPR1). Our data suggest a direct involvement of farnesylation in defense signaling. However, the protein targets of ERA1 involved in plant innate immunity have yet to be identified.

Endogenous small RNAs and host RNAi machinery added a fundamental layer of regulation in plant immunity
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Emerging evidence suggests that host endogenous small RNAs play an important role in plant immune responses. To identify and characterize pathogen-regulated small RNAs at the whole genome level, we performed high-throughput sequencing of small RNA libraries prepared from bacteria- and fungi-challenged Arabidopsis. We identified a diverse set of endogenous small RNAs, including miRNAs, nat-siRNAs, gene-targeting hc-siRNAs, and protein-coding gene-associated siRNAs. These small RNAs have different distribution patterns and distinct biogenesis pathways. Many of these small RNAs are either up- or down-regulated by various pathogens and may subsequently regulate target genes, thus contributing to gene expression reprogramming and fine-tuning in plant immune responses. Furthermore, we found that some of the RNAi pathway components are also important for regulating plant biotic stress responses. Our results suggest that host endogenous small RNAs and host RNA-silencing machinery represent a fundamental layer of control in plant immune responses.

Microbial Genomes Off the Beaten Path

Genome plasticity in the genus *Mycosphaerella*
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Most members of the genus *Mycosphaerella* grow very slowly in culture and have long latent periods *in planta*. Until recently, very little was known about their genetics or host-pathogen interactions. Genomes of two species have been sequenced: the septoria tritici blotch pathogen of wheat, *M. graminicola*; and *M. fijiensis*, the black Sigatoka pathogen of banana. The sequence of *M. graminicola* is complete; only one telomere and two internal gaps are missing. Among the 21 chromosomes of the 39.7-Mb genome, eight were smaller, had higher repetitive DNA contents, lower percent G+C and fewer genes compared to the essential chromosomes. These eight, representing 40% of the chromosomes and about 10% of the genome, could be missing in other isolates indicating an extraordinary degree of within-species genome plasticity. In contrast, the genome of *M. fijiensis* was 74 Mb in size but had a similar gene content. The increased size was due primarily to invasion of the genome by long-terminal repeat retrotransposons. The mitochondrial genome of *M. fijiensis* also was about twice the size as that of *M. graminicola*. Thus, extreme genome plasticity occurs both within and among species of the genus *Mycosphaerella*.

Why is *Ralstonia solanacearum* Race 3 cold tolerant? Using post-genomic analysis to explore strain-specific traits
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Phytopathology 99:S166

Most strains of the bacterial wilt pathogen *Ralstonia solanacearum* are tropical, but one group, Race 3 biovar 2 (R3b2), is adapted to cooler environments and causes disease in temperate zones and tropical highlands. We compared the growth and virulence of *R. solanacearum* strains GMI1000 (tropical, biovar 3) and UW551 (R3b2, temperate) at temperate and tropical temperatures. The two strains grew similarly in media at 20°C and 28°C. At 28°C, both strains wilted tomato plants rapidly in a naturalistic soil-soak virulence assay. In contrast, at 20°C UW551 was much more virulent on tomato than GMI1000, suggesting that interaction with plants is required for the temperate epidemiological trait of R3b2. To understand the mechanisms of R3bv2 cold tolerance, we studied global gene expression patterns of the two strains at 20°C and 28°C using genomic microarrays. In rich medium, the strains' expression profiles differed significantly with respect to both strain and temperature. In the four conditions, we found differential expression of genes involved in cell wall/membrane synthesis and function, carbohydrate transport and metabolism, transcription, replication, recombination and repair, and diverse unknown functions, including some specific to R3bv2. A hitherto cryptic quorum sensing system, SolIR, and a SolIR-dependent gene, aidA, were up-regulated in R3bv2 at 20°C. aidA, which was expressed around 12-fold higher at 20°C, is not present in the GMI1000 genome.

***Rhizoctonia solani* genome project; providing insight into a link between beneficial and plant pathogenic fungi**
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A consortium consisting of North American and international scientists in the *Rhizoctonia* community is actively involved in a collaborative project to obtain a high quality complete genome sequence of the soil fungus *R. solani* anastomosis group 3 (AG-3), strain Rhs1AP. This fungus is a competitive saprobe and an important pathogen of food crops in the plant family Solanaceae. In addition to its economic importance as a plant pathogen, the fungus and its closely related species can often form beneficial associations with early diverging land plants, lichens, and orchids. Sanger and 454 Titanium pyrosequencing methods have provided approximately 16X coverage of the genome with average read lengths of 709 and 383 bp, respectively. Estimates of genome size vary from 52.4 to 71.5 Mb depending on the method of analysis. Repetitive sequences in the genome have been identified and microsatellite-based genetic markers for population genetics studies are available to the community on the website.

Streptomyces scabies: Mapping a novel path to pathogenicity
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Phytopathology 99:S167

Streptomyces scabies is the dominant pathogenic species among the dozen or more streptomycetes that cause potato scab. Emergence of locally adapted potato scab species can now be attributed to horizontal gene transfer. The newly emerged pathogen *S. turgidiscabies* Car8 carries a 660 kb integrative conjugative element, encoding the thaxtomin biosynthetic pathway and other virulence loci. New genomic resources, including the genome of *S. scabies* 87-22 genome, are emerging that allow us to use comparative genomics to filter putative virulence genes from these large bacterial genomes. The picture revealed by this analysis is one of continuing evolution of pathogenicity islands in this genus, with acquisition of virulence genes found in other pathogenic bacteria and fungi, including animal pathogens. The importance of the cellulose biosynthesis inhibitor, thaxtomin, as a pathogenicity determinant has been confirmed. Thaxtomin is produced exclusively by scab-causing streptomycetes, but genome sequences reveal other virulence determinants that are shared with a diverse group of pathogens. Ongoing comparative and functional genomic analysis is providing substantial insight on the mechanisms by which these economically important and fascinating pathogens manipulate plant cells and continue to evolve in agricultural systems.

Evolutionary relationship of enteric plant pathogenic bacteria
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Pectobacterium is an *Enterobacteriaceae* plant pathogen that causes soft rot disease on diverse plant species. *Pectobacterium* species have wide host ranges, except for *P. atrosepticum*, which infects potato and a few other solanaceous species. We have found that the sequenced strain of *P. atrosepticum* is significantly less virulent on tubers and on the model plant, *Nicotiana benthamiana* than *P. carotovorum*. A comparison of genome sequences of two *P. carotovorum* subspecies and *P. atrosepticum* identified genes that could account for these differences. The *P. carotovorum* genomes were generated by 454 pyrosequencing and ordered by reference to the previously published complete circular chromosome of *P. atrosepticum* genome and each other. A tiled *P. carotovorum* microarray was designed based on the draft sequence and used to identify *P. carotovorum* genes that were highly expressed when the bacteria were grown in potato tubers. A highly expressed gene cluster present in *P. carotovorum* species, but absent from *P. atrosepticum*, was identified and we are currently testing these genes to determine if they affect virulence. We found that *P. carotovorum*, *P. atrosepticum*, and *P. wasabiae* differ in which T3SS genes are encoded and our gene expression and mutagenesis experiments suggest that the *P. carotovorum* T3SS is not important for infection of potato tubers, but is important for disease in plant leaves.

Molecular Mechanisms of Host Susceptibility

Victoria blight: A convergence of plant disease susceptibility and resistance?
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Victoria Blight is conditioned by a single dominant gene called *Vb* in oats. Virulence of the causal agent, *Cochliobolus victoriae*, depends on production of the "toxin", victorin. When a victorin-producing isolate encounters a host that carries the dominant allele, disease occurs. In all other cases, such as a homozygous recessive host, disease does not occur. In oats, numerous analyses have indicted that *Vb* is identical or closely linked to the *Pc2* gene, which confers crown rust resistance. Recently, we characterized a gene in Arabidopsis that confers both victorin sensitivity and susceptibility to *C. victoriae*. This gene, called *LOV*, encodes a CC-NB-LRR protein. We also found that LOV function requires the cytosolic thioredoxin, TRX5. Current analyses are directed at understanding the role of TRX5 and victorin in mediating LOV activity and the nature of the LOV-mediated response. Given that the primary role for CC-NB-LRR proteins is to confer disease resistance, results support the possibility that *Vb* and *Pc2* may be the same gene and indicate that a host resistance response can be exploited by *C. victoriae* to evoke disease susceptibility.

The Biotrophic Interfacial Complex and effector translocation during rice blast disease
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To cause rice blast disease, *Magnaporthe oryzae* sequentially invades living rice cells using specialized invasive hyphae (IH) that are enclosed in host-derived Extra-Invasive-Hyphal Membrane (EIHM). Little is known about how the fungus delivers effector proteins across the EIHM into the plant cell's cytoplasm to promote disease, or to trigger resistance in rice varieties with corresponding resistance genes. We performed coupled live cell imaging and microarray expression analyses of IH growing in first-invaded cells. The cellular analysis suggested that IH control rice membrane dynamics for growth in invaded cells and host pit fields for movement into neighboring cells. The expression analysis identified numerous novel Biotrophy-Associated Secreted (BAS) proteins as candidate effectors. Using live-cell fluorescence imaging, we identified a novel pathogen-induced structure, the Biotrophic Interfacial Complex (BIC), which accumulates fluorescently-labeled, secreted effectors AVR-Pita, PWL1, and PWL2 as well as some BAS proteins. Correlative light and electron microscopy identified the BIC as a complex aggregation of membranes and vesicles at a specific location in the EIHM matrix. We have shown that secreted effectors that accumulate in BICs are translocated into invaded host cells. Surprisingly, translocated effectors were also observed in uninvaded neighboring rice cells, suggesting that the fungus sends effectors to hijack host cells before entry.

Negative regulators of basal defense in cereal-fungal interactions
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Plants have evolved complex regulatory mechanisms to control the defense response against microbial attack. Both temporal and spatial gene expression are tightly regulated in response to pathogen ingress, modulating both positive and negative control of defense. BLUFENSIN1 (BLN1), a small peptide belonging to a novel family of proteins in barley, wheat, and rice, is highly induced by attack from the obligate biotrophic fungus, *Blumeria graminis* f. sp. *hordei*, causal agent of powdery mildew disease. BLN1 negatively impacts plant defense, is predicted to be secreted, and contains both structural and sequence similarities to knottins, small disulfide-rich proteins characterized by a unique disulfide through disulfide knot. To discern regulatory targets of BLN1, we conducted Barley1 GeneChip analysis of *Bln1*-silenced plants via *Barley stripe mosaic virus*-induced gene silencing (BSMV-VIGS). Sixty GeneChip hybridizations were performed, based on 5 replications of 12 BSMV-VIGS/host-pathogen interactions. Mixed linear model analysis revealed 36 highly significant new genes ($p < 0.0001$; FDR < 5%) that are suppressed together with *Bln1* (Contig12219_at; $p = 2.31E-07$), or induced when we compare BSMV:Bln1248 silenced plants to the BSMV:00 control. These candidates appear to have a role in R-gene mediated and innate immunity networks, thus, the functional identification of their precise roles will be a key step in understanding plant defense.

How oomycete effectors condition susceptibility to *Phytophthora sojae*
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Oomycete pathogens cause billions of dollars of damage to crops each year. The potato pathogen *Phytophthora infestans* and the soybean pathogen

Phytophthora sojae are among the most destructive oomycete pathogens. Genome sequencing of these and other oomycete pathogens has revealed large numbers of candidate effector genes, encoding proteins with the host cell entry motif RXLR. We have shown that oomycete RXLR motif binds to the phospholipids phosphatidyl inositol-4-phosphate and phosphatidyl-3-phosphate and that binding to these phosphoinositides enables the proteins to pass the plant plasma membrane into the cytoplasm. Effectors from fungal and apicomplexan pathogens also use this mechanism, which also allows entry into animal cells. Once effectors enter the plant cell they target the plant's defense response machinery. We have shown that the *P. sojae* effectors Avr1b and Avr1k can suppress effector (R gene)-mediated immunity via a site in the nucleus. The binding of Avr1b to a soybean U box protein may mediate suppression of effector-triggered immunity. Avr1b and Avr1k can also suppress immunity triggered by the PAMP elicitin, but the target for this action is located outside the cytoplasm. The *P. sojae* genome encodes around 400 effector candidates, and we have screened approximately half of these for the ability to suppress host defenses. Nearly all of those tested can suppress either effector- or PAMP-triggered immunity, and most can suppress both. The timing of expression of many effectors is staged so that those that suppress effector-triggered immunity are expressed earliest in order to prevent host defense responses to later effectors that suppress PAMP-triggered immunity.

Mechanisms of bacterial speck disease development in tomato: Functional role of a ubiquitin ligase and the chloroplast targeting virulence factor, coronatine

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Bacterial speck disease, which is caused by *Pseudomonas syringae* pv. *tomato*, is an economically important disease on tomato. Several pathovars of *P. syringae* produce a chlorosis-inducing virulence factor coronatine (COR), which functions as a phytohormone mimic of methyl jasmonate (MeJA) and JA-isoleucine (JA-Ile). A comparison of COR- and MeJA-regulated transcriptomes revealed that COR and MeJA share similar, but not identical activities and impact multiple phytohormone pathways in tomato. COR, by structurally mimicking JA-Ile, hijacks an ubiquitin E3 ligase of the SCFcoi1/jai1 complex to activate JA signaling and thereby suppress salicylic acid (SA)-mediated defense responses. Our results also demonstrate a role for COR-induced effects on photosynthetic machinery and ROS in modulating necrotic cell death. To identify new players in COR-induced chlorosis, we utilized Nicotiana benthamiana and virus-induced gene silencing (VIGS) as a genetic screen and identified a role for several chloroplast-localized genes and *sgt1* (suppressor of G2 allele of Skp1) in COR signaling. Collectively, the results of virulence assays, metabolic profiling, gene expression data and VIGS-based genetic screens indicate that COR targets the JA pathway to suppress SA-mediated defense responses and the chloroplast to modulate ROS homeostasis, thus promoting symptom development in tomato.

Breakdown of basal resistance in *Nicotiana benthamiana* and Arabidopsis against host and nonhost pathogens

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Plant nonhost resistance, a form of innate immunity, is the most common form of disease resistance exhibited by plants against the majority of potential pathogens in nature. We used virus-induced gene silencing in *Nicotiana benthamiana* to identify genes involved in nonhost resistance. Eleven genes were identified to be involved in type I and/or type II nonhost resistances by individually silencing ~4,000 genes from a normalized NbcDNA library. One of them encodes squalene synthase (*SQS*), a key enzyme catalyzing the first enzymatic step in sterol biosynthesis. Silencing *SQS* gene in *N. benthamiana* caused plant cell membrane leakage resulting in more nutrient accumulation in the apoplast. The Arabidopsis SQS1 RNAi lines were not only susceptible to nonhost pathogens, *Pseudomonas syringae* pv. *tabaci* and *P. syringae* pv. *syringae*, but also more susceptible to virulent pathogens, *P. syringae* pv. *tomato* DC3000 and *P. syringae* pv. *maculicola*, when compared with the wild-type Arabidopsis. We also discovered that a mutation in Arabidopsis *SMT2*, a gene encoding sterol methyltransferase (downstream enzyme in phytosterol biosynthesis), compromised nonhost resistance. Metabolite analysis indicated that, compared to the wild-type Arabidopsis, *SQS* RNAi lines and a *smt2* mutant produced less stigmasterol. Strikingly, the gene *AtCYP710A1* converting sitosterol to stigmasterol was dramatically induced and stigmasterol was significantly increased in wild-type plants upon inoculation with nonhost pathogens. Arabidopsis *cyp710a1* mutant and overexpressors of *AtCYP710A1* are currently being characterized and the results will be presented. Our data suggest that cell membrane components especially membrane associated stigmasterol plays an important role in plant innate immunity against bacterial infections.

The Balance is Tilting, Finding Resistance to Vascular Wilting

Breeding for resistance - new approaches and challenges

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Breeding resistance to soil-borne wilt diseases is challenging due to cumbersome testing methods and limited genetic variation for resistance. Verticillium wilt of lettuce caused by *Verticillium dahliae* can cause severe economic damage and threatens the existence of lettuce production in California; resistance is the only feasible control measure. Initial field trials identified resistance, however a decade of field selection developed resistant but commercially unusable germplasm. Research was conducted to accelerate breeding and meet the urgent need for resistant cultivars. Pathogen surveys and greenhouse assay development lead to the discovery of two races, and later demonstrated that known field-resistant cultivars were only resistant to race 1 (R1). A single gene for R1 resistance in the cultivar La Brillante was described and positioned on chromosome 5, and marker assisted breeding should produce R1 resistant cultivars within six years. Race 2 (R2) isolates are located in California, and may render R1 resistance obsolete. Screening germplasm for R2 resistance identified accessions with either reduced symptoms or disease incidence, but not complete resistance. Further, all tested germplasm can be colonized, even if a-symptomatic. A quantitative PCR assay for Verticllium *in planta* was developed to assess the usefulness of the reduced symptom phenotype for breeding, and populations are being created for genetic analysis and screening for transgressive segregants.

Molecular mechanisms of resistance - functions of major R genes

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Vascular wilt pathogens have evolved a diverse arsenal of toxic proteins and compounds, termed effectors, designed to promote pathogenesis and facilitate suppression of basal host defenses. However, as is the case with most phytopathogens, certain plant hosts have developed a corresponding set of resistance (*R*) genes that encode receptors capable activating strong defenses upon recognition of specific pathogen effectors. Research efforts over the past 20 years have resulted in the identification and cloning of *R* genes for a wide variety of pathogens from many plant hosts. The identification of *R* genes and their incorporation into cultivated crops becomes particularly important when dealing with pathogens such as vascular wilts, where chemical control options are typically very limited. This presentation will include an overview of genes conferring resistance to vascular wilt pathogens and an update on research results related to mechanisms of pathogen recognition and resistance activation.

Secondary metabolites and toxins - what is causing disease symptoms?

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Fusarium species produce a variety of phytotoxic metabolites. Of the races of *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) that attack cotton, alfalfa, and okra, many secondary metabolites are derived *via* a polyketide biosynthetic pathway. The recent discovery of new pathotypes not previously found in the U.S. is of particular concern to the cotton industry. In addition, a virulent *Fov* biotype has been identified in Australia that can cause >60% plant mortality. In 2002/2003, several shiploads of fumigated/live cottonseed were imported into the U.S. from Australia as feed for dairy cow. We isolated 17 *Fov* isolates from about 17,000 of these seeds. The most virulent belonged to race 3 based on molecular phylogenetic analysis and it was vegetatively compatible with the Australian biotype. Indel analysis of the EF gene also revealed a close relationship to the Australian biotype. While the Australian biotype has not

been found in U.S. fields, a newly discovered Fov race 4 is of increasing concern due to losses of Pima cotton in California. Race 4 and the Australian biotypes both attack cotton seedlings, and produce prodigious quantities of fusaric acid (FA) when grown on Czapek media. We confirmed that FA is derived in part *via* a polyketide synthase (PKS). We are searching for the corresponding PKS genes, and investigating the role of FA in the pathogenicity of these *Fov* isolates. Results from these studies and generation of FA knockout mutants will be presented.

Molecular interactions between *Fusarium oxysporum* and Arabidopsis
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Fusarium wilt of Arabidopsis is a model pathosystem for studying vascular disease. Three distinct *formae speciales* of *Fusarium oxysporum* from crucifer hosts, cabbage (f. sp. *conglutinans*), radish (f. sp. *raphani*) and stock (f. sp. *matthioli*), instigate disease symptoms in Arabidopsis that are indistinguishable from those observed in the natural host of each. Among Arabidopsis ecotypes, considerable variation in susceptibility is observed. We are identifying the genes and gene interactions responsible for the quantitative variation of resistance. We are also studying the enhanced resistance or susceptibility of Arabidopsis mutants, which implicates well-characterized plant processes as well as poorly characterized plant genes in disease progression. Our ability to study Fusarium genes in vascular disease has improved considerably as genetic transformation of *F. oxysporum* has become routine and because full Fusarium genome sequence is now available.

Molecular responses to quantitative bacterial wilt resistance in tomato
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Host resistance is the only practical control for bacterial wilt of tomato, caused by *Ralstonia solanacearum*, but wilt resistance is horizontal, polygenic and its basis is not understood. We found that tomato plants infected with *R. solanacearum* Race 3 biovar 2 strain UW551 upregulated genes in both the ethylene and salicylic acid signal transduction pathways. However, in response to *R. solanacearum* infection the horizontally wilt-resistant tomato line H7996 activated expression of these defense genes faster and to a greater degree than did susceptible cultivar Bonny Best. Interestingly, results suggest different roles for the virulence factor extracellular polysaccharide (EPS) in resistant and susceptible hosts. Wild-type UW551 induced a lower defense response in susceptible tomato than did an *eps* mutant, supporting the idea that EPS can shield *R. solanacearum* from recognition. In contrast, the *eps* mutant induced significantly lower defense responses in resistant H7996 than the wild-type strain, weakening the "cloaking" hypothesis. The *eps* mutant also induced noticeably less accumulation of the defensive reactive oxygen species hydrogen peroxide in resistant tomato leaves, despite attaining similar cell densities *in planta*. Further, cell-free purified EPS from UW551 triggered significant defense gene expression in resistant but not in susceptible tomato plants. Collectively, these data suggest that H7996 specifically recognizes EPS from *R. solanacearum*.

Plant Disease Management

Carboxylic Acid Amide Fungicides (CAA) FRAC Group 40

Molecular and genetic aspects of CAA mode of action and resistance
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Carboxylic acid amide (CAA) fungicides comprising the commercial compounds dimethomorph, flumorph, iprovalicarb, benthiavalicarb and mandipropamid are specifically active against pathogens of the oomycetes such as *Phytophthora*, *Plasmopara*, *Pseudoperonospora* and *Bremia* species but not against *Pythium* species. The biochemical mode of action was claimed to involve inhibition of membrane or cell wall biosynthesis. Resistance to CAAs was detected in field populations of *P. viticola* in Europe and *P. cubensis* in USA and some Asian countries but not in *P. infestans* and *B. lactucae*. In crosses of sensitive and CAA resistant isolates of *P. viticola*, all F1 progeny isolates were sensitive; after crossing F1 isolates, CAA resistance segregated with an average r : s ratio of 1 : 9 suggesting a recessive inheritance pattern of resistance involving genes with one or both loci being heterozygous. Resistance co-segregated for all CAA fungicides but not for mefenoxam. Sequencing the four known genes involved in cellulose biosynthesis revealed mutations in *cesA3* of CAA resistant isolates suggesting that the mechanism of resistance is linked to cellulose biosynthesis which probably represents also the site of CAA action. Based on field observations, the molecular and genetic results and the different biology of the pathogens, resistance risk to CAA fungicides is estimated as low for *P. infestans* and moderate for *P. viticola*.

Activity of CAA fungicides against *Phytophthora infestans* and *Bremia lactucae*
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The CAA fungicides, mandipropamid (MPD), dimethomorph (DMM), benthiavalicarb (BENT) and iprovalicarb (IPRO) are effective inhibitors of spore germination of *Phytophthora infestans* and *Bremia lactucae* and hence, of infection. MPD had the highest intrinsic activity against spore germination and IPRO the lowest. Cystospores of *P. infestans* exposed to CAAs showed extensive internal deterioration within 1 h. CAA did not hamper F-actin rearrangement in germinating spores of *B. lactucae*. Spores that were exposed to CAAs for 1 h and then washed with water retained their germination and infectivity. Curative application at 1 day post inoculation required higher concentrations of CAAs to prevent infection. Trans-laminar protection, although achieved with higher doses, was more effective with MPD than with the other CAAs. Natural resistance against CAAs occurs in *Plasmopara viticola* and *Pseudoperonospora cubensis* but not in *P. infestans* or *B. lactucae*. We were able to mutate sporangia of *P. infestans* for resistance against the phenylamide fungicide mefenoxam but failed to select mutants with stable resistance against CAAs. *B. lactucae* was mutated for resistance against CAAs but stability of resistance and pathogenic fitness of the mutants are yet unknown. In a mini-epidemic conducted in a closed plastic house, two mutant isolates survived for three months on CAA treated lettuce plants. Their persistence on untreated plants is yet unknown.

Mandipropamid and dimethomorph baseline sensitivity distribution and resistance monitoring
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The baseline sensitivities to the carboxylic acid amide (CAA) fungicides (Group 40) mandipropamid and dimethomorph of some *Phytophthora* and downy mildew species have been established. The mycelia sensitivity (ED_{50} values) to mandipropamid of 75 isolates of *P. capsici* ranged from 0.0056 to 0.0265 mg/L with a mean of 0.014 mg/L. The mycelia sensitivity of 40 isolates to dimethomorph ranged from 0.068 to 0.333 mg/L with a mean of 0.226 mg/L. The sensitivity to mandipropamid for the control of late blight on detached leaves of 122 isolates of *Phytophthora infestans* ranged from 0.025 to 2.98 mg/L. The mandipropamid sensitivity of 15 isolates of *Bremia lactucae* tested on lettuce seedlings ranged from 0.001 to 0.01 mg/L with a mean of 0.003 mg/L. Sensitivity monitoring for the early detection of resistant isolates is in place for several CAA target pathogens. At this time no CAA resistant *P. infestans* isolates have been detected in the USA or Europe. However, resistant isolates of *Pseudoperonospora cubensis* were detected in the USA in 2007. Resistant isolates of *Plasmopora viticola* have been detected in Europe for several years but no practical product failures have been reported when CAAs were used in mixture with contact fungicides. The fungicide resistance action committee (FRAC) has classified the CAA risk for resistance development as low for *P. infestans* and moderate for *P. viticola* and *P. cubensis*. CAA sensitivity monitoring helps with the early detection of resistance and its proper management.

Biological effect of some carboxylic acid amide fungicides on growth and sporulation of three species of Phytophthora and the diseases they cause
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The effect of dimethomorph, a carboxylic acid amide (CAA) fungicide, was evaluated on the growth, sporangium formation, and germination of encysted zoospores of *Phytophthora capsici*, *P. citrophthora* and *P. nicotianae*. Of these parameters, mycelial growth was most sensitive to the fungicide, with a 50% reduction in mycelial growth (EC_{50}) for the three pathogens being

achieved with concentrations in the range of <0.1 to 0.38 µg/ml. The EC_{50} value for sporangium formation for the three *Phytophthora* spp. was <1.0 µg/ml. Suppression of encysted zoospore germination required somewhat higher concentrations of dimethomorph, with EC_{50} values for *P. capsici*, *P. citrophthora* and *P. nicotianae* of 3.9, 3.3 and 7.2 µg/ml, respectively. Following application of dimethomorph to the outer bark surface of tangelo trees, inhibition of lesion development after placement of *P. citrophthora* or *P. nicotianae* mycelium on the bark cambium 30, 60 and 90 days after fungicide application was 60, 46 and 42%, respectively for *P. citrophthora* and 74, 59 and 41%, respectively for *P. nicotianae*, compared to nontreated bark. In greenhouse trials highly favorable to disease development using soil naturally infested with *P. capsici* and treated with dimethomorph or another CAA fungicide mandipropamid, bell pepper plant mortality after 2 months was 42 and 48% respectively, values significantly lower than the 72% plant mortality in nontreated soil.

Dimethomorph efficacy studies and resistance management
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Key application timings of fungicides (including dimethomorph) to limit further infection of potato foliage by *Phytophthora infestans* were determined. Programs initiated 72 h before and after inoculation with *P. infestans* delayed development of late blight but later timings were similar to the untreated control. When dimethomorph was tank-mixed with protectant fungicides and integrated into a chlorothalonil-based late blight control program, all programs were effective. The sensitivities of 11 isolates of *P. infestans* to dimethomorph at all stages of the asexual life cycle were examined on potato leaf disks. Zoospore encystment and cystospore germination were highly sensitive to dimethomorph. The degree of dimethomorph sensitivity in all stages of the asexual life cycle was positively and significantly correlated, except cystospore germination. Dimethomorph insensitive mutant strains of *P. infestans* were made using ethidium bromide/ultra-violet light. Mutagenesis created two strains of *P. infestans* with resistance factors >20, i.e. the ratio of the EC_{50} of the mutant strain to that of the wild-type. Regardless of the induction treatment, reduced fitness was common for all *Phytophthora* spp., indicating a biological cost associated with dimethomorph insensitivity. Based on these results, the development of field resistance to dimethomorph of *P. infestans* and other species is unlikely with the currently employed dimethomorph resistance management schemes.

Challenges for Managing Insect Vectored Diseases

Changes in the epidemiology of Pierce's disease in California due to the introduction of the glassy-winged sharpshooter
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Pierce's disease is caused by the xylem limited bacterial pathogen, *Xylella fastidiosa*. This pathogen is vectored by xylem feeding insects and has been present in California for more than 100 years. Within California there are three management regions that have different key vectors: the North Coast, the Central Valley, and Southern California. On the North Coast the key vector is the native blue-green sharpshooter (*Graphocephala atropunctata*). In the Central Valley, there are two native vectors and one exotic vector. The two native vectors are the green sharpshooter (*Draeculacephala minerva*) and the red-headed sharpshooter (*Xyphon fulgida*). Both are widely distributed in the Central Valley. In contrast, distribution of the exotic glassy-winged sharpshooter (*Homalodisca vitripennis*) is limited to southern portions of the Central Valley and throughout Southern California where it is the sole key vector. Two epidemics of Pierce's disease occurred approximately 10 years after introduction of the glassy-winged sharpshooter into California. The first occurred in Southern California and was followed by an epidemic in the southern portion of the Central Valley. These epidemics resulted in losses typically not observed with native vectors. Factors hypothesized to contribute to these losses were aspects of glassy-winged sharpshooter ecology, the cropping landscape in Southern California, climate, and the absence of a management program for a newly introduced vector.

Expansion of *Xylella fastidiosa* into blueberries in Georgia and Florida
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Koch's postulates were applied in 2006 to confirm *Xylella fastidiosa* as the cause of bacterial leaf scorch (BLS) of blueberry, predominantly a problem on southern highbush cultivars (*Vaccinium corymbosum* interspecific hybrids). Symptoms include a marginal leaf scorch, leaf drop, yellowing of stems, and eventual plant mortality. In 2008, a survey was initiated to determine the prevalence of BLS in Georgia, as well as whether field resistance is present among some *V. corymbosum* cultivars. In Aug, Sep, and Oct, BLS was identified in the field based on symptoms. Samples were taken for confirmation by ELISA, and an estimate of disease incidence was collected for each site. Of 45 farms surveyed, 71.1% were positive for BLS in at least one field; of 167 fields surveyed, 41.9% were positive. Field resistance or tolerance was observed among some cultivars, and initial results indicate that Windsor, Emerald, Millennia, Southern Belle, and V5 either had no or minimal BLS symptoms (<1% incidence). In contrast, Star, O'Neal, and particularly FL 86-19 had a high incidence of infection and therefore appear susceptible. Based on regression analysis relating disease incidence to plant age, ~75% of FL86-19 plants will be symptomatic within 10 years of planting, whereas ~30% of Star plants will be diseased within the same time frame. These data provide good evidence that the solution to bacterial leaf scorch will involve resistance breeding and selection.

Transmission and management of Cucurbit yellow vine, caused by the bacterial pathogen *Serratia marcescens*
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Phytopathology 99:S170

Cucurbit yellow vine disease (CYVD), caused by the phloem-inhabiting bacterium, *Serratia marcescens*, is a periodic threat to watermelon, squash, and pumpkin production throughout parts of the United States. Squash bugs, *Anasa tristis* (Hemiptera: Coreidae), are the only known primary field vectors, transmitting the bacterium with a range of 5–25% efficiency for single inoculative insects. The mode of transmission is likely propagative as nymphs retain the ability to transmit after molting to the adult stage. Feeding behavior studies using electrical penetration graph (EPG) technology show that the squash bug displays different feeding profiles on squash as compared to watermelon, but most probing activity occurs with stylets in contact with the xylem. Thus far, a specific phloem-ingestion EPG waveform has not been identified, a result consistent with a hypothesis that bacteria may be deposited in plant tissue other than phloem. Comparisons of squash bug EPG parameters on potentially resistant germplasm suggest that differences in probing activity are quantitative and not qualitative. Control of CYVD in watermelon is best achieved by controlling the vector squash bug population early in the season either by timed insecticide treatments or by the use of squash trap crops.

Management of Curly top virus in vegetables
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Curtovirus infection is due to a complex of related viruses ie. *Beet curly top virus*, *Beet mild curly top virus*, *Beet severe curly top virus*, that are vectored by the beet leafhopper (*Circulifer tenellus*). The viruses and leafhopper have very large ranges of dicot hosts and are endemic throughout much of the western USA. Disease losses are routinely reported from vegetables such as tomatoes, peppers, melons, and beans, in addition to losses in sugarbeets. In New Mexico, curly top causes substantial, but unpredictable, losses to chile peppers. Management of curly top in vegetables has traditionally included cultural and insecticide sprays. However, the inability to spray insecticides for leafhopper control for the last few years in some areas has limited control to plant resistance and cultural methods. In New Mexico, a system for predicting the magnitude and timing of curly top infection based on environmental factors is being developed from research results on the primary overwintering weed host of the virus and leafhopper populations and this has allowed growers to plan for disease. Cultural methods such as weed control and kaolin sprays have also been used successfully for disease control in peppers. Characterization of plant resistance in several vegetables will provide additional sustainable management strategies. An integrated approach to control of the insect vector is the basis for curly top disease management in vegetables.

Managing whitefly vectors of three cucurbit viruses new to Florida

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In recent years, three whitefly-transmitted viruses have become a serious problem for growers of cucurbits, especially watermelon, in Florida. *Squash vein yellowing virus*, an ipomovirus transmitted in a semi-persistent manner, was identified in 2005 as the causal agent of watermelon vine decline (WVD), a particularly devastating disease. Since 2007, *Cucurbit leaf crumple virus*, a begomovirus, and *Cucurbit yellow stunting disorder virus*, a crinivirus, have also been identified in cucurbits in southwest Florida. Until resistant varieties are developed, the best option for managing these viruses is more intensive management of the whitefly vector, *Bemisia tabaci*, biotype B. Multiple tactics are being explored because of the very real concern about development of insecticide resistance. In one study, the use of reflective mulch and a soil application of a neonicotinoid insecticide combined with foliar applications of spiromesifen reduced fruit symptoms of WVD. Evaluations of many other insecticides showed reductions in disease severity in research plots, although final disease incidence was not affected. Current efforts focus on integration and further development of management strategies and development of virus and whitefly monitoring tools. Area-wide surveys will identify "hot spots" and reservoir crops for whiteflies and viruses and will help evaluate effects of current and future control strategies on virus incidence in commercial fields.

Cucurbit Downy Mildew: Re-Emergence of a Historical Disease

The history and re-emergence of cucurbit downy mildew

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Cucurbit downy mildew (DM), caused by *Pseudoperonospora cubensis*, was first described in 1868 in Cuba. It is among the most well-known downy mildew diseases in the world, attacking cucurbits wherever they are grown, especially in humid climates. It is also one of the most notorious pathogens for developing resistance to fungicides. Several research programs in Israel, the U.S.A., Japan, India and the Czech Republic have contributed to an extensive knowledge-base currently in place. Breeding efforts in cucumber in the 1950s and 60s led to the development of resistance levels sufficient to control the disease without the use of fungicides. Although this resistance was not as effective in other parts of the world, the U.S. enjoyed approximately 35 years of effective control against the disease. This changed in 2004 when the cucumber crops from North Carolina to New Jersey and beyond were destroyed by DM. The disease is now a major problem on cucurbits throughout much of the eastern half of the U.S., especially on cucumber. This led to the re-establishment of cucumber breeding efforts for DM resistance as well as research programs in pathogen biology and epidemiology. These programs are approaching an old problem using new tools.

Chemical control of cucurbit downy mildew: A summary of field experiments in the U.S.

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Phytopathology 99:S171

Cucurbit downy mildew (DM), caused by *Pseudoperonospora cubensis*, is controlled mainly through the use of host plant resistance and the application of fungicides. Currently, host plant resistance must be accompanied by fungicide application in order to achieve adequate levels of disease control. Each year fungicide performance against DM is evaluated under field conditions in several locations throughout the eastern half of the U.S., where the disease is most common. These experiments are generally funded by the agricultural chemicals industry and performed by extension specialists at land-grant universities. A portion of these field evaluations are published as Fungicide and Nematicide Tests (known as Plant Disease Management Reports since 2006). For example, from 1999 to 2007, 121 reports on DM were published from 17 states in the U.S. on 8 cucurbit hosts, 34% of which used cucumber as the host crop. A total of 1440 treatments (mean = 12 treatments/experiment) were evaluated. These reports are representative of the collective experience of industry and academia on chemical disease control, yet there is little attempt to systematically summarize the information they contain to gain a broad view of fungicide performance. Although fungicide performance may vary by location, year and host there is great value in summarizing performance from many experiments. Meta-analysis will be discussed as a tool for this purpose.

Fungicide resistance in cucurbit downy mildew

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Since 2004 downy mildew caused by *Pseudoperonospora cubensis* has become an increasing problem in the production of several cucurbit crops in the United States. The development of resistance to various site-specific fungicide classes has limited the diversity of fungicides available for control of *P. cubensis*. Resistance to the phenylamide class of fungicides (e.g., metalaxyl and mefenoxam) was first reported in Israel in 1980 and in the USA was observed in 1987 in Florida. Phenylamide sensitivity surveys conducted in the USA over the past 3 years have shown that resistant populations have become widespread across the main cucurbit production areas. Resistance has also been reported in *P. cubensis* to QoI (quinone outside inhibitors) fungicides, first in Europe, then in Japan and then in the USA. Recent resistance monitoring surveys in the USA on the QoI fungicides azoxystrobin and pyraclostrobin have shown a high frequency of resistant isolates. In 2007, isolates of *P. cubensis* resistant to the CAA (carboxylic acid amides) fungicides dimethomorph and mandipropamid were detected in the USA. To date, resistance has not been reported to other fungicides with different modes of action currently used to control downy mildew such as zoxamide, fluopicolide, cyazofamid, propamocarb and cymoxanil. The resistance risk for zoxamide, propamocarb and cymoxanil is considered low to medium, for cyazofamid medium to high and for fluopicolide is unknown.

Forecasting long distance movement of *Pseudoperonospora cubensis* and the Cucurbit ipmPIPE

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The dynamics of cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, were analyzed in 22 states in the eastern U.S. in 2008 based on disease reports collected as part of the Cucurbit ipmPIPE downy mildew surveillance program. Independent experiments were also conducted in the summer at Raleigh, NC, to quantify the effects of exposure to solar irradiance on the survival of *P. cubensis* sporangia. The rate of temporal disease progress averaged 1.1 new cases per day and was higher in sentinel plots than in nonsentinel cucurbit plots. Disease cases occurred sporadically before the start of June but increased rapidly thereafter. The median nearest-neighbor distance of spread was about 110 km, with 14% and 20% of the distances being below 40 km and 210 km, respectively. The average rate of disease spread on cucumber was about 7.5 km per day. A linear model, provided a significant (R^2 = 72.1%, $P < 0.0001$) quantitative description of the effect of solar irradiance dose on the percentage of *P. cubensis* sporangia that germinated. Based on this linear function, a solar and UV dose of 23.5 MJ/m^2 and 1.2 MJ/m^2, respectively, will completely inhibit sporangia germination and this dose can be accumulated in as few as 10 to 11 h of exposure. The linear function of sporangia survival and exposure to solar radiation will be incorporated into an aerobiological model to provide cucurbit growers in the U.S. and Canada with more accurate downy mildew forecasts.

Genetic and pathogenic relatedness of *Pseudoperonospora cubensis* and *P. humuli*: Implications for detection and management

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A stable nomenclature for plant pathogens is critical for pathogen identification, development of disease management tactics, and plant quarantine issues. Downy mildew pathogens typically display low morphological complexity and species concepts increasingly rely on inferences of reproductive isolation based on molecular evidence. However, a stable nomenclature for many downy mildew pathogens remains elusive. Recent efforts to clarify phylogenetic and systematic relationships between *Pseudoperonospora cubensis* and *P. humuli* based on sequence data of the internal transcribed spacer (ITS) region of the ribosomal DNA and morphological characteristics have

suggested these pathogens are synonymous. Extensive sequencing of the ITS, cytochrome oxidase cluster, beta-tubulin, and NADH gene regions provided higher resolution of the genetic relatedness of these pathogens, and revealed conserved polymorphisms among the 30 isolates tested that reliably differentiate *P. cubensis* and *P. humuli*, as confirmed by phylogenetic analyses. Host range studies conducted on universally susceptible cucurbit hosts and hop cultivars also demonstrated that these organisms have distinct pathogenic capabilities. Together, these data suggest that biologically relevant characteristics of these pathogens integral for detection and management of downy mildew on hop and cucurbit hosts may be obfuscated by reducing *P. humuli* to the status of a taxonomic synonym of *P. cubensis*.

Epidemiology of downy mildew: A regional and molecular approach
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The occurrence of a downy mildew, incited by *Pseudoperonospora cubensis*, in Michigan represents a significant threat for cucurbit growers in general and cucumber growers in particular, as well as pickle processers and allied industries. To address the immediate short-term needs of the industry, a statewide disease warning system for downy mildew has been established. During the growing season, the atmospheric sporangia concentrations in five Michigan counties are monitored and results posted on a webpage. Growers use this information in conjunction with weather conditions and confirmed disease outbreaks in neighboring regions as a guide for applying fungicides. Fungicide efficacy trials have been conducted in collaboration with cooperators. To develop a successful, sustainable long-term management strategy, molecular characterization of the pathogen is necessary. Using genome sequencing and bioinformatic approaches, we have identified a suite of effector proteins from *P. cubensis*, and have characterized these based on in planta localization, putative function, and in part, contribution to overall pathogen virulence. In total, our analysis has focused on determining the role of RxLR and QxLR effectors from *P. cubensis*. In short, our data suggests an evolutionary divergence, as well as a possible functional divergence, when compared to effectors from other downy mildews. Functional characterization of RxLR and QxLR effectors will be discussed.

Finding an Exotic Pest—What Do I Do Now?

A national perspective of the detection of and response to exotic pests
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APHIS Plant Protection and Quarantine (PPQ) works with Federal agencies, State, tribal and local governments, industries, and stakeholders to implement coordinated actions designed to contain, control, or eradicate invasive plant pests newly introduced into the United States. In addition to biological and ecological considerations, PPQ and cooperators evaluate several other key factors, including environmental, economic, and international trade implications, in selecting the most appropriate response strategies. This presentation summarizes the framework currently being used in response to invasive pest outbreaks in the United States.

The role of the Technical Working Group in new pest detections
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Phytopathology 99:S172

The USDA Animal Plant Health Inspection Service Plant Protection and Quarantine (PPQ) is authorized to respond to plant health emergencies. The Center for Plant Health Science and Technology (CPHST) provides scientific support to PPQ for these plant health emergencies. One of the roles of CPHST is to provide leadership in the development and implementation of Technical Working Groups (TWG) to facilitate the transfer of useful information to regulatory personnel in a timely and efficacious manner. This presentation will describe how TWGs are formed and the role that scientists play in the TWG.

The role of the NPDN in the detection of and response to exotic pests
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The National Plant Diagnostic Network (NPDN) was formed to support and coordinate plant diagnostics among diagnostic laboratories in the U.S. Member laboratories and a network of first detectors serve a passive surveillance role by recognizing newly emerging and unusual outbreaks as revealed by field observation and diagnostic confirmation. The NPDN works closely with the USDA APHIS-PPQ, the Forest Service and state departments of agriculture to establish chain-of-custody and chain-of-communication standard operating procedures (SOP). These procedures cover the processes of confirmation of a suspect regulatory sample and the notification of those results through appropriate channels. The NPDN has collaborated with APHIS in conducting over 60 exercises of the communications SOP in all 50 states. APHIS, USFS, FBI, CIA, state and local law enforcement, and the NPDN also have partnered in full-scale incident command and other types of exercises on the response following a detection. The NPDN plays a critical role in providing triage and surge capacity support, and provides diagnostic expertise during an outbreak of a regulatory or non-regulatory but high consequence plant pest or disease. Critical to the success of the Network is engagement of associated laboratories early in the process to plan for and conduct early detection and accurate diagnoses and to assist in the response and recovery activities.

The detection of and response to exotic pests in Florida
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Florida's agricultural, horticultural and native plant resources are under an ever increasing attack and burden by a diverse array of exotic plant pests. The globalization of exotic plant pests has resulted in an unprecedented invasion that threatens the very core of Florida's plant foundations. The exotic pest challenge has led to the formation and implementation of a substantive array of "weapons" that are wielded by Florida's regulatory agricultural body, the Florida Department of Agriculture and Consumer Services. Many strategies are used by the department to foster early detection of a newly established exotic pest and once found, a much larger process is initiated to deal with the new invader. Key to Florida's success in detection and response are regulatory authority, a well-seasoned work force and response infrastructure, logistical resources, informational outreach and a strong relationship with its stakeholders at the public, private agency and institutional levels. This presentation describes the many facets of the Florida program directed to the ever growing onslaught of exotic pests that have reached its shores.

The detection of and response to exotic pests in California
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Phytopathology 99:S172

Finding an exotic pest in a nursery setting can cause a financial burden on growers, especially if the pest becomes established. This presentation will provide an overview of the role that grower play in the event that a new pest or pathogen is detected.

Finding an exotic pathogen: An industry perspective
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Phytopathology 99:S172

A grower's reaction to finding a new pest is not necessarily the same as a scientist's reaction would be! Will there be a quarantine? Will I lose my entire crop this year? What will my banker say? If I just let this one go, will it really matter? Agriculture wants to be protected from new pests and pathogens ... and is often the front line of defense. What are the options for encouraging industry cooperation and participation in that effort? How can growers and industry be enlisted as collaborators, rather than adversaries? And how can we work better together, to find science-based, yet practical solutions to new (and old) problems?

Methyl Bromide Alternatives Research: Plant Pathology Outcomes

The ozone hole: Anthropogenic sources of methyl bromide and recent data on atmospheric methyl bromide levels

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Phytopathology 99:S173

Methyl bromide (MeBr) is classified as a Class 1 ozone depleting substance (ODS) under the Montreal Protocol on Substances that Deplete the Ozone Layer and the U.S. Clean Air Act and, therefore, use has declined over the past decade. MeBr has been the focus of scientific and political controversy that stems from the role of MeBr in stratospheric ozone depletion and its toxicity to humans, contrasted with its value as an agricultural fumigant. Updated measurements show that anthropogenic ODSs have declined by 12% in the troposphere from their peak values in 1992–1994. This decline is due in large part to the shorter-lived gases such as MeBr. MeBr abundance decreased in the troposphere by over 18% from 1997–2008. This decline is greater than was originally forecasted but is attributed to decreased anthropogenic uses. Whereas tropospheric abundance data are encouraging, stratospheric bromine levels have yet to show a decline. MeBr is responsible for slightly less than half the bromine reaching the stratosphere today and is very efficient in depleting ozone. The Montreal Protocol does seem to be working. Outside of the polar regions, the decline of stratospheric ozone depletion has not continued and the ozone layer has shown some signs of recovery. Atmospheric scientists appreciate the role agricultural scientists have contributed to finding MeBr alternatives in crop production.

Current status of chemical alternative technologies for managing soil borne diseases

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Phytopathology 99:S173

Field research continues to focus on a co-application approach of different fumigants, herbicides, and other alternative tactics to achieve pest control efficacy and crop growth response similar to that of methyl bromide. Different application rates, technologies and grower practices are under evaluation in anticipation of potential risk mitigation tactics to reduce buffer zone distances and personal protective equipment requirements being proposed by EPA fumigant reassessments. Both chisel and drip applied fumigant application technologies are being evaluated in conjunction with high barrier, virtually impermeable mulch films to reduce air emissions and fumigant use rates. Drip fumigation procedures under evaluation continue to focus on factors affecting both water and gas phase movement of the different fumigants in soil, including chemical injection period, fumigant concentration, and numbers of drip tubes per bed. Problems with fumigant application and unsuitable environmental conditions appear to be responsible for significant yield and pest control inconsistencies with alternative fumigants. Treatment costs and returns to investment will likely be important economic considerations determining grower use decisions and transition strategy to the alternative fumigants.

Towards reduced dependence on fumigants for management of Prunus replant problems: Opportunities and challenges

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Phytopathology 99:S173

In California, successive orchard generations of almond, peach and other *Prunus* spp. are subject to replant problems that suppress tree survival, growth, and productivity. Prunus replant disease (PRD; associated with a microbe complex, can affect virtually all of the acreage) and root-parasitic nematodes (RPN; can affect <1/3 of acreage) are the dominant replant problems, and both are managed with preplant soil fumigation. Spot treatment technology using GPS-controlled shanks or buried drip emitters to deliver fumigant to planting sites was developed and controlled PRD with reduced fumigant rates (8 to 15% of broadcast rates). The "spatial economy" of spot treatments is amenable to diverse non-fumigant treatments; spot treatments with fungicides, soil amendments, and steam are being examined. Partial remediation of PRD was achieved with fallowing and crop rotation, but the treatments were much less effective than fumigation. The potential for RPN populations to build along with root systems reduces likelihood that spot treatments can effectively control them. For RPN, reducing dependence on fumigants probably will require improved, broad rootstock resistance to ring, root knot and lesion nematodes integrated with long-term cultural and/or biological strategies for suppressing the pest populations. Advances in microbial ecology will be central to comprehensive understandings of PRD etiology and sustainable management of all replant problems.

Development of a regional transitions program: From discovery research to extension in strawberry production

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Phytopathology 99:S173

Execution of a regional multi-disciplinary MeBr alternatives program generated multi-tiered outcomes. Stakeholders (growers, fumigant applicators and suppliers, county-level extension and other consultants, land-grant specialists and decision makers) were engaged in identification of priority issues and evaluation of alternatives in order to develop an infrastructure of knowledge and experience. Research and extension goals were realized through: Phase I experiments that evaluated new products, methods of applications or novel farming systems on research stations; Phase II projects that were primarily discovery on-farm-research experiments; Phase III projects that were large scale regional on-farm trials. Initial research efforts documented that one of the main production constraints was Black root rot, due to a complex of fungi including diverse *Pythium* species and *Rhizoctonia fragariae* strains belonging to several anastomosis groups. Molecular tools were developed to monitor key pathogens. Alternatives developed included tactic substitutions for growers who sought chemical-based alternatives; tactic diversification for growers who complimented fumigation with IPM tools and tactic development for growers who sought alternative farming systems that were biologically-based including use of cover crops and compost. Technical and economic analysis provided growers with decision tools and regional train-the-trainer programs enhanced extension impacts.

Advances in microbial ecology and farming systems as a replacement for methyl bromide to manage soil borne diseases

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Phytopathology 99:S173

Several unrelated developments have contributed to an undercurrent of renewed interest in biologically based approaches for managing soil borne diseases in crop production systems traditionally reliant upon methyl bromide soil fumigation. From a commodity perspective, difficulties competing in global markets when production costs remain local have led to the examination of value added products such as certified organic or pesticide free. Concomitantly, increased regulatory pressure restricting the application of broad spectrum soil fumigants has raised doubts over their long-term availability. From a scientific perspective, advances in the technology and methods used to delineate microbial communities (genomic fingerprinting) and in the nonparametric multivariate statistical methods used to characterize their community structure and link them to environmental variables have made microbial ecology studies more accessible to applied plant pathologists. From an individual farming system perspective, advances in GPS technology used to mark and track discrete field locations coupled with the increasing use of locally generated organic soil amendments has created new opportunities to implement and monitor field experiments jointly with grower cooperators. Many challenges remain including the linkage of soil microbial community attributes to ecosystem function within the context of plant disease suppression.

Perceptions of Risk, Risk Aversion, and Barriers to Adoption of Decision Support Systems and IPM

A brief history of Plant Disease Risk Assessment: Successes and challenges

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Phytopathology 99:S173

Plant disease epidemiology provides a foundation for developing the management strategies that reduce the impact of plant diseases on global food production. Predicting the risk of plant disease epidemics is a natural application of the knowledge gained through epidemiology. During the past 15 years, more than 54 journal articles have addressed the development and application of disease prediction models. These reports indicate that considerable progress has been made in developing new models, adapting models to new environments, and verifying model performance as part of practical disease management. However, additional efforts are needed to

quantify the impact of risk assessment models on the decision process and odds of making appropriate management decisions. Sustaining the deployment of prediction models remains a significant challenge. Models appear to have a natural life cycle of development, verification, application and atrophy. Given this natural cycle, it seems unlikely that long-term deployment of prediction systems is the best indicator of success. More useful measures of success may be related to the educational objectives of a modeling effort. Using this criterion, the success of a model could be quantified by how effectively it helps decision makers acquire the skills needed to identify useful indicators of disease risk and take appropriate action without the future aid of the model.

Barriers to IPM adoption in developed and developing countries
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Intensive, participatory IPM extension programs seem universally to advance IPM adoption. Quantitative analysis of outcomes including effects on crop yields and the impacts of pesticides on human health and the environment all reveal significant benefits of the IPM programs that farmers adopt. These benefits however rarely amplify to larger scales and rates of IPM adoption may decline over time. Barriers to adoption may include limitations to the up-scaling inherent in current models for extension, low availability and use of monitoring data to reinforce the benefits of adoption, weaknesses in the development and validation of pesticide alternatives and other IPM practices, mass marketing of other models for pest management and economic constraints. This paper will review evidence for a suite of potential barriers and discuss ways forward.

The sociology of uncertainty, risk, and change
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Phytopathology 99:S174

Bayesian decision theory allows subjective probabilities to be used to model individual decision-makers' perceptions of risk. We review how such perceptions are influenced by a range of social factors including, socio-demographic factors, cultural (contextual) factors, associated benefits, psychometric paradigm dimensions of risk, the role of the media, initial impressions, and trust in different sources of information. Specifically among farmers, a range of studies have investigated the factors that influence perceptions of risk and levels of risk aversion, perceptions that influence decision-making on topics such as technology adoption and management practices. Factors which have been found to be important include: farmer age and education, and farm business characteristics such as farm size, ownership structure, gross farm income, solvency, and enterprise type. As a specific case study, we report on a postal questionnaire conducted with barley farmers in Scotland. Two variables were found to be related to whether or not farmers would consider using decision support tools for disease management. These were the region of Scotland in which they were farming and whether or not they expected to pass on the farm to the next generation of their family. We discuss these findings in relation to previous studies of farmer decision-making and the recent interest in Bayesian decision theory in plant disease management.

The cost of making decisions in plant disease management
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Phytopathology 99:S174

In making decisions about possible epidemic outbreaks (or the need for control interventions), there are, at a minimum, two possibilities: either predicting an epidemic or predicting no epidemic. Each decision can be correct or incorrect, which leads to four possible outcomes. With results from a sample of epidemics and non-epidemics, one can thus determine the: true positive proportion (proportion of true epidemics correctly predicted; sensitivity or TPP), true negative proportion (proportion of true non-epidemics correctly predicted; specificity or TNP), false positive proportion (FPP), and false negative proportion (FNP). These proportions, which are estimates of conditional probabilities of correct and incorrect decisions, define the accuracy of a predictor. Given the (fixed) costs of incorrect decisions and the prior probability of an epidemic (estimated, for example, by the overall prevalence of epidemics in a given region), the expected cost of disease prediction (EC) can be estimated. Using a regret graph of Hilden and Glasziou and the skill/value function of Briggs and Ruppert, which are based on EC, one can determine if disease prediction is more or less cost effective as always predicting an epidemic or always predicting a non-epidemic. The skill/value function can further be used to choose the operating point of a predictor (i.e., the threshold of an indicator variable for the risk of an epidemic) which minimizes the cost of using the decision tool.

The challenge of assessing uncertainty and risk in weather-based decision support tools
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Phytopathology 99:S174

Many disease management decision aids rely on weather inputs to calculate an indicator for disease hazard. Uncertainty in the weather inputs may be propagated from raw data through indicator and model calculations to management decision recommendations. Sources of uncertainty in weather data may include sensor placement, time resolution, spatial interpolation and weather forecasting. The extent to which errors in weather inputs affect the quality of the final management outcome depends on a number of aspects of the disease management context, including the use of thresholds in the disease indicator, combined with whether management consists in a single dichotomous decision or in a multi-decision process extending over the cropping season. Quantitative risk analyses used for dichotomous decisions are problematical in multi-decision management, not only because of the perennial difficulty of assigning prior probabilities for disease events, but also because the total management effort (of which the decision aid is a part) is composed of events overlapping in time. In practice the accuracy of the disease hazard indicator is periodically assessed by reference to actual disease development, and management is adjusted for maximum benefit/cost. Effects of uncertainty in weather-based, multi-decision disease management aids will be illustrated with a simulation model for stem rust in ryegrass seed fields, and powdery mildew indices for grape and other perennial crops.

Development of management strategies for ray blight of pyrethrum: A case study of successful diffusion
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Phytopathology 99:S174

Ray blight, caused by *Phoma ligulicola* var. *inoxydablis* is an important disease affecting pyrethrum production in Tasmania, Australia. Since regular and severe spring epidemics were first noted in 1999, research programs have developed and refined management strategies for this disease. Current recommendations for disease management were released to industry in 2002. Currently, over 90% of growers have adopted these recommendations for ray blight management. The recommendations consist of improved seed treatments to reduce seed-borne inoculum, and two to three foliar applications of a suite of fungicides coinciding with initial stem elongation. Key to the successful uptake of these recommendations were on-farm trials clearly demonstrating the benefits of fungicide application. These results were also expressed, following yield comparisons, to growers in financial return per hectare with calculation taking into account the costs associated with the fungicides and their application. Complementary to on-farm demonstration trials was effective communication of these findings using grower workshops, the extensive field extension network of the pyrethrum industry, and informative extension bulletins. All of these secondary tools were directed at the benefits of rapid implementation of management for ray blight as measured by return upon investment (i.e., fungicide application costs).

Primum Non Nocere: Risk Assessment for Biological Control

Beyond efficacy: Challenges in the selection of safe bacterial biological control agents
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Phytopathology 99:S174

The search for new biological control agents often begins with screening *in vitro* for activity against target pathogens, followed by greenhouse or field

assays. Physiological, biochemical, and phylogenetic analyses frequently are not undertaken until much later, after considerable investment already has been made in the candidate agent, and only then are many promising new isolates found to carry "baggage" due to their real or perceived affiliation with known plant or animal pathogens. Here we consider representatives of three complex genera (*Pseudomonas*, *Burkholderia*, and *Bacillus*) and the features that make them attractive as BCAs. We review current phylogenetic relationships within each of these genera and identify features that may prove useful in evaluating risks associated with representative strains.

Minimizing risk associated with applications of microbes for biological control
D. P. Roberts
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Phytopathology 99:S175

Certain effective biological control agents with unique biotechnological traits are also opportunistic human pathogens. These microorganisms typically are producers of antimicrobial metabolites, resistant to multiple antibiotics, and highly competitive and versatile with regard to resources such as nutrients. These traits are desired in biological control agents but also may allow colonization of immunocompromised or otherwise challenged human subjects. Excellent examples come from the *Burkholderia cepacia* complex where isolates with effective disease suppression properties were registered for agricultural use by the EPA and marketed for suppression of plant disease. Subsequent concerns regarding impacts of the use of these strains in agricultural settings led to their removal from the market. Strategies are needed and being developed to harness unique biotechnological traits from these concern organisms for disease suppression while reducing the risk to human health.

***Fusarium* spp. as biocontrol agents**
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Phytopathology 99:S175

Fusarium spp. are among the most effective and consistent biocontrol agents for use against diseases caused by soilborne plant pathogenic fungi, particularly Fusarium wilt diseases. Biocontrol strains of *Fusarium* are presumed nonpathogenic because disease symptoms do not occur on hosts tested. Concerns have been raised about the possibility that biocontrol strains might either be pathogenic on hosts not yet identified or that through widespread application the biocontrol strain might become pathogenic over time. Genetic sequence data and functional genomics provide information for informed decisions about the risks involved in the use of *Fusarium* as a biocontrol agent. Sequence information is providing insight into the relationships among pathogenic, biocontrol and saprophytic strains of *Fusarium*. Functional genomics can identify genes associated with pathogenicity, or with biocontrol ability. Functional genomics also provides detailed information on mechanisms of action of biocontrol agents. Knowledge of mechanisms is useful in assessing risk as well as mitigating risk. Our laboratories recently sequenced 25 N-terminal amino acids of a protein produced by the biocontrol strain *F. oxysporum* CS-20 which elicits resistance in tomato. The sequence contained many basic amino acid residues and is cysteine rich. Use of an elicitor rather than a living organism may eliminate the need to release a living biocontrol agent into the environment.

U.S. EPA's approach to risk assessment and regulation of biopesticides
G. Tomimatsu
Biopesticides and Pollution Prevention Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC
Phytopathology 99:S175

Biopesticides may be distinguished from conventional chemical pesticides by their specificity to target species, natural occurrence, unique modes of action, or low volume use. For the organic farmer, they offer very attractive and environmentally-benign pest control options in IPM strategies. The U.S. pesticide registration process is rigorous and complex; it involves comprehensive review and assessment of science and economic data or information to support federal pre-market approval and periodic evaluations of labeled uses for manufacturers, distributors and users in the agricultural, industrial, structural, municipal, commercial, residential and recreational sectors. The Office of Pesticide Programs in the U.S. Environmental Protection Agency uses the standard tiered risk assessment paradigm of analyzing hazards and exposures to assess pesticidal risks to human health and to the environment. Within this tiered structure, potential risks are determined first from estimates of acute hazard and limited exposures under worst-case scenarios. Studies at the lower tiers are essentially screening tests—simple in design, but broad in scope. Higher tier testing may encompass field testing and sub-chronic or repeated dose exposures. A risk assessment is developed from a synthesis of results from tiered testing, intended uses and the open literature to fulfill data requirements addressing the primary disciplines of product characterization (i.e., microbial product analysis or biochemical product chemistry) and manufacturing, mammalian health and ecological or environmental effects. The presentation will provide an overview of a risk assessment and the registration process for biopesticides, based on over 30 years of regulatory experience.

Improving the risk assessment of beneficial plant pathogens for biological control of weeds: Yellow starthistle and Russian thistle pathogens
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Phytopathology 99:S175

The objectives of risk assessment are to learn about whether a candidate agent would be safe to use in the environment where release is planned, and to present such information in a clear, understandable format to regulators, stakeholders, and the public. Plant pathogens evaluated for biological control of weeds are, by definition both plant pests and pesticides, and for each of these categories there is a unique set of regulations. Risk assessment, therefore, is central to development of plant pathogens for weed management, particularly if the candidate is of foreign origin. Both objectives were achieved when permit was issued in 2003 for the release of *Puccinia jaceae*, a rust fungus collected in Turkey, for biological control of yellow starthistle (*Centaurea solstitialis*), an invasive plant of major importance in the Western U.S. Risk assessment approaches that led to this achievement had been defined over 30 years ago. Recently, statistical and molecular tools have become readily available to scientists in biological control that should facilitate development, analysis, interpretation, and communication of data concerning candidate agent safety. The application of molecular information and the use of mixed model analysis are being applied currently to risk assessments of Russian thistle (*Salsola tragus*) pathogens.

Professionalism/Service/Outreach

Can You Hear Me Now? Expanding Plant Pathology Coverage With Diverse Delivery Tools

Entering the digital world: How to go from being a newbie to an internet maven
J. H. LaFOREST, C. Bargeron, D. Moorhead, G. K. Douce
Center for Invasive Species and Ecosystem Health, University of Georgia, Tifton, GA
Phytopathology 99:S175

For many people, entering the world of computers is intimidating, scary and confusing. This is extremely problematic when a person's job requires them to share information since electronic resources are widely used and frequently requested. Fortunately, there are many resources, helpful groups and patient individuals that can make the entry into the Digital World relatively painless. This talk will highlight the path and some of the helpful tools that will take you from being a rookie on the computer to a wizard on the World Wide Web. It will also provide insight into one of the first steps of developing your online presence. The Bugwood Image Database will be featured along with other resources for sharing and managing images online.

Online outreach: Phytophthora training for nursery growers
J. L. PARKE (1), J. Pscheidt (1), R. Regan (1), J. Hedberg (2), N. Grunwald (3)
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Phytopathology 99:S175

Phytophthora spp. cause some of the most important diseases of nursery crops nationwide. The quarantine pathogen *P. ramorum*, the cause of ramorum blight and sudden oak death, is of special concern. In partnership with the Oregon Department of Agriculture, we created an online course to teach

growers how to reduce the incidence of *Phytophthora* diseases in their nurseries. This free, non-credit course includes three modules that focus on 1) biology, symptoms and diagnosis, 2) disease management, and 3) *Phytophthora ramorum.* Both English and Spanish language versions of the course are available. After completion of the course, nursery growers can take an optional online exam for a fee. If they pass the exam, they earn a certificate of mastery from Oregon State University Extended Campus and qualify for pesticide recertification credits. The course may be accessed at http://ecampus.oregonstate.edu/phytophthora. The development and efficacy of online training courses for grower education will be discussed.

Adobe connect: What can it do for you?
G. Snyder
Dept of Communications, Kansas State University, KS
Phytopathology 99:S176

Save time, travel, and communicate with people across the globe by using a simple Web browser and Webcam. Adobe Connect is a full features on-line video conferencing system. K-State is utilizing Connect for live meetings, research collaboration, eLearning, on-line presentations, seminars (Webinars), and even accredited courses. Connect allows you to use live video, audio, the ability to view PowerPoint presentations, post agendas, share documents, and offers interactive features such as polls, whiteboards and active chat. This session highlights several examples from the K-State Research and Extension Connect system. It will also cover many of Connect's features, offer tips, resources, and ideas on how to get started.

ipmPIPE: Legume PIPE new option for generating, summarizing, and disseminating real-time pest data to stakeholders
H. F. SCHWARTZ (1), M. A. C. Langham (2), S. A. Tolin (3), J. Golod (4), J. LaForest (5), K. F. Cardwell (6)
(1) Colorado State University, Fort Collins, CO; (2) South Dakota State University, Brookings, SD; (3) Virginia Tech, Blacksburg, VA; (4) Pennsylvania State University, University Park, PA; (5) Bugwood Network, University of Georgia, Tifton, GA; (6) USDA-CSREES, Washington, DC
Phytopathology 99:S176

The Integrated Pest Management Pest Information Platform for Extension and Education (ipmPIPE) began as a dynamic, integrated national warning system for soybeans (and other legumes) that would promote efficient and coordinated IPM decision making for Soybean Rust and Soybean Aphid management. In 2007, the Risk Management Agency (RMA) requested that the ipmPIPE help determine causes of disease and insect losses in other legumes including fresh and dry peas and beans, chickpeas, lentils, lima beans, and cowpeas. Thus, the Legume ipmPIPE evolved from the ipmPIPE to address multiple pests on related legume hosts. Specialists from nearly 30 states devised sampling protocols and developed diagnostic assays for legume diseases including viruses in 2007. National mapping of this information on a public website began in 2008. Legume ipmPIPE enhances the role of extension specialists in IPM by providing near real-time access to observations, model output, pest management information, and diagnostic images at http://legume.ipmpipe.org. Communication tools also allow specialists to customize information for dissemination to state and regional crop consultants and growers. The diversity of pathogens, pests and hosts are uniquely suited to demonstrate the value of the ipmPIPE as a "one-stop shop" for legumes where educators and stakeholders can easily obtain information on pathogens and pests identified in a specific area or general region.

eXtension.org - how to use it to deliver your information
T. Meisenbach
eXtension Initiative, Rancho Mirage, CA
Phytopathology 99:S176

eXtension takes the traditional technology transfer role of Cooperative Extension to a new level with an opportunity to reach millions of Americans who use the Internet for information and education every day. Faculty and staff in universities throughout the nation are finding the collaborative opportunities offered by eXtension's wiki environment an excellent base for developing and delivering content on myriad topics from beef cattle to fire ants to consumer horticulture to organic agriculture and more. Multi-state, multi-disciplinary teams or communities of practice work together to create "best of the best" educational resources with science- and research-based content enhanced by Web 2.0 tools. And, America's borders do not limit the reach of eXtension. Nearly 20 percent of all eXtension traffic comes from outside the United States. eXtension provides training for Cooperative Extension faculty and staff and collaborators in new technologies to enhance learning, explores delivering educational experiences through Second Life, an interactive environment finding great success in the corporate training world, and uses Moodle for development of courses for professional development and public consumption. eXtension's Frequently Asked Questions and Ask an Expert features allow consumers to search a database of more than 8,000 peer-reviewed questions and connect directly to experts for more specific answers when needed.

Prepare for your Future: Career Opportunities After Graduate School - Option 1. Industry

Dispelling the myths of working in industry
P. Kuhn
Syngenta Crop Protection, Inc.
Phytopathology 99:S176

Of the career paths open to recent graduates in plant pathology, positions in the private sector may be given less consideration than options in the public sector (academia, USDA, etc.). In part, this may reflect a higher comfort level with the more familiar settings of universities or state/federal laboratories, but probably in many cases also a lack of firsthand knowledge of what is involved in working in industry. The disinclination to consider careers in the private sector may be reinforced by peers and advisers when they present an unfavorable perspective of life on the other side of the fence. To compound this state of affairs, graduates of plant pathology may also associate working in the private sector with a number of myths that are discouraging. This presentation will explore some popular misconceptions, giving candidates a more informed view of what a position or career in the private sector involves and of the associated advantages.

Acquiring the skills to get the job you want
B. Olson
Dow AgroSciences LLC
Phytopathology 99:S176

Graduate and post graduate research in plant pathology provides scientists with the basic understanding of the science and knowledge of plant health, scientific method, experimental design, data interpretation and analysis. In addition to an academic degree there are several non-technical skills and attributes individuals need to develop and demonstrate over the course of their research to ensure successful careers. These attributes are important building blocks to an individuals' personal and professional effectiveness throughout their career. The non-technical skills are transferable from one job or project to another and serve as strong discriminating factors when evaluating candidates from a large pool of well qualified researchers. These attributes are rarely taught or discussed in graduate school and instead are each individual's responsibility to develop. These include: leadership and initiative; creativity and problem solving; communication; research productivity and quality; teamwork; and accountability. Often in industry these attributes are evaluated during the candidate interview process using a tool known as behavioral interviewing. Regardless of the position an individual may have in academia or industry the non-technical skills developed by an individual will be transferable to all of their various roles and responsibilities and will allow them to be effective, productive, and respected scientists.

Putting it together - getting the job you want with the right resume
R. Kaiser
Valent BioSciences Corp.
Phytopathology 99:S176

Landing the job you want in today's economic environment isn't easy, but it can be done with planning and perseverance. Step 1 is a serious examination of your skills and aspirations. Step 2 is developing a marketing plan that sells your unique skills and personality to potential employers. You should list three key strengths and develop supporting evidence (three examples for each strength) that demonstrate how you have produced results using these strengths. Developing your resume' as a short advertisement of your achievements is Step 3. Future employers pay for results, not just knowledge or skills. Your resume should reflect your abilities in leadership, communication and problem solving by giving examples of your achievements. Your employer will teach you any new necessary skills, but in the selection process they look for candidates with a track record of producing results. Steps 4 and 5 are utilizing networking techniques and

preparing for the interview. This presentation will focus on Steps 1–3 and help candidates recognize their strengths and develop strong results-orientated statements.

A year in the life of a field scientist
R. Bounds
Syngenta Crop Protection, Inc.
Phytopathology 99:S177

Industry field scientists are in integral part of research and development efforts to deliver new technologies to agricultural producers. The activities of field scientists, from project planning to experiments to results, will be described from the perspective of an agrochemical industry field scientist.

A year in the life of an agricultural consultant
C. Becker
BAAR Scientific LLC, Romulus, NY
Phytopathology 99:S177

Consulting in agriculture is an increasing employment option. Extensive applied experiences and an advanced degree are the typical requirements for the resume. The diversity of options include pest management, contract research, nutrient management, yield optimization, and marketing. Scouting for pest management requires little capital input, but also generates limited financial returns. Whereas contract research may yield increased income potential, however, it require extensive agricultural equipment, followed by extensive marketing of your companies services. Also personal experiences with contract research have showed extreme diversity with agricultural projects every year. In addition to drawing from education and experiences in the applied sciences, most professionals consulting in agriculture need to understand business and insurance issues, must communicate with a diverse audience, and benefit from efficient management of information. Experiences from 6 years of conducting knowledge based pest management in grapes and conducting contract research on the diverse crops in the Northeastern U.S. will be discussed.

Pathology roles in disease resistance discovery and implementation
P. Himmel
Seminis Vegetable Seeds, a division of Monsanto
Phytopathology 99:S177

'An inoculum cook at the mercy of plant breeders in an environment lacking innovative research' is perhaps the most common misconception of the role a plant pathologist plays in a vegetable seed company. Competition between seed companies that requires strict confidentiality and legal protection of research exacerbates this misconception. Disease resistance is an important component of commercial vegetables and often determines whether or not a variety can be released into key markets. Staying ahead of the evolving disease landscape requires innovation, focus and teamwork between pathologists, plant breeders, geneticists and molecular biologists. Today, there are opportunities in both molecular and applied pathology to discover, develop and deploy new disease resistances and speed deployment of existing resistances. Essential to these diverse pathology roles is the ability to communicate with growers as well as our non pathology colleagues in research, seed production, sales and marketing. A clear understanding of disease resistance helps set realistic performance expectations for the vegetable varieties we sell. A composite of pathology roles and responsibilities in research and seed production will be presented.

Developing your career in industry
L. Fought
Bayer CropScience LP
Phytopathology 99:S177

Obtaining a job in industry marks the beginning of a lifetime of learning and professional development opportunities. The skills developed prior to entering industry serve as the basis for continued growth in an environment that depends greatly on innovation, commitment, and personal standards of excellence. A career in industry may not take a straight path, and there are many opportunities to stretch and strengthen skills and learn new ones. A sampling of the opportunities available for career development in industry will be presented.

Regulation of Pests and Pathogens—Where Are We?

Update on APHIS PPQ 7 CRF 330 regulatory changes
J. L. White, S. Wager-Page
USDA APHIS, Riverdale, MD
Phytopathology 99:S177

USDA, APHIS, Plant Protection and Quarantine is committed to improve customer service for plant pathogen and nematode permitting (form 526). E-Permits, our on-line application process, has improved service significantly by reducing lost paperwork, prompting applicants to reauthorize expiring permits, and electronically copying existing permits for the reauthorization process. Efforts to streamline permits for domestic pests this year has focused on fungi and nematodes. The first widely prevalent fungi list by state was submitted by APS (http://prevalentfungi.org/). PPQ consulted with all States and territories on whether permits for widely prevalent fungi could be expedited (i.e., organisms and conditions are preapproved by the States). Forty-five States agreed to expedite many widely prevalent fungi. These States had agreed previously to expedite certain viruses and bacteria. The list of States participating, the organisms, and the standard permitting conditions are available at http://www.aphis.usda.gov/plant_health/permits/organism/wpp/index.shtml. APS and PPQ expect that the widely prevalent nematode list by State will be completed by the end of 2009 and the expedited list by July 2010. PPQ envisions that changes in epermit software will allow organisms on the expedited list to be fully used without applicant knowledge of the organisms on the list or participating States. PPQ plans to propose these revisions to the plant pests and microbial biocontrol regulations (7CFR330) in the near future. This will address whether certain widely distributed pest or biocontrol agents will require an e-permit.

The APS Public Policy Board: Pulse on Policy Issues

APS and public policy
K. Eversole
Eversole Associates
Phytopathology 99:S177

Public policy impacts virtually all aspects of the diverse scientific enterprise from providing education and training for careers in science to funding research projects to applying results from fundamental research to the field. While many scientists believe that it is difficult if not impossible for them, personally, to have an impact on calls for proposals, regulations, funding decisions, or even Presidential budget requests and Congressional appropriations, exactly the contrary is the reality. One person can make a difference and a group of scientists have a variety of mechanisms by which to influence the policies that affect them. The APS has a diverse tool kit for understanding the interrelationship between public policy and the science of plant pathology as well as for providing advice, suggestions, and assistance to improve the conditions under which scientists must operate. An overview of some of the methods for approaching public policy will be presented along with several examples of how individual scientists can influence public policies that affect the conduct of science.

The APS Early Career Internship
A. Records
Texas A&M University, College Station, TX, USA
Phytopathology 99:S177

The APS Public Policy Board (PPB) Early Career Internship presents a rare opportunity for a graduate student, post-doctoral associate, or junior professional to learn how science policies are shaped. As the second PPB intern, I worked with the PPB for a year, gaining hands-on experience in public policy and learning about APS initiatives. Specifically, I became most involved in the Genomics initiative, because funding for plant and plant-pathogen genome sequencing has had a significant impact on my research and that of so many plant pathologists. The highlight of the internship was the annual governmental outreach meeting in Washington, DC, where I joined the PPB in talks with key policy makers and visits to several federal agencies. I will present an account of my internship experience, with special focus on the meetings in Washington, DC. In addition, I will discuss the impact that the APS PPB internship has had on my professional development and direction.

The future of plant pathology education
J. MacDonald
UC Davis
Phytopathology 99:S178

In a recent survey of plant pathology students, graduate program heads, and employers, concerns surfaced about the shrinking ability of universities to provide students with educational experiences related to the applied or field-oriented aspects of plant pathology. Graduate program heads also expressed concerns about shrinking numbers of students with an interest in studying plant pathology. These two concerns actually extend well beyond plant pathology, having surfaced in virtually every discipline related to the plant sciences…and beyond that to most of the agricultural sciences. Indeed, at the November, 2008 meeting of the American Association of Agricultural Scientific Societies, which represents approximately 50,000 scientists from academia, government and industry, "education concerns" were listed among the groups five priorities for focused attention. For its part, the APS Public Policy Board and the ad hoc Committee on the Future Education of Plant Pathologists, hosted a national workshop on The Future of Education in Plant Pathology and Related Disciplines in March, 2009. This workshop brought together leaders of plant pathology and other plant science-related disciplines, students, and representatives of government and private sector employers. The workshop sought to identify the educational challenges facing our disciplines, what employers need for the future, and strategies for working together to address what is coming to be viewed as a looming crisis in national and international food systems. This presentation will summarize the major outcomes of the workshop and the anticipated next steps.

The culture collection resource of the future
S. Gold
University of Georgia
Phytopathology 99:S178

The PPB has undertaken an initiative, in partnership with the USDA-ARS and USDA-APHIS, to establish a plan for a National Plant Microbial Germplasm System (NPMGS). This is intended to be a culture collection system for the long-term and stable preservation of important plant associated microbial resources. Two workshops to develop this plan will have been carried out by the time of the 2009 APS meeting. Participants in these workshops include scientists from APS, industry, university and government. The first workshop was held in November 2007 and produced an outline document defining the critical components of the system. The second, to be held in January 2009, will focus on perfecting this plan and producing an executive summary document for distribution to policy makers. The second workshop will include international participants involved in major culture collection systems around the world with the intent of gaining from their experiences and determining how best to integrate a U.S. system with other major collections. The structure of the system as currently envisioned involves a central hub repository and a system of taxonomic expert spoke centers. The central repository will capture orphaned collections and store backups from spoke centers. A NPMGS steering committee will be established charged with developing policies regarding (among others) inclusion, distribution and funding for the system. Copies of the NPMGS executive summary plan will be available.

Plant pathology contributions to food safety
J. Barak
University of Wisconsin
Phytopathology 99:S178

Bacterial pathogens, both plant and human, colonize crop plants and can share environmental niches. The colonization of plants by enteric animal pathogens causes a unique public health concern, and recent impacts of fresh produce-linked epidemics of foodborne illness have reached the tens to hundreds of millions of dollars for each industry implicated. Plant pathologists, who bring collective knowledge and experience to unravel the synergy between the plant and human pathogens on plants, have begun to address the biology of these interactions, revealing mechanisms used by the bacteria to attach to and colonize plants, as well as factors that impact the nature of such interactions. An APS-Colorado State University workshop, "Human Pathogens on Plants," in October 2008 served as a stimulating interface for plant pathologists, food safety specialists, and industry and agency representatives to learn about ongoing research in each others' disciplines, to understand the overlapping contributions from each discipline, to discuss and prioritize future research efforts, and to identify ways in which our diverse communities can work together to enhance and direct that research in a meaningful way. Joint efforts planned as outcomes of the workshop will include white papers directed at policy makers and funding agencies, and a future APS workshop to continue these productive multi-disciplinary linkages.

Perspectives from the APS-OSTP Fellow
J. Sherwood
University of Georgia
Phytopathology 99:S178

The Office of Science and Technology Policy (OSTP) is in the Executive Office of the President and serves to advises the President on issues in science and technology on domestic and international affairs; on major policies, plans and programs of the Federal Government; and leads interagency efforts to develop and implement sound science and technology policies and budgets. With support from the Public Policy Board of APS, the APS Foundation and the University of Georgia, Dr. Sherwood had the opportunity to work in OSTP for the six month period of November 2008 through beginning of May 2009. This was a significant time in regards to potential impacts on policy in regards to agricultural research as there was a significant transition in leadership for the Federal Government and in policy as the National Insitute for Food and Agriculture was becoming established, and the Agriculture and Food Research Initiative was implemented under the new Farm Bill. Dr. Sherwood will speak on his experiences and activities during this period, and possible impacts on the field of plant pathology.

Reflections from the Early Career PPB Intern
M. Abril
Louisiana State University
Phytopathology 99:S178

I have had a unique opportunity to serve as the third APS Public Policy Board (PPB) Early Career Intern this year. This experience has allowed me to be involved first hand in issues that have direct impact on current and relevant issues in agriculture at the national level. I have participated in several discussions addressing critical issues that directly impact agriculture through monthly conference calls with my fellow Board members. My first course of action was to become familiar with the current APS initiatives that include Biosecurity, Culture Collections, Education, Food Safety, Industry, Genomics, General Agricultural Funding, and Regulatory Issues. From there, my personal interest let me to join the Education Initiative and to participate actively on a symposium that will assess the current status of plant pathology educational programs nationwide scheduled concurrently with the APS Annual Meeting to be held next year in Portland, OR. I have also been engaged in making decisions about numerous current concerns in the field of plant pathology from the demise of faculty and research positions to Farm Bill Implementation. The APS PPB internship available to APS members (graduate students, post-docs, or members with 10 years of receiving a degree) members within 10 years of receiving their degree offers an invaluable experience to those interested in getting first-hand insight of policy making at the national level. Having the opportunity to be directly involved on the process of decision-making on relevant aspects concerning my field of interest has certainly rendered this experience gratifying and rewarding. The internship has made me aware of the impact to the economy of the country of funding allocations for advances in science and technology. Attention to biotechnical research applied to agriculture should be considered a priority to all parties involved, since it represents an opportunity to improve the nation's resources for generations to come. Furthermore, now I am aware of the crucial role that scientific societies such as APS have with respect to providing leadership to address immediate needs in agriculture that are of a national scope, such as the development of resistant plant cultivars and the training of plant pathologists.

Working With Genetically Engineered Plant Pathogens in the Modern Regulatory Environment

Shipping genetically engineered pathogens
D. Stiger
Federal Aviation Administration, Portland, OR
Phytopathology 99:S178

The presentation will cover an overview of United States Department of Transportation hazardous materials regulations concerning shipping of infectious substances. The new classification criteria and packaging requirements will be covered and then compared to the international standards for shipping of infectious substances by air. In addition, the session will cover the extensive difference between United States transportation regulations and international transportation regulations of Genetically Modified Micro-Organisms (GMMOs) and Genetically Modified Organisms (GMOs).

Culture collections: An important partner in establishing and enforcing regulations on research with genetically engineered plant pathogenic micro-organisms

K. McCluskey
UMKC, Kansas City, MO
Phytopathology 99:S179

Culture collections have a unique perspective on the regulatory requirements for working with genetically engineered micro-organisms. While many researchers never face the questions of how to achieve regulatory compliance, culture collections by their very nature encounter these questions frequently. Because the scientific understanding of genetic manipulations has increased in recent years, regulations have needed to evolve to promote a culture of compliance in academic and commercial research laboratories. As the significant majority of strains in genetic research are now genetically engineered, nearly every researcher will be regulated by government agencies regarding the sharing of research strains. Culture collections can serve to promote both awareness and development of those regulations.

2008 Pacific Division Meeting Abstracts

Abstracts presented at the APS Pacific Division meeting in Jackson Hole, Wyoming, June 25–27, 2008. The abstracts are arranged alphabetically, by first author's name.

Evaluation of a forecast model for tomato powdery mildew (*Leveillula taurica*) in central California

B. J. AEGERTER (4), C. Stoddard (3), M. Le Strange (6), C. Mickler (5), R. Davis (2), C. J. Rivara (1)
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Phytopathology 99:S180

A model for predicting when to apply fungicides was developed by Guzman-Plazola et al. in 1995. In this project, the model was evaluated in commercial fields at ten locations in the San Joaquin Valley during 2006 and 2007. At each location, an automated weather station was placed within the field to record temperature, relative humidity and leaf wetness within the canopy. In replicated plots in each field, the fungicides myclobutanil (Rally) and pyraclostrobin (Cabrio) were rotated in applications timed according to a calendar schedule (14 to 21 day intervals), or according to model recommendations. Control plots received no fungicide applications. At the end of the season, severity of powdery mildew was evaluated in each plot. In 2006, two locations had no powdery mildew, while at five other locations there was a range of disease pressure from low to high. In 2007, there was an epidemic of powdery mildew in the Central Valley and all three field locations had moderate to high disease pressure. Over the ten trials, the calendar treatment averaged four sprays per season, while the model treatment averaged 2.5 sprays. At six of the eight locations where powdery mildew appeared, the calendar and model treatments provided a similar level of control, whereas at the other two locations the calendar treatment provided better control. At selected locations, we deployed a second set of sensors or a second weather station from a different manufacturer. The data suggest that using the model can reduce the number of fungicide applications but may not always provide the same level of control to that of calendar spray applications. Furthermore, we found that the model output is very sensitive to differences in the weather data, such as microclimate differences between nearby fields, sensor placement (in-canopy versus above), and type of weather station.

Screening different Brassica spp. germplasm for resistance to *Rhizoctonia solani* AG-2-1 and AG-8

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Phytopathology 99:S180

Poor stands of canola seedlings in Pacific Northwest (PNW) have been associated with *Rhizoctonia solani* AG-2-1 and AG-8. A total of eighty five genotypes of *Brassica napus*, *B. rapa*, *B. carinata*, *B. juncea* and *Sinapsis alba* were evaluated in the growth chamber for their resistance to both *R. solani* AG-2-1 and AG-8. The percentage of seedling emergence after seven days, survival of seedlings after twenty one days, shoot length, root length and disease severity were used as criteria for evaluation. *R. solani* AG-2-1 was highly pathogenic compared to AG-8. None of these genotypes exhibited complete immunity or complete resistance, but significant differences in susceptibility levels were observed. Three varieties: two hybrids from Germany (Flash and Sitro) and one open pollinated from Dekalb (CWH688), performed significantly better than others.

Effect of Ca on pink rot infection in potato

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Phytophthora erythroseptica causes a disease known as pink rot in potatoes, which is responsible for substantial pre and post harvest tuber loss. Multiple factors such as nutrition, temperature, moisture, pH, cultivar susceptibility, and isolate resistance to fungicides contribute to yield loss. Many nutrients including Ca are associated with plant disease development and severity. The amount of Ca in the soil effects plant health and ability of *P. erythroseptica* to infect the host. Ca can act as a means to prevent infection and understanding the relationship of Ca on *P. erythroseptica* infection is essential to preventing disease outbreak. Russet Norkotah potatoes were grown hydroponically in association with the disease. The degree of infection was assessed by means on quantitative RT PCR. The effect of Ca on disease development was statistically significant at $P = 0.0396$. The pattern between Ca level and infection increased as Ca levels were reduced from 120 µmol to 1 µmol. A substantial decrease in infection was observed at 120 µmol.

Revus and Inspire: New fungicides for disease management

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Phytopathology 99:S180

Revus and Inspire fungicides, with the active ingredients mandipropamid and difenoconazole, respectively, received US federal registration in early 2008. Revus is a carboxylic acid amine (CAA, FRAC group 40) fungicide registered for use in numerous vegetables and grapes for control of late blight and downy mildew diseases. Revus is a reduced-risk product that offers excellent preventive activity by inhibiting spore germination. Difenoconazole is a de-methylation inhibitor (DMI, FRAC group 3) fungicide with current registrations in tomatoes, potatoes, sugarbeets, and apples. Inspire is highly active against powdery mildew, leafspot, and rust pathogens. Premixture products that include difenoconazole are in development for several crops, including vegetables, tree nuts, and grapes.

Residual effectiveness of fungicides in protecting rhododendron leaves from *Phytophthora ramorum*

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Phytopathology 99:S180

Over 20 fungicides have been tested in the last 3 years to determine their residual effectiveness in protecting Rhododendron × 'Nova Zembla' foliage from *P. ramorum*. Following application, leaves were periodically collected from fungicide-treated and untreated container-grown rhododendron plants for up to 16 weeks. Detached leaves were inoculated with suspensions of zoospores from an NA1 lineage rhododendron isolate by pipetting three 10-µl

drops of zoospore suspension onto the lower leaf surface on each side of the leaf midrib. The leaf tissue was injured beneath 3 drops on one side of the leaf midrib using an insect pin. The tissue beneath the drops on the other side of the leaf were left unwounded. Checks included inoculated and non-inoculated leaves from untreated plants that had been sprayed with water. Leaves were then incubated for 7 days at 19°C. Fungicide efficacy was quantified by measuring the areas of the resulting leaf spots using ASSESS. No disease developed on any of the non-inoculated checks. The size of the leaf spots on fungicide-treated leaves was compared to the size of leaf spots that developed on the inoculated check leaves after each inoculation test. Results indicate that residues of some fungicides, such as captan, had very limited residual activity. On the other hand, residues of other fungicides such as cyazofamid significantly reduced disease development up to 12 weeks after application. Overall, the residual effectiveness of fungicides was greater on unwounded leaves.

Graduate A+: A new post-harvest decay control tool for citrus

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Phytopathology 99:S181

Azoxystrobin and fludioxonil are newly registered 'reduced risk' fungicides for the postharvest management of green mold caused by *Penicillium digitatum*, the most important decay of citrus fruit, and some other decays. Both compounds are very effective by themselves against green mold. The high risk of resistance development in the pathogen against single-site mode of action compounds, as has been experienced with all of the older fungicides, however, instigated our evaluation of fungicide mixtures. In experimental packingline studies using inoculated lemon fruit, mixtures at 600 mg/L of each active ingredient were more effective in reducing decay as compared to single-fungicide treatments at 593 and 1000 mg/L, respectively. Decay was reduced from 86% incidence in the control to 0 to 3% in treated fruit. In addition, the mixture was very effective in inhibiting sporulation of the pathogen on infected fruit. This is critical, because decay will less likely spread during the sometimes long-term storage of lemon fruit and additionally, fewer propagules are exposed to resistance selection. A formulated pre-mixture under the brand name Graduate A+ is currently under development with an anticipated federal registration in 2009. This mixture product will represent a highly efficacious alternative in postharvest decay control of citrus fruit and represents an effective anti-resistance management strategy.

Novel delivery IPM tools in real time for decision support - pull

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Phytopathology 99:S181

The delivery of pest models for IPM is evolving rapidly, and should allow users to routinely access the data (pull approaches), or utilize tools that deliver the data to them (push approaches). The IPPC at OSU utilizes a pull approach to deliver a broad set of weather driven products that can be freely accessed by informed decision makers for all agricultural commodities. We have integrated 12,000+ weather stations with very-near real-time data ingest and delivery, dozens of insect, plant disease, weed, and crop models, PRISM based interpolation of temperatures and degree-days for the 48 state coterminous US, and Fox Weather LLC site-specific forecasts for OR, S. WA, and W. ID tied to 1,300+ locations. By combining actual and forecasted weather, we now have prototype virtual weather stations that are being tested to serve as a substitute for real weather stations, and for filling in missing weather data to mitigate data outages. The OSU IPPC system emphasizes end-user responsibility to examine model input data for quality assurance, and to understand which models they might need and how to use them. It serves in part as a test bed for new technologies developed by the Western IPM Weather Workgroup, for NW IPM needs, and for National Plant Diagnostic Network epidemiology needs.

Novel approaches to spatial and temporal estimation of diverse western meteorology

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Phytopathology 99:S181

A main goal of the Western Weather Workgroup is to foster the development of state-of-the-science weather and climate-based systems for agricultural decision making and other societal purposes. The western US, with its spatially complex weather patterns and sparse data density, presents unique challenges to this goal. Building on the unique capabilities of group members, the Western Weather Workgroup has taken novel approaches to the problem of providing accurate and timely simulation and forecasting of meteorological conditions at specific sites. Three approaches, addressing past, present, and future aspects of weather, respectively, are discussed here. The first approach is the recognition that there are repeatable patterns in the spatial complexity of western weather. The effects of terrain and other physiographic features leave an indelible climatological "fingerprint" on day-to-day weather. Therefore, accurate spatial data sets that represent long-term climatic conditions can provide critical guidance in mapping weather conditions, especially where data are sparse. The second approach is the idea that a "virtual weather station" can be created to simulate current meteorological conditions at a site, without the need for an actual observing platform. This requires a spatial climatological base, effective and timely ingestion of meteorological observations, and accurate interpolation of deviations of current observations from climatology. The third approach recognizes that effective planning and decision-making by weather-sensitive segments of society requires accurate and timely site-specific forecasts several days in advance. This requires a forecast system that can ingest coarse-grid forecast model results and downscale them to spatial scales that match the needs of agricultural users. Ideally, the system would have a strong climatological basis, but be sensitive to subtle meteorological patterns that make up today's weather patterns. Examples of each of these three approaches will be presented.

Initial assessment of genotypic diversity of *Phytophthora ramorum* associated with Washington state ornamental nurseries

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Phytopathology 99:S181

Isolates (65 total) of *P. ramorum* were obtained with help of Washington Department of Agriculture from 13 nurseries, two streams, and one landscape situation in Washington state and genotyped using 4 previously developed microsatellite markers (Prospero, 2007). Three previously described lineages, known as EU1, NA1 and NA2, (Ivors, 2007) were detected in Washington nurseries. The NA1 lineage was the most common, occurring in seven retail nurseries, one wholesale nursery, one landscape situation, and both streams. The NA2 lineage was detected in three retail and one wholesale nursery, while the EU1 lineage was detected at a single wholesale nursery. At one nursery, both the NA1 and EU1 lineages were isolated from different branches on the same rhododendron plant. DNA fingerprinting identified six unique genotypes among the NA1 lineage isolates and two unique genotypes among the NA2 lineage isolates. High heterozygosity coupled with the clonal population structure, suggests that *P. ramorum* has not undergone sexual recombination in Washington state nurseries. High levels of genotypic diversity observed at three nurseries and lack of sexual recombination suggests multiple introduction events have occurred at Washington nurseries. The fingerprinting results have also provided some insights to potential sources of inoculum that infested the two streams. In one instance isolates obtained from an infested stream matched isolates associated with an infested nursery on the stream. However, in the other situation the isolate collected from an urban stream had a rare fingerprint and the source of the stream infestation is uncertain.

Increased sustainability of potato and verticillium control with green manures of corn

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Phytopathology 99:S181

Field studies involving 3 years of Russet Burbank potato showed that green manures of corn (*Zea mays* cv. Jubilee sweet corn) were effective for suppressing verticillium wilt caused by *Verticillium dahliae* Kleb. Further studies showed that once a suppressive effect had been established, a green manure treatment for a single season rather than 2–3 years of successive treatments were sufficient to either maintain or re-establish the control of verticillium wilt. Results also showed that with continued use of green manures, yields increased over time even though soilborne inoculum levels of *V. dahliae* had increased by >3-fold from 50 to 150 cfu per g of soil. Similarly, following 3 years of Russet Burbank potato, the wilt incidence became significantly lower even though soil inoculum levels had increased by

>3-fold. Throughout the 3 years of potato cropping, the colonization of *V. dahliae* in potato stems was positively correlated with wilt incidence. Although there were highly significant increases of *Pratylenchus* spp. with the cropping of sweet corn, there was no indication of adverse effects as a result of this. Measurements of microbial activities in soil were shown to be negatively correlated with wilt incidence and *V. dahliae* root infections. When nutritional variables were taken into account, they were shown to be secondary towards relationships of cause and effect for disease suppression. Consistently fallow treatments showed lowest yields and significantly more wilt compared with green manure treatments. All data from this study showed Jubilee sweet corn to contribute positively to the sustainability of Russet Burbank potato while also providing a yearly crop of sweet corn between potato crops.

Relative roles of tuber and soil-borne inoculum in the development of Verticillium wilt in potato

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Verticillium dahliae, the cause of Verticillium wilt (VW), persists for years in soil as microsclerotia and can be carried in the vascular tissue of potato tubers used for seed. The effects of soil-borne and tuber inoculum on VW symptoms were compared in the greenhouse. Naturally-infected and *V. dahliae*-free tubers were grown in *V. dahliae*-infested and non-infested potting mix. Area under the disease progress curves (AUDPC) were calculated from disease severity index ratings and stems and progeny tubers were assayed for *V. dahliae*. Mean AUDPC did not differ for infected and non-infected tubers grown in non-infested soils. Plants from infested soils had higher AUDPC than those from non-infested soil but mean AUDPC did not differ for plants from infected and non-infected tubers grown in infested soils. *V. dahliae* was isolated 30 cm up the stem from 96% of plants from infected and non-infected tubers grown in infested soils and 8% of plants from infected tubers grown in non-infested soil. Mean vertical microsclerotia colonization of stems was 50% total stem height for non-infected and infected tubers in infested soil and 0.5% for the infected tuber/non-infested soil treatment. *V. dahliae* was recovered from 15% of progeny tubers from plants grown in infested soil and 0% of progeny tubers from plants grown in non-infested soil. Tuber infection did not contribute to VW symptoms, indicating that efforts to reduce initial inoculum should focus on reducing the populations of the pathogen in the soil.

Evaluation of Thermal Pest Control technology for insect and disease control and harvest quality parameters in Romaine lettuce

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Phytopathology 99:S182

Thermal Pest Control (TPC) uses a machine with a propane burner to force heated air onto plants as it is pulled through the field by a conventional tractor. Anecdotal evidence from Chile, Argentina, Brazil, New Zealand and Spain suggests that TPC can be substituted for traditional insecticide and fungicide applications for pest control and improves the quality of the harvested crop. In Romaine lettuce, TPC application every 7 days was compared with conventional insecticide and fungicide application and an untreated check for control of aphids and downy mildew and effect on harvest parameters. Harvest parameters included yield, tissue nutrient levels, and taste test. Incidence of downy mildew was low in all treatments on all ratings dates. Aphid populations remained low until just prior to harvest. Nutrient levels were similar among all treatments and there were no treatment differences in consumer preference according to the taste test. This was the first replicated field trial of TPC technology. TPC for pest control and crop quality needs further research prior to drawing well-supported conclusions.

Risk assessment of Verticillium wilt in organic production of strawberry in California

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Phytopathology 99:S182

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a severe problem in organic strawberry production in California. This fungus overwinters in the form of microsclerotia which survive for many years in the soil and serve as a major source of inoculum for subsequent crops. Currently, the threshold for a high risk of Verticillium wilt in strawberry is based on growers' experience and data gathered from other crops such as cotton. However, there has been no specific relationship determined for inoculum level of *V. dahliae* and effects on strawberry. A functional model for rapidly assessing the risk of the disease will allow growers to make informed, science-based decisions in order to minimize losses. To establish the relationship between inoculum and disease level, plots in fumigated commercial strawberry fields were artificially infested with microsclerotia of *V. dahliae*. Plots consisted of ten plants each, with six replications of eight inoculum levels (representing 0, 1, 3, 5, 10, 15, 20 and 30 microsclerotia per gram of soil), repeated at two separate sites. Soil samples were taken from each plot and plated on NP-10 media using the modified Anderson air sampler technique to quantify actual inoculum levels. Each plant was measured (height and width) and rated for disease severity at regular intervals throughout the harvest season to monitor disease progression. Marketable fruit yield was recorded at every harvest. Results show that as few as five *V. dahliae* microsclerotia per gram of soil are enough to cause significantly higher disease severity and have a significant impact on marketable yield, with an average of 24% reduction in yield compared to controls. Inoculum levels as low as three microsclerotia per gram of soil resulted in moderate disease severity, and ten or more microsclerotia per gram of soil resulted in yield losses of 47–63%.

Next steps on the horizon for weather and climate-based decision support systems

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Phytopathology 99:S182

The Western Weather Workgroup is developing the conceptual, research, and implementation framework for weather information systems to support agricultural decision making and other societal purposes. Building on its current momentum, the workgroup seeks to expand its membership to encompass a broader geographic area and multi-disciplinary team. Objectives for the next three years include developing approaches to reduce known sources of uncertainties in weather-driven IPM decision-aids, refining site-specific estimated weather data and forecasts, and establishing linkages with national IPM decision support and biosecurity systems. Within three to six years, the workgroup aims to develop and deliver an operational system with downscaled, canopy-corrected estimated weather data. The system will include weather forecasts and links to pest model outputs. Central to achieving these objectives are distribution of products to IPM personnel and stakeholders, and identification of stable funding for long-term sustainability and system maintenance. Meeting these goals will enable integration of multiple systems nationally across diverse geographical and climatological regions, and provide opportunities for expansion into non-agricultural settings where site-specific weather and climate data currently are absent.

Sampling and detection based management of grapevine powdery mildew

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Phytopathology 99:S182

A polymerase chain reaction (PCR) assay employing species-specific primers was developed to differentiate *Erysiphe necator* from other powdery mildews common in the northwest United States. During field studies, this PCR assay facilitated the detection of *E. necator* inoculum in air samples within hours of sample rod collection and prior to disease onset. The initial PCR detection of *E. necator* of the season occurred during seasonal ascospore releases caused by precipitation events between bud burst and the prebloom period during the three years of the study. Detection ceased for 7 to 11 days following ascospore release and then resumed several days prior to the observance of microscopic symptoms and signs of powdery mildew in the field. Results of this qualitative study were used to initiate fungicide programs in experimental and commercial vineyards and in both cases resulted in a reduction of fungicide usage and significant economic and environmental benefits.

Novel delivery IPM tools in real time for decision support - push

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Phytopathology 99:S182

Washington State University's AgWeatherNet (AWN) is a state-supported network comprised of about 100 regional weather stations and a support staff of 9 professionals. In addition to providing time-sensitive weather observa-

tions and raw data, AWN also has rapidly evolving internal and external value-added product portfolios that include insect models, disease models, water management tools, and cold hardiness information for perennial crops. Included in the external portfolios (powered by AgWeatherNet weather data) are commodity-specific decisions aids (http://das.wsu.edu), integrated production and business management software in production by the private sector (http://wine.tools4ag.net), and proprietary "internal" decision aids in development by the private sector. Both internal and external product portfolios include standard web-based delivery and but also utilize "push" technologies that include automated email and text messaging. A decision aid tool for wine grapes includes MS SharePoint UDAL (user defined alert layer) where the client can choose a variety of value-added products (e.g., mildew warnings, critical temperature, wind speed, or other weather parameter warnings, frost alerts, accumulated ET values, etc.) for delivery via email, text messaging, or synthesized voice at intervals specified by the subscriber.

Metabolites from *Pseudomonas chlororaphis* O6 differentially inhibit growth of *Fusarium graminearum* and *Fusarium oxysporum*
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Phytopathology 99:S183

Pseudomonas chlororaphis O6 suppresses growth of the wilt pathogen, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, and fungi causing wheat and barley scab, *F. graminearum*. Metabolites produced by *P. chlororaphis* O6 that could be involved in fungal suppression include phenazines, hydrogen cyanide, siderophore, and pyrrolnitrin. To determine the effective compounds thin layer chromatographs of ethyl acetate extractions of secreted metabolites were overlaid with the fungi. *F. graminearum* was inhibited by pyrrolnitrin but both phenazines and pyrrolnitrin inhibited *F. oxysporum*. These results were supported by TLC bioassay of the extracts from the *phzA* mutant, which lacked production of phenazines, and the pyrrolnitrin-deficient *prnA* mutant. Production of these antimicrobial agents was sensitive to the nutrients supplied to the bacterium and required functional GacS and RpoS regulation. These factors may in part account for the variability of biocontrol under field conditions.

Control of seedborne *Helminthosporium solani* on specialty potatoes
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Phytopathology 99:S183

Increasingly, silver scurf (SS)-infected seed lots are planted in rain-fed and semi-arid areas of the PNW. To determine whether seed fungicides control SS in contrasting environments, naturally-infected seed of Cascade White (CW), Chieftain (CH) and Yukon Gold (YG) were treated with 5 fungicides (fl oz/cwt) and planted near Mt. Vernon (NWREC) and Hermiston (HAREC). Checks included susceptible Russet Norkotah (RN); no seed treatment (nst); and, SS-free pre-nuclear seed (except YG). Field plots were planted in May in a split plot design with 4 reps, and maintained for 10 wk by typical practices. For each cultivar, emergence and yield among treatments was more uniform at NWREC (91–100%; 2.0–2.7 lb/plant) than at HAREC (49–97%; 3.5–6.0 lb/plant) despite planting the same seed lots. Following harvest, SS severity on NWREC tubers (2–34% surface w/lesions) was significantly reduced relative to nst for: Dynasty (.38) and Dynasty+Maxim (.38+.08 of D+M) on CW; all except Mertect (.021) on RN; and Maxim (.16), Dynasty, and D+M on YG. SS severity on HAREC tubers (0–3.3%) was significantly reduced due to Dynasty and D+M on RN, and for all except Maxim (.8) on YG. SS incidence in pre-nuclear seed treatments was unexpectedly high at NWREC (68–99% sporulating tubers) but low at HAREC (1–5%), suggesting infection in the field before harvest.

Survival of *Colletotrichum acutatum* on common strawberry nursery cover crops
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Phytopathology 99:S183

Strawberry anthracnose is a serious disease caused by *Colletotrichum acutatum*. Nursery transplants can carry inoculum and new transplants have developed symptoms in fruiting fields. At what point this fungal pathogen enters the nursery production system, and from what source, remain unclear. Volunteer strawberry plants in nursery rotation fields have been identified as sources of inoculum for infections occurring in adjacent fields. In addition, the rotation crop itself may provide a suitable substrate for survival and sporulation of the fungus, thus providing another source of inoculum. To better understand the disease cycle of *C. acutatum* on strawberry in California, common nursery cover crops including Austrian winter pea, bell bean, clover, hairy vetch, Merced rye, oats, Sudan grass, triticale, and wheat were evaluated for their role in the epidemiology of the disease. Additional species representing ten plant families were evaluated for their potential use as novel rotation crops. Strawberry (cv. 'Albion') was included as a positive control. Plants were inoculated with conidial suspensions in growth chambers. Isolations and sporulation assays showed the fungus is able to asymptomatically colonize all plant species tested, remain viable in dry plant debris for at least one month, and sporulate upon exposure to moisture, with significant differences among plant species. *C. acutatum* can be re-isolated from at least 80% of some crop tissues (e.g. sunn hemp, potato and wheat); while onion (13.2%), corn (16.7%), and bell bean (24.3%) are more difficult to re-isolate from. Sporulation assay results indicate the fungus can produce at least 7×10^4 conidia/mg debris (dry weight) on plant species such as basil during one week at 100% RH. On other species, like tomato, significantly less sporulation was detected. These results suggest risk of *C. acutatum* inoculum propagation may be reduced in rotation fields by planting less-susceptible crops.

Strategies for controlling macadamia quick decline
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Phytopathology 99:S183

Quick decline of macadamia (*Macadamia integrifolia*) trees continues to be a serious problem in Hawaii. Initial signs and symptoms include bleeding, the presence of Ambrosia beetles and orange fruiting bodies of *Nectria* sp. followed by yellowing and browning of leaves within the tree canopy. Isolations from diseased branches have yielded *Phytophthora capsici*. A zoospore suspension of *P. capsici* was injected into branches of cultivar "HAES 344" to incite macadamia quick decline (MQD) signs and symptoms. Two branches showed for the first time MQD bleeding, powder posts and *Nectria* in an inoculation proving Koch's postulates and also indicating that *P. capsici* may be the primary causal agent. Control methods for MQD with the fungicide Fosphite were investigated on the cultivar "HAES 333". Soil drenching proved ineffective and was abandoned for a trunk injection method for delivering the fungicide. Initially, a passive trunk injection method was utilized. A 10 ml syringe containing concentrated Fosphite was used to dribble the fungicide into a 1½" deep hole that was drilled at a downward angle into the trunk of the tree. Unfortunately, only 25% to 50% of the label rate was applied. A more efficient and reproducible method utilized a pressurized injection system (Arborjet Tree I.V. System). With this system, the entire amount of fungicide was delivered into 12 trees at the label rate. The Fosphite control vs. no control field was monitored every two weeks for signs of MQD. MQD trees with no control died within an average of 205 days or 6.8 months after first signs of infection. Fosphite-treated trees have survived thus far for an average of 614 days. With treatment, trees to date have lived an additional 409 days. The trunk injection method may provide the macadamia industry a well needed control method for MQD.

Quantitative real-time PCR detects and quantifies colonization activity of *Trichoderma* spp.
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Trichoderma spp. parasitize sclerotia and are potential biocontrol agents of *Sclerotinia sclerotiorum*. Traditional methods (plating, microscopy) to quantify growth and colonization by *Trichoderma* spp. in natural environments are labor-intensive, with limited resolution. Our objective was to develop quantitative real time PCR methods to detect and measure colonization of sclerotia by *Trichoderma*. Specific PCR primer/probe sets were developed for *Trichoderma* spp. and *S. sclerotiorum*. A total of 180 ITS1 (internal transcribed spacer) and ITS2 sequences from different *Trichoderma* species were aligned, and consensus sequences determined. Six candidate primer sets were based on conserved regions, and the specificity of each nucleotide sequence was examined using BLAST (Basic Local Alignment Search Tool). Primer sets were tested on genomic DNA of *T. harzianum* strain ThzID1-M3, six *Trichoderma* isolates from soil, and genomic DNA of *S. sclerotiorum*. The optimum primer/probe set (TGP4) successfully amplified genomic DNA of all *Trichoderma* isolates tested, showing high precision and reproducibility over a range of 8 orders of magnitude (85 ng - 8.5 fg of genomic DNA). TGP4 did not amplify *S. sclerotiorum* DNA. PCR primer/probe set TMSCL2 was developed for *S. sclerotiorum*, based on the calmodulin gene sequence. TMSCL2 did not amplify *Trichoderma* DNA. Quantitative real-time PCR with these primers was used in experiments to evaluate effects of two soil moisture levels (–50 kPa, –1000 kPa matric potential) on colonization of *S. sclerotiorum* by indigenous *Trichoderma* spp. Periodically over 40 days, *Trichoderma* and *S. sclerotiorum* DNA in sclerotia were quantified by PCR with appropriate primers. More than 90% of sclerotia were colonized by indigenous *Trichoderma* at 1000 kPa, vs. 60% at 50 kPa. Real-time PCR allowed measurement of the extent of colonization, which was significantly greater in the drier soil. This method provides a sensitive

detection and measurement tool to evaluate colonization of sclerotia by *Trichoderma* spp.

Downy mildew resistance in four breeding lines of quinoa
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Quinoa (*Chenopodium quinoa* Willd.) is a staple crop in the Andean highlands of South America exhibiting a high nutritional content and drought tolerance. The most significant disease of quinoa is downy mildew caused by the pathogen *Peronospora farinosa* f. sp. *chenopodii* Byford. It is endemic to Bolivia, Colombia, Ecuador, and Peru, and decreases yields by 33–58% and up to 99% in certain genotypes. The most effective means of managing downy mildew is through the development of resistant cultivars, as fungicides are expensive and difficult to obtain for subsistence quinoa growers. Genotypes KU2, 0654, NL6 and Chucapaca were grown under greenhouse conditions, inoculated and evaluated for disease resistance in a growth chamber. Genotypes 0654 and NL6 showed moderate levels of resistance, while Chucapaca showed high levels of susceptibility. Pathogen movement through host tissue of resistant and susceptible genotypes was also observed using scanning electron microscopy (SEM). Presence of the mildew was verified with amplification of the ITS region using PCR. SEM observation showed that sporangiophores create an appressorium, enter through stomata, colonize tissue intercellularly, and sporulate through stomata.

Overview of the Western IPM Weather Workgroup - Diverse collaboration to meet challenges
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The Western IPM Weather Workgroup is a dynamic group of climatologist, meteorologists, entomologist, and plant pathologists from both the private and public sector who are actively engaged in the development and delivery of management tools that utilize weather and forecast data. The group grew from numerous individual efforts into a collaborative team due in part to funding from the CSREES Western IPM Program on workgroups. The group's mission is to improve crop management decision-making abilities by developing new approaches to access, synthesize, distribute, and use weather data. There is an emphasis on integrating climatological and weather information to produce estimates of current and forecasted weather, and on approaches for delivering the information to decision-makers. In pursuit of this mission the group has identified impediments and potential solutions to the use of IPM tools reliant on access to weather data. We have sought funding for and initiated several research projects to test new approaches for spatializing weather and forecast data, with the goal of developing the methods for delivering estimated, site-specific weather and forecast data with a resolution of one km or less. We welcome collaborations with anyone having similar interests.

Virus complexes: Unraveling the mess and implications in disease management
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Recent work with virus diseases of strawberries, raspberries and blackberries have shown that in most cases diseased plants in the field are infected with more than one virus and that many 'severe' strains of viruses in these crops are actually due to mixed infections. In these complexes, there are usually several viruses that are critical for disease development and there may be several that are not important in the disease. It is necessary to identify the viruses in the complex that are critical for the disease to develop. At least 13 viruses have been identified in blackberry plants exhibiting yellow vein symptoms and dieback. However, in any one area there is only a subset of these viruses present and a single virus, Blackberry yellow vein associated virus is always present. However, BYVaV is symptomless in single infections, as are many of the other viruses in the complex. The same is true for strawberries showing symptoms of decline, different complexes in different areas and most viruses symptomless in single infections. The key to control is to identify the viruses in the complex that contribute to the disease in the field and then determine which is the easiest to manage in that region. Virus management requires knowing the vector for each of the important viruses, something about the biology and ecology of the vector and the sources of inoculum for each of the viruses. Vector management is key for control of viruses in perennial crops. The application of molecular detection methods to the production of virus-free plants and to virus management in nurseries and fruit production fields will be discussed.

Effect of rootstock and lemon variety on development of brown wood rot by *Antrodia sinuosa*
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In 1992 and 1997 respectively, *Coniophora eremophila* and *Antrodia sinuosa* were first reported to be associated with a brown heartwood rot occurring on lemon trees in Yuma, Arizona. Subsequent research revealed that the optimum temperature range for growth of both wood-rotting fungi was 30–35°C and development of wood decay columns was greatest from May through October. Also, the rate of wood decay caused by *A. sinuosa* was significantly higher than that caused by *C. eremophila*. In recent years, virtually all new occurrences of brown wood rot on lemon trees in Yuma are caused by *A. sinuosa*. To evaluate the possible effect of rootstock and lemon variety on the rate of development of brown wood rot caused by *A. sinuosa*, a planting of five different lemon varieties (Corona Foothills, Eureka, Frost, Limoneira 8A, and Prior) was established on three different rootstocks (*Citrus jambhiri*, *C. macrophylla* and *C. volkameriana*) in 2003. In July of 2006 and 2007, branches on these trees approximately 3 to 4 cm in diameter were inoculated by inserting a small wooden dowel colonized with the fungus into a hole drilled into each test branch. Inoculated branches were removed eight months later and the length of wood decay columns were measured. For all lemon varieties on *C. macrophylla* rootstock, the mean length of wood decay columns in the 2006 and 2007 trials was 12.7 and 9.2 cm respectively. These values were significantly greater than those recorded for lemon varieties on *Citrus jambhiri* (6.4 and 6.6 cm) and *C. volkameriana* (8.3 and 6.3 cm) during the same respective years. There were no consistent differences among the different lemon varieties in the rate of brown wood rot development in these two trials.

Examining the association between cold therapy of Pierce's disease-infected grapevines and viability of cultured *Xylella fastidiosa* cells *in vitro*
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Pierce's Disease (PD)-infected vines can be cured of infection after exposure to cold temperatures; therefore PD does not occur in colder regions of North America. To better understand this phenomenon, PD disease severity, curing rates and biochemical changes in Pinot Noir (PN) and Cabernet Sauvignon (CS) grapevines grown in 4 field locations and in 4 cold chamber temperatures were compared to the viability of *Xylella fastidiosa* (Xf) cells cultured *in vitro*. Xf viability was evaluated in water, grapevine xylem sap, various media and buffers at different pH values. Xf cell suspensions were exposed to various temperatures between –20°C and 28°C. Viable colony forming units were counted daily for one week to determine the effect of each temperature treatment. Xf survival was best in PD3 medium compared to other buffers at temperatures between –5°C and 28°C. No culturable Xf was recovered from any of the media, buffers, xylem saps or water after 24 hours at –10°C or at –20°C. Differences between Xf viability *en planta* and *in vitro* were observed. PD severity was lowest and curing rates were highest for infected vines exposed to the coldest temperatures. Differences in Xf survival between the field and in culture suggest that cold curing is impacted by the physiology of the grapevine. The field collected xylem sap varied in pH from 5.5–6.2, whereas the buffer experiments show that Xf survives best at pH of 6.6–6.8. Osmolarity of PD3 media, used to grow Xf, is 113 mmol/kg, whereas the osmolarity of extracted xylem sap is 25–45 mmol/kg. Trends will be discussed as they relate to potential factors that mediate cold therapy.

Characterization of resistance to powdery mildew in grapevine progeny
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The progeny of three sources of resistance to powdery mildew (PM), all backcrosses to *Vitis vinifera* were evaluated: *Vitis romanetii*; 2) *Muscadinia rotundifolia*; and 3) *Vitis aestivalis*. We evaluated PM prevalence and severity among segregants in the greenhouse and microscopically examined the resistance reactions. The progeny population of the *V. romanetii* backcross segregated into two main groups: highly resistant or highly susceptible to PM. The progeny of the *V. aestivalis* backcross and those of *M. rotundifolia* backcross segregated into many categories of resistance. Histological examination included segregants with various degrees of susceptibility to PM.

Hydrogen peroxide (H_2O_2) accumulation, which is often associated with a signaling role in the cell to initiate secondary defense mechanisms including hypersensitive cell death, was evaluated 48 h after inoculation. Positive H_2O_2 reactions were verified by diaminobenzidine stain. We identified three types of H_2O_2 reactions in each segregant population: 1) localized (reaction was present in a small area just below appressorium); 2) moderate reaction (reaction extended beyond the appressorium area); and 3) whole-cell reaction (100% of the cell area was stained, often with several surrounding cells affected). Whole-cell reaction, which is the symptom of hypersensitive cell death, was rare and was identified only in a few cases in PM-susceptible and PM-resistant segregants. Spearman's rho coefficient showed no correlation between PM susceptibility in the greenhouse and positive H_2O_2 reactions, suggesting that in evaluated populations resistance to PM is conferred by some other mechanism.

Temporal and spatial effects of long-term floor management on the bacterial and nematode communities in a Salinas Valley, California grape vineyard

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California grape producers traditionally manage vineyard floor vegetation to control weeds and vine vigor, and prevent erosion. The attitude towards vineyard floor management of some has changed from one of preventing disease and weeds to promoting plant and soil-borne microbial diversity. In the final year of a long term vineyard floor management field study, we examined both the bacterial and nematode communities present in the row middles and berms across six different floor management strategies. Samples were assayed for total bacterial and nematode populations in addition to identifying individual members of these communities. An analysis of the culturable bacteria community found weed management and cover cropping practices had no significant quantitative effect on bacterial populations. Qualitatively, however, bacterial populations were altered as a function of cover crop and weed management practices. The grapevine rhizosphere bacterial populations were greater than populations in the row middles bulk soil during the grape root flush in the spring. During harvest and dormancy, bulk soil bacterial populations were greater in the rows than on the berm ($P < 0.05$). The nematode community in the row middles was significantly affected by both weed management and cover crop practices. In the berm, nematodes were not affected quantitatively or qualitatively under the six management regimes. Moreover, the nematode community of the berm was different from that found in the row. These data suggest changes in the microbial community in the row do not influence the microbial community of the berm where the roots of the vine are concentrated.

Differences in plant defense gene expression during Fusarium crown rot infection in susceptible and partially-resistant wheat seedlings

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Fusarium crown rot caused by *F. culmorum* remains a constant problem for dry land wheat production in Southeastern Idaho. We designed a laboratory study to investigate the genes involved in plant defense against *F. culmorum* infection in wheat seedlings of the partially-resistant Australian line '2-49' and the susceptible wheat variety 'Puseas'. A transcriptional analysis of both genotypes at 10 days post-inoculation with *F. culmorum* was done using the Affymetrix wheat gene chip. Five plant defense genes with differences in expression were chosen for further characterization at 1, 5, and 10 days post-inoculation using real-time quantitative RT-PCR. One–way ANOVAs were used to test pair-wise comparisons between 2-49 and Puseas for inoculated and non-inoculated treatments at each time point for each gene. The real-time quantitative RT-PCR analysis of plant defense genes chitinase 1, oxalate oxidase, WIR 1, stress-response protein, and xylosyltransferase showed that induction of chitinase 1 and WIR 1 occurred in the inoculated treatment of Puseas at 10 days post-inoculation. Oxalate oxidase was expressed at a higher overall level in 2-49 than in Puseas. Stress-response protein was expressed at a higher overall level in Puseas than 2-49. Xylosyltransferase showed significant induction in the inoculated treatment of 2-49 at 5 days post-inoculation. The differences in expression between the two genotypes showed that resistant and susceptible wheat seedlings have distinctly different genetic defense responses to *F. culmorum* infection. Further characterization of these genes and the pathways that they are involved in are needed to understand the mechanism of partial seedling resistance in wheat.

Uncertainties in modeling and weather estimation - Conceptually unique case studies

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The output of a pest model decision aid is based on an interconnected chain of estimates and calculations, from weather element values through model inputs to model performance. Error, or uncertainty, in each step of the process affects the uncertainty of the final output. In order to prioritize efforts at improving accuracy of the final output, it is useful to know which steps are currently most in need of improvement, and how sensitive the final output is to error at each step. In field studies to partition this uncertainty, weather elements (e.g., temperature, leaf wetness) were measured at field locations in several crops and used to run various types of disease management models (e.g., simulation, disease warning index). We compared model outputs to those obtained by using estimated (rather than actual) weather data as model inputs, and compared both to observed levels of disease in the fields. In initial trials in 2007 we noted that temperature (e.g., daily max, min, average) was well portrayed by the weather estimation procedures (monthly average error < 1C), but moisture (e.g., precipitation and leaf wetness) estimates were subject to greater error. Weather estimation errors also were greater in some months of the year than others. Estimation of canopy-level weather elements (used for disease model inputs) from standard-height measurements was another source of error whose magnitude was affected by time of year. Effects of these errors on the management recommendations produced by disease models differed with the type of model. Uncertainties estimates and model performance for a stem rust simulation model and powdery mildew risk index are presented and discussed as conceptually unique case studies.

"Following the genes that make resistant plants: Shared tools for breeding and pathology"

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Although plant pathology and breeding are distinct disciplines with unique perspectives, they frequently share a common goal: that of identifying and understanding durable resistance, the kind of resistance that will not be overcome quickly and will remain effective against a wide array of isolates. While pathologists strive to discover the sources of resistance, it is the breeder's function to deploy and make them useful to the agricultural community. This function has become of paramount importance for improving the productivity and sustainability of agriculture and reducing its environmental impact. Two of the main challenges breeders face are time and diversity. Crop variety development is a lengthy process; it may take up to 20 years to introgress a single gene into a commercial variety. The limited durability of most R-genes makes it even more important to identify and deploy new sources of resistance rapidly. Pathogen diversity also plays a major role in plant breeding. In pathosystems with a wide array of isolates, or subject to fast pathogen evolution, the deployment of single resistance genes may not be as functional as the use of multilines or pyramiding genes. The identification of the correct phenotypes is crucial during the breeding process, but it can be difficult and time consuming, especially when desirable and detrimental genes are linked. However, the use of molecular markers such as AFLPs, RFLPs, SSRs and SNPs can accelerate the process of surveying the genome for the correct array of resistance genes in a breeding progeny, making it more efficient than the traditional method of inoculation for phenotyping and substantially shortening the breeding time. Molecular markers have become increasingly popular in the search for major R-genes, QTLs and even those genes involved in resistance pathways. To date, a wide array of major R-genes have been mapped, characterized and cloned. Structural similarities between R-genes have allowed for identification of resistance clusters, making it easier to recognize areas of the genome desirable for breeding. This, in turn, has the potential to enhance the durability of resistance as it has been shown that linked R-genes tend to act synergistically. Furthermore, when resistance clusters are mapped in model pathosystems, synthenic areas can reveal the location of resistance in related species, giving clues of chromosomal segments that may be important to explore for breeding.

Ringspot leaf symptoms on *Sorbus scopulina* Greene associated with virus-like particles

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Sorbus scopulina Greene (Greene's mountain ash), is a native shrub of Alaska that is widely distributed in south central Alaska in natural ecosystems and as an ornamental in public and residential landscapes. In spring, emerging leaves

frequently contain noticeable chlorotic ring spots, vein-clearing that often develop into oak-leaf patterns. Erinea and gall forming eriophyid mites were often present on leaves of affected plants. Ultra-thin sections of leaf mesophyll revealed single to several spherical structures (average diameter 50 nm) enclosed within a membrane. Amorphous inclusions similar to viroplasm-like structures were readily visible. Although particles were not detected from purified "virus" preparations, a prominent protein ~32 kDA was consistently present from only symptomatic leaves. The 32 kDa protein did not have a serological affinity with universal potyvirus antiserum or with European mountain ash ringspot-associated virus antiserum on western blots. Double-stranded RNA and "virion" RNA extraction procedures did not result in distinct bands on agarose gels, and may have been precluded by interfering leaf polysaccarides and tannins. Polymerase chain reaction using universal potyvirus primers and cDNA from total RNA extracts did not generate products with distinct bands. Symptoms and the associated 32 kDa protein were not detected on the following plants that had been inoculated with sap from affected leaves: *Sorbus scopulina*, *S. aucuparia*, *Nicotiana benthamiana*, *Chenopodium amaranticolor*, and *C. quinoa*. The presence of a 32 kDa protein, membrane bound spherical particles, chlorotic leaf symptoms, an association with eriophyid mites, and difficulties with nucleic acid identity, suggests that the causal agent of diseased *S. scopulina* Greene is similar to a group of unclassified tentative viruses with a unique morphology of membrane bound particles.

Application of real-time PCR for quantification of soilborne pathogens

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Soilborne pathogens can be particularly difficult to quantify. Unlike foliar diseases, symptoms caused by soilborne pathogens such as *Pythium* and *Rhizoctonia* spp. are not readily observable, making it difficult to estimate pathogen populations. *Pythium* and *Rhizoctonia* present an additional problem in cereal production systems. Rather than the diseases being caused by a single species of each genus, multiple species may be present in the same field and even on the same plant. In eastern Washington, the species prevalence and diversity of each of these pathogens can vary greatly from one region to another. Due to limitations of traditional agar media-based quantification methods, real-time PCR assays were developed for multiple species of *Pythium* and *Rhizoctonia*. Soils were collected over a large geographic region of eastern Washington in 2005, 2006, and 2007. Total DNA was extracted from these soils and species-specific primers for three species of *Rhizoctonia* and three to nine species of *Pythium* were used with a Roche LightCycler to quantify pathogen DNA in these soils. The prevalence of Pythium species is favored by higher precipitation zones. The diversity can also very greatly with as many as nine or as few as one species being detected in a single soil sample. Conversely, *R. solani* AG-8 is quantified in low amounts in the higher precipitation zones and favors areas with less than 300 mm of annual precipitation. *Rhizoctonia oryzae* is less affected by precipitation, being prevalent in most regions. This work has also revealed correlations between the presence of certain species of these necrotrophic root pathogens with specific host plants. For example, *R. solani* AG-2-1 is favored by rotations with brassica crops. Using these real-time PCR assays, disease risk models are being created to develop this procedure into a preplant tool for improved disease management.

Effect of 1-Methylcyclopropene (1-MCP) on reducing postharvest decay of tomatoes

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1-Methylcyclopropene (1-MCP), which is marketed as SmartFresh™ technology, is an ethylene antagonist and has been shown to delay ripening in tomatoes. We evaluated 1-MCP for its efficacy at reducing tomato decay caused by *Alternaria alternata*, *Botrytis cinerea*, and *Fusarium* spp. on tomato cvs Quality 33 and Seminis 35. 1-MCP was applied at two rates (600 ppb for 12 h and 1000 ppb for 6 h) on tomatoes with or without artificial inoculation of *Alternaria alternata* and *Botrytis cinerea*. Fusarium rot occurred from natural inoculum. Postharvest decay was evaluated in 1-MCP treated and untreated tomatoes at 7–10 day interval for 31 to 42 days during storage at 15°C. The test was repeated twice on each cultivar at green or pink developmental stages. No differences existed in cumulative disease incidence or severity between treatments of 600 ppb and 1000 ppb 1-MCP, with or without inoculation of the pathogens. Cumulative incidence and severity of decay in 1-MCP treated tomatoes was significantly reduced compared to that of the corresponding controls for both cultivars. Disease incidence and severity of individual diseases in 1-MCP treated fruits was also significantly reduced compared to that of the untreated controls, except in one inoculated test where severity of Alternaria rot in 1000 ppb treated fruits were significantly higher after extended storage of 42 days, while incidence was significantly lower than its corresponding control. In two tests, either inoculated or non-inoculated, incidence of Alternaria rot was significantly higher in 1000 ppb treated fruits than that treated with 600 ppb. Our results indicate that 1-MCP can reduce postharvest decay in tomatoes.

Detection of *Xanthomonas hortorum* pv. *carotae* on and in carrot with loop-mediated isothermal amplification (LAMP)

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Detection of chromosomal DNA of *Xanthomonas hortorum* pv. *carotae* (*Xhc*) by a loop-mediated isothermal amplification protocol (LAMP) was evaluated in laboratory and greenhouse assays on carrot plants and seed. LAMP amplifies target DNA rapidly (1 hour), isothermally (65°C), and with high-specificity based on four primers designed to recognize six independent sequences of target DNA. A positive reaction results in a cloudy white precipitate of magnesium pyrophosphate in a PCR tube. With whole cell suspensions, our LAMP protocol had a detection limit of 5 to 25 colony forming units, which is similar to the sensitivity of nested PCR. The LAMP primers did not react with suspensions of other bacteria obtained from seeds or whole carrot plants at densities ranging from 10^3 to 10^7 CFU/g. In experiments with whole carrot plants, inoculation with a rifampicin resistant selection of *Xhc* at 3×10^3 CFU/ml resulted in positive detection over a 4 week sampling period from symptomless plants. LAMP reactions on extracted DNA from commercial seed sample washes resulted in 100% detection for infested seed (ranging from 10^4 to 10^6 CFU/g) and 0% detection on seed not infested with *Xhc*. Overall, detection of *Xhc* on plants or carrot seeds using LAMP technology has the potential for enhancing management of this disease in the field.

Fremont cottonwood dieback in California caused by *Cryptosphaeria* species

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In 2005, we detected severe limb and twig dieback of Fremont cottonwood trees (*Populus fremontii*) in several counties in California including Napa, Sonoma, Solano, Merced, Sacramento, Yolo, Stanislaus and El Dorado. Symptoms in the wood consisted of brown discoloration and decay in both sapwood and heartwood. Symptoms were often associated with the presence of fungal fruiting bodies originating from the surface of dead bark. Occasionally the disease or the fungus was observed on additional cottonwood species including *P. nigra* and *P. deltoides*. Isolations from cankers and preliminary diagnostic work allowed us to recognize a new *Cryptosphaeria* species associated with the disease. Perithecia of this fungus were typical of Diatrypaceae, ascospores were brown, slightly curved, 12–16(–18) × 4.5–5 µm. Colonies had irregular margins, slow growth and color varied from orange to yellow on PDA. The anamorph resembled the form-genus *Cytospora* Ehrenb.:Fr. However, California isolates could be distinguished from the formerly documented *C. chrysosperma* by having longer conidia, (7.5–)10–16(–18) × 2–2.5 µm and representing a separate phylogenetic lineage. Phylogenetic analyses using ITS region showed that most *Cryptosphaeria* isolates from California formed a unique clade, which separated from all previously described *Cryptosphaeria* spp. Results of one year pathogenicity test showed that each of two *Cryptosphaeria* spp. tested were pathogenic to Fremont cottonwood. Fungi were recovered from all inoculated saplings after one year incubation period. Cankers developed in the wood and the extent of cankers varied from 50 to 63 mm for the various isolates, canker length in the control was only 22 mm. Taxonomy of *Cryptosphaeria* species occurring in California remains unclear and more work has to be done to fully characterize all putative fungal species. Work also is continuing to clarify fungi biology and disease cycle.

Double pruning as a potential method to control Bot canker disease of grapes and duration of susceptibility of grapevine pruning wounds to infection by *Botryosphaeriaceae*

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Grapevine canker diseases, resulting from infected pruning wounds, are one of the main factors limiting vineyard longevity and productivity. Consequently, knowledge of low risk infection periods and pruning wound susceptibility are critical in deciding appropriate timing for pruning and wound treatment. Bot canker disease, caused by at least 9 different *Botryosphaeriaceae* species, has been recently identified as the most common canker disease in California vineyards. Double pruning of grapevines, which allows for more final pruning

in late winter, has been shown to reduce infections caused by *Eutypa lata*, the causal agent of Eutypa dieback, because infections on prepruning wounds do not develop further than the final pruning point. In this study we evaluated the efficacy of double pruning to reduce infections caused by *Botryosphaeriaceae* species, a much more rapidly colonizing fungus than *E. lata*. Chardonnay and Cabernet Sauvignon grapevines were prepruned and separately inoculated with a spore suspension of *Lasiodiplodia theobromae* and *Neofusicoccum parvum* from mid October to February with a final pruning in March. Pruned-off canes were examined and length of vascular discoloration measured from the point of infection in order to determine whether fungal infection developed beyond the point of final pruning. Duration of susceptibility of pruning wounds to infection by *Botryosphaeriaceae* was studied in the same site. Vines were pruned from mid November to February and inoculated with the same fungal species at 12 day intervals after pruning during 50 days. Percentage of infected pruning wounds for each treatment was determined by isolation of the pathogens from necrotic margins of cankers and/or vascular discoloration. Results from both double pruning and grapevine pruning wound susceptibility to infection by Botryosphaeriaceae experiments will be presented and discussed at the meeting.

Characterization of broad spectrum Potato virus Y resistance in an *Solanum tuberosum* ssp. *andigena*-derived population and select breeding clones using molecular markers, grafting, and field inoculations

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Potato virus Y (PVY) causes yield loss in potato and PVY necrotic strains can cause potato tuber necrotic ringspot disease (PTRND) resulting in quality loss. Breeding for resistance to PVY can be achieved by incorporating the Ryadg gene from *Solanum tuberosum* ssp. *andigena*. Resistance obtained from the Ryadg gene has been shown to provide extreme resistance, defined as resistance to all strains. Past work in Europe has shown that Ryadg-based resistance has conferred resistance to PVYO and PVYN/NTN strains, but the resistance has not been tested against a new strain, PVYN:O, detected in North America. Molecular markers tightly linked to Ryadg have been developed and are being utilized for marker assisted selection (MAS) in potato breeding. Three molecular markers linked to Ryadg were used to screen a NY241-7 (PVY resistant) × GemStar Russet (PVY susceptible) breeding population and a set of 53 clones/cultivars in the USDA-ARS Aberdeen Potato Breeding program. The breeding population was mechanically and graft inoculated with three isolates each of PVYNTN, PVYN:O, and two PVYO isolates Results show a 1:1 segregation ratio of resistant to susceptible confirming that PVY-resistant parent, NY241-7, is simplex for Ryadg. Resistant progenies were resistant to all PVY strains and had the presence of markers linked to Ryadg whereas susceptible progenies were lacking the diagnostic markers. Use of the markers on the set of clones/cultivars shows that when positive results were obtained from all three markers, resistance was present, but differences between markers were noted in four resistant clones, three of which have *S. stoloniferum* background and one which has *S. t.* ssp. *andigena* background. Use of these markers shows that while some discrepancies exist, they are useful in MAS for PVY resistance against all PVY strains and their use can increase the selection efficiency for PVY resistance in breeding programs.

Residual effects of fludioxonil and pyrimethanil on blue mold in Red Delicious apple fruit

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Fludioxonil and pyrimethanil are reduced-risk fungicides and were recently registered for postharvest use on pome fruits. Blue mold caused by *Penicillium expansum* is a common postharvest disease of apples. In 2005–06 and 2006–07 seasons, we investigated residual activity of fludioxonil and pyrimethanil in apple fruit against *P. expansum*. Organic Red Delicious fruit harvested from a commercial orchard were either not treated or drenched with fludioxonil, pyrimethanil, or thiabendazole at the label rates prior to storage and then stored in controlled atmosphere at 0°C for 5 and 7 months, after which time the fruit were removed from storage and subjected to washing and brushing during packing. Fruit were then wounded and inoculated with conidial suspensions of *P. expansum*. Inoculated fruit were treated either with sterile water or fungicides. Fruit were stored at 0°C for 8 weeks and at room temperature for one additional week after cold storage. During the 2-year study, no decay or up to 26% blue mold incidence was observed on fludioxonil-drenched fruit that were not treated with fungicides at packing. No decay or less than 4% blue mold incidence was observed on pyrimethanil-drenched fruit that were not treated with fungicides at packing, whereas 65–99% blue mold incidence was observed on thiabendazole-drenched fruit that were not treated with fungicides at packing. The results indicate that residual effects of fludioxonil and pyrimethanil applied prior to storage on blue mold in Red Delicious fruit can last for at least 7 months under apple-storage conditions.

Surfactants for crown gall management in walnut nurseries: Efficacy of benzalkonium chloride and cetyl trimethylammonium bromide on grafting tools

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The dominant rootstock in California walnut production is Paradox (*Juglans regia* × *J. hindsii*). This rootstock is precocious, hardy in marginal soils, and resistant to Phytophthora diseases, although highly susceptible to crown gall caused by *Agrobacterium tumefaciens*. Recently, serious outbreaks of crown gall have occurred in walnut nurseries with crop losses approaching 100%. Galls are not only appearing on roots and crowns, but also at grafting and bleeding wounds made during the grafting process. In greenhouse trials, *A. tumefaciens* was readily transmitted from plant to plant via sequential wounding of *Datura stramonium* plants with a cutting tool. To investigate sanitizing options, *A. tumefaciens* was challenged by two cationic surfactants, benzalkonium chloride (BC) and cetyl trimethylammonium bromide (CTAB). After a 30 minute exposure, 5 ppm of BC or CTAB is required for 100% bacterial mortality of an aqueous suspension of *A. tumefaciens*. When compared to quaternary ammonium and sodium hypochlorite, both surfactants were less affected by organic matter and less corrosive to metal tools. These characteristics, along with low phytotoxicity, make BC and CTAB promising materials for use in most systems where pruning or grafting tools are potential inoculum vectors.

Abstracts of the VI International Scientific Seminar on Plant Health

Abstracts presented at the VI International Scientific Seminar on Plant Health, September 22–26, 2008. The abstracts are arranged alphabetically, by first author's name.

Alternatives for the management of *Pseudoperonospora cubensis* Berk & Curt Rostow of cucumber (*Cucumis sativus* L.) in green houses

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Pseudoperonospora cubensis is one of the diseases that most frequently attack cucumbers (*Cucumis sativus* L.) planted in green houses in Cuba. The effectiveness of some systemic fungicides was evaluated: Fluopicolide 6.25% plus propamocarb hydrochloride 62.5% (0.09 + 0.94 kg a.i./ha, respectively); fenamidone 7.5% plus propamocarb 37% (0.5 + 0.74 and 0.18 + 0.92 kg a.i./ha, respectively); dimetomorf 9% plus mancozeb 60% (0.23 + 1.5 kg a.i./ha, respectively) and azoxystrobin 25% at the rate of 0.25 kg a.i./ha applied alone or alternating with mancozeb 80% at 2.4 kg a.i./ha. In a design of long parcels, the percentage of damaged foliar area was evaluated. All pesticides were effective in reducing the foliar damage caused by *P. cubensis*. Fluopicolide 6.25% plus propamocarb hydrochloride 62.5% (0.09 + 0.94 kg a.i./ha, respectively) and fenamidone 7.5% plus propamocarb 37% (0.18 + 0.92 kg a.i/ha, respectively) were the best treatments when alternated with mancozeb.

Determinación de genes relacionados con la patogenicidad en aislados de *Passalora fulva* (Cooke) U. Braum & Crous en Cuba

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El moho de las hojas, causado por *Passalora fulva* (Cooke) U. Braun & Crous es la enfermedad más común y destructiva del tomate creciendo bajo condiciones de cultivo protegido en el mundo. En Cuba, constituye el principal problema fúngico que incide en estas instalaciones de cultivo protegido, por esto el objetivo del presente trabajo fue determinar los genes relacionados con la patogenicidad (*Avr* y *Ecp* en 36 aislados de *P. fulva* procedentes de diferentes regiones del país. Para ello, se utilizó la técnica de Reacción en Cadena de la Polimerasa (PCR) y el empleo de cebadores específicos descritos en la literatura científica. Los resultados mostraron la variabilidad existente en *P. fulva*, en las condiciones de Cuba. Además, las diferencias detectadas en las amplificaciones de los genes de avirulencia (*Avr*) y genes que codifican para las proteínas extracelulares (*Ecp*) en los aislados determinados como raza 0 evidenciaron subdivisiones dentro de la misma. Estos resultados constituyen una primicia para Cuba y sientan las bases para futuros estudios de interacción tomate-*P. fulva* y mejoramiento genético.

Diagnostic and biological characterization of a *Papaya ringspot virus* isolate (PRSV-P) from Cienfuegos, Cuba

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Papaya ringspot potyvirus is considered the main cause of economic losses in papaya growing regions. In Cuba, although widely distributed among production areas, only two isolates have been biologically characterized. This is a crucial issue for the efficient setting of management strategies. In order to identify and characterize this viral disease, samples with symptoms of papaya var. Maradol roja were collected and the viral particles isolated (PRSV-CF). Inoculation on healthy plants was carried out using Carborundum (600 mesh). A true isolate of PRSV-VC served as control. The virus was identified by means of DAS-ELISA with polyclonal antibodies (Agdia) as well as RT-PCR reactions. Analyzed samples were positive to serological diagnostic, and the presence of an approximately 800 bases pairs band was also detected. Plants infected with the PRSV-CF isolate showed a retard in the appearance of the symptoms. The PRSV-CF isolate seems to be less virulent respect to other isolates already characterized in the country.

Integrated pest management in tobacco crop systems

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Phytopathology 99:S188

An efficient IPM program was developed for Cuban tobacco farmers, based on a) pest identification by labs of the surveillance plant health system; b) scouting of pathogens based on population dynamics (presence and density in fields including risk analysis of potential plant pathogens that help estimate economic losses related to disease incidence in fields) and c) coordination with effective control measures that keep pathogen levels as low as possible. Also used are physical and biological control methods such as crop rotation and natural enemies. Chemical control is used based on an understanding of the life cycle of the pathogen that allows us to apply the products when pathogens are most vulnerable. We started a breeding program to obtain new genotypes through hybridization without the use of artificial DNA manipulation techniques and continued with evaluation and selection of superior superior genetic combinations. New promising varieties were evaluated in different locations for production, processing, storage and market qualities.

Determinación de actividades enzimáticas implicadas en la patogenicidad y la virulencia de cepas de *Pectobacterium carotovorum* subsp. *carotovorum* y *Dickeya chrysanthemi* de papa

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Pectobacterium carotovorum subsp. *carotovorum* y *Dickeya chrysanthemi* causan daños severos en papa y otros cultivos de importancia económica. El desarrollo de la enfermedad se debe a la acción de enzimas extracelulares producidas por estas bacterias que degradan la pared celular de las plantas. En este trabajo se evaluó la patogenicidad y la virulencia de cepas de ambas especies en tubérculos de papa de la variedad Spunta; y se determinó la presencia cualitativa y cuantitativa de las enzimas pectato liasa, poligalacturonasa, celulasa y proteasa. Las bacterias fueron aisladas de campos de papa en provincia Habana, donde ocurrieron afectaciones del 2002 al 2005. Todas las cepas causaron maceración en los tubérculos pero difirieron en el grado de afectación. Las más virulentas ocasionaron mermas superiores a 14% en el peso de los mismos. Para *D. chrysanthemi* la mayor virulencia fue vinculada con una elevada actividad pectato liasa y celulasa de 2,4 a 4,6 U y de 0,24 a 0,3 U respectivamente; mientras que para la otra especie se relacionó con una alta actividad poligalacturonasa, de 1,1 a 1,7 U. La mayor actividad proteasa, con halos de hidrólisis superiores a 18 mm, correspondió a dos cepas de *D. chrysanthemi* de elevada virulencia. El resto de las cepas presentó valores similares, con excepción de una en la que no fue detectada esta enzima.

Identificación y determinación de grupos de anastomosis de miembros del complejo Rhizoctonia por comparación de secuencias de la región ITS del ADNr nuclear

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El complejo Rhizoctonia está formado por un grupo de hongos filamentosos con fase asexual no productora de conidios. El número de núcleos y la anastomosis hifal son los caracteres diagnósticos que permiten identificarlos,

este último requiere cepas patrones representantes de cada grupo de anastomosis (AG), las cuales no están disponibles en Cuba. El objetivo de este trabajo es identificar los grupos de anastomosis para aislamientos cubanos de *Rhizoctonia* spp. utilizando la zona ITS del ADNr nuclear. Se extrajo el ADN cromosomal de 32 taxones, se amplificó la región ITS1-5.8-ITS2 y se secuenció automáticamente. Las secuencias obtenidas se compararon con las reportadas en el GenBank mediante un BLAST y fueron sometidas a un análisis de máxima parsimonia utilizando el programa MEGA3.1. Un total de 17 aislados son binucleados y presentan más de 90% de homología con el género *Ceratorhiza* (teleomorfo: *Ceratobasidium* spp.), de estos, ocho se asociaron con el AG-G, y nueve se agruparon con el AG-F y AG-P. El resto de los aislamientos son multinucleados y mostraron más de 95% de homología con *Rhizoctonia solani* (teleomorfo: *Thanatephorus cucumeris*), cuatro de ellos se agruparon con el AG-3, dos con el AG-5, dos con el AG-2-2IIIB, uno con el AG-4HGIII y seis con el AG-4HGI. El género *Ceratorhiza* se reconoce por primera vez para Cuba.

Molecular detection of *Potato yellow vein virus* in leaves and shoot-tubers of *Solanum phureja* from Colombia

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PYVV (*Crinivirus*/*Closteroviridae*) from the Andean countries is phloem limited, with tripartite ssRNA genome and two coat proteins (CP). Symptoms include leaf yellowing starting from apex up to full leaf. PYVV is transmitted by the whitefly *Trialeurodes vaporariorum*. Many grown varieties are susceptible and its incidence is high. In this work, PYVV was detected by RT-PCR (retrotranscriptase PCR) in leaves and shoot-tubers from symptomatic *S. phureja* plants, using dsRNA as template with primers for the major CP gene. Bands of 756 pb and 296 pb were seen in symptomatic samples, but not in negative controls. SSCP tests showed at least four different patterns in 50 samples, suggesting variability. In Colombia, potato crops are important since 161.000 ha of potato are grown per year. The relevance of this work is to provide a molecular detection method for PYVV from leaf and tuber samples useful in seed certification and variability studies.

Emergencia de virus transmitidos por mosca blanca en Cuba

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Virus transmitidos por mosca blanca causan gran pérdida en cultivos de regiones tropicales y subtropicales. En la última década diferentes especies de los géneros crinivirus y begomovirus se identificaron en tomate, pimiento y papa. A partir de 1980 en Cuba se identificaron 8 especies de begomovirus y particularmente en tomate se identificaron TYLCV-Is, ToMHV y TTMoV, predominando TYLCV-Is. Recientemente hemos detectado el crinivirus de la clorosis del tomate (*Tomato chlorosis virus*) en tomate de invernadero, con altos niveles de mosca blanca y síntomas de clorosis intervenal, severo mosaico amarillo y perdida de color. Tambien tomamos muestras con síntomas de begomovirus en tomate, pepino, pimiento y diferentes especies de malezas (*Euphorbia heterophylla* L., *Rhynchosia minima* (L.) DC y *Walteria indica*). Se usó la reacción en cadena de la polimerasa con cebadores genéricos para begomovirus. En muestras con begomovirus se usó amplificación por círculo rodante para amplificar el genoma viral íntegro para después clonar y secuenciar. Existen begomovirus no descritos antes lo que sugiere que nuevas especies virales están surgiendo por la interacción entre malezas, cultivos y la mosca blanca como vector.

Description of microbiota associated to the false Broomrape manifestation in tobacco crop in Cuba

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False Broomrape disease is an important phytosanitary problem to the tobacco crop in the central provinces of Cuba. Nowadays its causal agent is unknown. Many researches have suggested that this disorder is caused by a biotic agent that's present in the soil of the affected tobacco field. The aim of this work is to describe the microbiota associated with the false Broomrape manifestation. The isolation and purification of tumor's microbiota was done. Bacterial groups were arranged in basis of colony's pigmentation. The isolated fungi were grouped in a single group. Susceptible tobacco plantlets were inoculated with different combinations of bacterial groups. Other set of susceptible tobacco plantlets was inoculated with the only fungi group. These biological tests were complemented with morphological studies of the bacterial cells associated with the symptoms. Only the plants inoculated with white bacterial colonies were symptomatic. Members of the gram-positive bacteria were found in this colony group. It is the first time this illness is associated to the rhizospheric bacteria.

Registros de patógenos fúngicos en algunas plantas ornamentales de Cuba

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En Cuba, las plantas ornamentales han ganado en importancia en los últimos años, por lo que se han incrementado las áreas de siembra y la aparición de hongos patógenos, principales microorganismos que afectan a estos cultivos. Debido a su importancia se realizaron colectas de plantas enfermas algunos municipios de Ciudad de la Habana, La Habana y Santiago de Cuba, estas fueron procesadas mediante cámara húmeda, siembra en medios de cultivos, y técnica de cebo, según correspondió en cada caso. La identificación de las especies de hongos se realizó según caracteres morfológicos, en el caso del Complejo Rhizoctonia, se utilizaron también técnicas moleculares. Se analizaron muestras de 20 plantas ornamentales, donde se encontraron 35 hongos patógenos, de ellos 29 son nuevos registros para los hospedantes donde fueron detectados. Especies de los géneros *Colletotrichum*, *Alternaria* y *Curvularia* fueron las más frecuentes, además son comentados los síntomas y las patologías causadas por estos organismos a las plantas afectadas.

Tissue culture and transformation for introducing genes useful for fungal diseases management in J-104 rice cultivar

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Fungal diseases affect severely J-104, the most important rice cultivar in Cuba. Breeders have developed strategies to control fungal attack, but a higher level of resistance is needed. Pathogenesis related proteins glucanase and chitinase are components of plant defence mechanisms against fungal pathogens. In this work we established *in vitro* culture of J-104 rice cultivar and its transformation with chitinase-glucanase genes. We proved the effect of 2.4-D, agar, proline and glutamine for callus induction and plant regeneration. The highest frequency of callus induction was obtained with 2.5 mg/L 2.4-D and 0.8% agar. Proline and glutamine promoted callus growth. Morphology of regenerated plants revealed the simultaneous occurrence of somatic embryogenesis and organogenesis. We used *A. tumefaciens* for genetic transformation with pCAMBIA 1300 harbouring chitinase gene from bean and glucanase gene from tobacco. Co-cultivation conditions determined the transformation success. PCR analyses confirmed the presence of transgenes in regenerated plants.

Caracterización del hongo nematófago cepa IMI SD 187 de *Pochonia chlamydosporia* var. *catenulata* (Kamyscho ex Barron y Onions) Zare y Gams

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La cepa IMI SD 187, del hongo *Pochonia chlamydosporia* var. *catenulata* es un eficaz agente de control biológico de nematodos formadores de agallas. No obstante, se desconocen aspectos de su comportamiento parasítico, estabilidad genética y potencialidades. El objetivo de este trabajo fue la caracterización bioquímica de la cepa IMI SD 187 y su interacción con cepas de la variedad *chlamydosporia*. Se estudió la producción de enzimas relacionadas con el parasitismo, en medios con inductores y hospedantes diferentes, de la cepa IMI SD 187. Esta cepa se caracteriza por alta producción de esterasas y escasa inducción de VCP. La cepa es estable a través de diferentes subcultivos seriados. Se estandarizó la PCR en Tiempo Real para el monitoreo del hongo en el suelo, liberado como sustrato colonizado o clamidosporas puras, con una sensibilidad de 100fg y especificidad del 100%. Es factible la aplicación de ambas formas de inóculo y los efectos de una aplicación son duraderos durante seis meses. La interacción de diferentes cepas de *Pochonia* en la rizosfera de plantas de tomate, demostró la posibilidad de la aplicación de

productos de composición mixta. Estos resultados apoyan la selección de esta cepa para su producción y aplicación masiva y contribuyen a su registro como ACB.

Efecto de un propóleos de abeja en el control postcosecha de la Antracnosis *Colletotrichum gloeosporioides* en mango

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La Antracnosis ocasiona crecientes pérdidas económicas en mango (*Mangifera indica*). Se están estableciendo estrategias para el combate del agente causal *Colletotrichum gloeosporioides* Penz Sacc, estudiándose las propiedades que tiene el propóleos de abejas (*Apis mellifera* L.), al cual se atribuye efectos antifúngicos. Se investigó el efecto en frutos de mango en proceso de maduración. Los tratamientos aplicados fueron diluciones de propóleo al 1%, 5% y 10% en forma preventiva (aplicación del propóleos y luego inoculación con el patógeno), curativa (inoculación con el patógeno y luego aplicación del propóleos). Se cuantificó el número de lesiones producidas, así como el porcentaje de frutos manchados. Se observó la efectividad del propóleos, con mayor expresión en el tratamiento preventivo al 10%. El propóleos ejerció control sobre el desarrollo del patógeno en frutos en etapa de postcosecha, retardando la aparición y crecimiento de manchas necróticas.

Molecular variability of begomovirus isolates affecting pepper crops in Cuba

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In Cuba, the presence of begomoviruses in pepper crops as reported for first time in 2002, with the detection of *Tomato yellow leaf curl virus*. Later, a infection by a bipartite begomovirus with 94% identity with *Cabbage leaf curl virus* was determined. To investigate the molecular diversity of these viruses, a survey was carried out in 2007 in areas of pepper production in Cuba. We collected 212 pepper samples showing typical symptoms of begomoviruses infections. The samples were tested by PCR using begomovirus-universal primers (Palv1978/PARc496), and 30% were positive to infection. DNA from positive plants was used as a template for full-length circular genome amplification and the products were characterized by restriction analysis, which allowed the detection of four distinct patterns in the analyzed samples. These results indicate the possible presence of additional viruses, besides the previously begomovirus species reported, and constitute evidence of the wide molecular variability of these pathogens in Cuba.

Micobiota asociada al deterioro de granos de Maiz *Zea mays* almacenados en silos metálicos para consumo humano en Cuba

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El maíz *Zea mays* L. constituye uno de los principales alimentos en la dieta humana a nivel mundial. Desde el 2006 en Cuba se almacenan en silos metálicos (SM) la mayoría de los granos que forman parte de la materia prima para las harinas y piensos, por lo que se hace necesario conocer todos los factores que actúen contra las buenas condiciones de almacenamiento de estos productos que son consumidos por humanos y animales. El objetivo de este trabajo es determinar los hongos responsables del deterioro de granos de maíz almacenados en SM. Se colectaron 20 muestras durante los meses de febrero a mayo de 2007, en SM de las provincias Matanzas, Cienfuegos, Holguín y Guantánamo. El análisis micológico se realizó utilizando los métodos de cámara húmeda y siembra en medio agarizado. La determinación de los géneros y especies, se realizó consultando las claves taxonómicas de identificación. Se detectaron siete géneros de hongos, siendo los más frecuentes *Aspergillus* y *Penicillium*.

Selección de aislamientos de *Trichoderma* spp. para el biocontrol del Tizón de la Vaina en Arroz en condiciones de campo

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El Tizón de la Vaina, es considerada la segunda enfermedad de importancia en Cuba y el mundo en el cultivo del arroz, la misma se incrementa cada año. Por lo que se hace necesario la búsqueda de nuevas alternativas para su control. Se han obtenido resultados positivos con *Trichoderma* spp. como antagonista de *Rhizoctonia* spp. El presente trabajo tuvo como objetivo seleccionar aislamientos promisorios de *Trichoderma* spp. para el biocontrol de *Rhizoctonia* spp. en campo. El experimento se montó con aislados promisorios para el control del patógeno en condiciones semicontroladas y se evaluaron 7 aislamientos en canteros tecnificados de 1 m^2, con inoculación del patógeno y el antagonista, sobre la variedad Perla susceptible al patógeno. De estos fueron seleccionados 3 (17, 75, 78) aislados para la evaluación en campo abierto en parcelas de 25 m^2, con inoculación natural del patógeno; donde se determinó el momento de aplicación y los mejores aislados en dependencia de su efectividad técnica (ET). Resultando como mejores momentos para la aplicación el trasplante, inoculando las posturas y al suelo en el momento del primer estrés hídrico a una concentración de 10^{11} ufc/ha. Los aislados 17 y 78 resultaron promisorios para el control de la enfermedad con una (ET) del 80%.

2008 Northeastern Division Meeting Abstracts

Abstracts presented at the APS Northeastern Division meeting in Newport, Rhode Island, October 8–10, 2008. The abstracts are arranged alphabetically, by first author's name.

Transformation of apple for disease resistance: Evolving strategies
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Apple is highly heterozygous and self-incompatible, has a long generation time and a large plant form. Therefore, development of disease-resistant cultivars with competitive quality by conventional breeding is excessively long-term and costly. rDNA technology was recognized as an attractive alternative approach. For resistance to the two most important diseases of apple in humid climates, fire blight (caused by the endobacterium *Erwinia amylovora*, Ea) and apple scab (caused by the ascomycete *Venturia inaequalis*, Vi), rDNA strategies initially used heterologous genes with direct effects on the pathogens, lytic proteins vs. Ea, and chitinases vs. Vi. Ea-resistant lines with normal quality were obtained with each type of gene. Transgenes from Ea (hrpN, and dspF) and phages (lysozyme, depolymerase) also effectively increased resistance to Ea. However apples with genes from other organisms were judged to be less acceptable to consumers than apples with added or silenced Malus genes. An additional copy of the apple transcription facilitator gene, MpNPR1, significantly increased Ea resistance in susceptible apple cultivars, and also moderately increased resistance to Vi and cedar apple rust. Silencing of the DIPM pathogen-protein receptor genes, and of the HrpN-interacting protein (HIPM), resulted in increased Ea resistance in some apple lines. The cloned R genes, Vfa1 and Vfa2, from the wild Malus floribunda have increased Vi resistance when transferred into susceptible apple cultivars. The preferred strategy now is to alter expression of native apple genes, using plant promoters, without using antibiotic or herbicide resistance selectable marker genes, in order to facilitate approval by regulatory agencies, and acceptance by consumers and apple growers.

Prospects for precision agriculture to manage aerially dispersed pathogens in a patchy landscape
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Precision application of disease control measures for an aerially dispersed pathogen depends on the spatial scale and temporal dynamics of pathogen spread and host development. These dynamics can be expressed in terms of basic biological and physical properties, viz., latent period, infectious period, basic infection rate, dispersal distance and survival time scales, host phenology, and the level of acceptable risk. A mean waiting time for new infections to appear on discrete patches of host plants a certain distance from a focus of disease is defined in terms of these basic parameters. We illustrate how this waiting time can be used to help establish guidelines for minimizing application of fungicides, while maintaining acceptable yield. We examine the following questions: 1) Once disease or the pathogen is detected locally, can a safety zone around a focus be protected without spraying the whole field? 2) When can small fields separated by a given distance be treated as separate management units? 3) For hosts distributed on the regional or landscape scale, can we define waiting times that allow us to forgo or delay control measures in a neighboring region?

Efficient generation of RNAi mutants of apple using multi-vector transformation
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Apple RNAi mutants for determination of function of candidate genes in resistance of apple to *Erwinia amylovora* (fire blight) were produced using an efficient transformation system. M.26 apple was transformed with a mixture of five RNAi EST-silencing vectors in each transformation experiment to allow selection of up to five types of mutants from a single experiment. RNAi-silencing constructs were created using ESTs associated with response to *E. amylovora* which were identified by bioinformatics analysis. These constructs were transferred to *Agrobacterium tumefaciens* strain EHA 105pCH32. The five silencing constructs were mixed, and the mixture used to transform leaf-slice explants. Regenerants were selected on M.26 regeneration medium with 100 mg/L kanamycin and screened by PCR using universal primers for the presence of a silencing construct. In almost all lines PCR showed only single genes had been inserted. Because amplicons from some transgenics co migrated, to better determine the identity of the ESTs contained in the silencing-insertion, the PCR fragments were cut with 4-cutter restriction enzymes. Thus far ESTs from genes in six functional categories, general metabolism (1), photosynthesis (2), nucleic acid metabolism (1), protein metabolism (3), signaling (1), and defense/stress (4), have been subjected to this protocol. To assay their resistance phenotype, young plantlets were inoculated with *E. amylovora*, and bacterial populations and reaction symptoms determined. This project is supported by a National Research Initiative Competitive Grant 2005-35300-15462 from the USDA Cooperative State Research, Education, and Extension Service.

Development of an eco-label program in support of IPM for apples in the Northeast
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Since 2005, the University of Massachusetts, Cornell University, Red Tomato (a nonprofit produce marketing corporation) and the IPM Institute of North America have been developing a protocol for producing and marketing "Eco Apples", an eco-label for apples in the Northeast. The goal has been to create a market niche for apples grown using a well-defined integrated pest management program that will result in premium prices and access to high quality markets; in return for using relatively more expensive and less toxic

management methods, growers should receive higher returns. There has been a significant increase in both participating growers and sales, starting with six growers and approximately $130,000 in sales in 2004 and increasing to 13 growers and approximately $1,470,000 in sales in 2007. In the Eco Apple protocol, pesticides are classified into three categories: green, use with justification; yellow, use when green materials are not available or effective; and red, do not use, based on potential toxicity. To date, disease and other pest control in Eco Apple orchards was generally as effective as that in orchards using standard production methods. Fungicides are the most commonly used type of pesticide in Eco Apple orchards, and of these, almost all (96%) are classified "yellow", primarily captan and ethylenebis (dithiocarbamates).

Discovery of a new species of *Fusarium* from *Spartina alterniflora* and the influence of drought on its ability to cause plant mortality

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The rapid loss of the salt marsh plant, *Spartina alterniflora* (SA), has been termed Sudden Vegetation Dieback (SVD). SVD has occurred along the Atlantic and Gulf Coasts since 1999. Isolations from SA from SVD sites along the Atlantic Coast yielded over 200 isolates of *Fusarium* spp. Eighty-eight percent fell into two morphospecies (MS1 & MS2) that could not be readily identified. Inoculation into stems of healthy SA plants showed that isolates from MS 1 were virulent whereas isolates from MS2 were slightly virulent to avirulent. Growth rates on PDA also distinguished the two MS. Partial DNA sequences from the TEF1-alpha, beta-tubulin, and calmodulin genes revealed that the MS1 isolates were distinct, closely related, and clustered in the trichothecene-producing clade of the *Fusarium* section *Sporotrichiella*. Isolates in MS2 had more variation and belonged to the *F. incarnatum-equiseti* species complex. When isolates of MS1 were mixed into soil, planted with seedlings of SA, and subjected to drought, normal watering, or flooded conditions, the presence of drought and *Fusarium* resulted in more plant mortality than drought without *Fusarium* ($P = 0.02$). Compared to controls, *Fusarium* infection of SA in normal or flooded conditions reduced plant weights, and increased root disease, but did not cause mortality. Increasing salinity did not increase disease. These findings suggest that drought and this undescribed *Fusarium* species could cause significant mortality of SA.

Virginia creeper as a reservoir for inoculum of grape powdery mildew

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Virginia creeper (*Parthenocissus quinquefolia*) is a native perennial woody vine which can grow up to 60 feet in length. Since the plant needs some sunlight, it usually grows along the edge of a woodlot either as a ground cover or climbing into trees along the edge of the forest. This is exactly the ecological niche occupied by wild grape vines and these two plants often grow side by side. Vineyards in the northeastern United States are most often flanked by wooded areas, so that the ubiquitous virginia creeper is often in close proximity to commercial vineyards. This plant is in the grape (*Vitaceae*) family and is susceptible to grape powdery mildew (*Erisyphe necator* syn. *Uncinula necator*). Sampling of virginia creeper adjacent to infected vineyards have indicated high levels of disease incidence (25%–50%) by mid-summer (July) which produce cleistothecia prolifically until frost. For this reason, the removal of this vine around vineyards may lower the springtime flush of primary inoculum of this damaging disease.

Mixing biorationals to optimize grape powdery mildew control

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The objective of the study was to investigate the potential of mixing biorational compounds in order to obtain greater control of powdery mildew (*Unicinula necator*) on 'Riesling' vines. The research combines a topical and a systemic acquired resistance (SAR) inducing fungicide to investigate whether greater control of powdery mildew can be obtained than with either material used separately. Monopotassium phosphate and potassium bicarbonate were applied individually, as well as in combination, then given 24 hours to stimulate resistance mechanisms, or "prime" the plants. These treatments were compared with the conventional fungicide combination of boscalid and pyraclostrobin. On days 1, 3, 5, and 7 the percent of conidial germination and percent conidia killed were evaluated, as well as lignin and callose formation as indicators of the SAR response. Potassium bicarbonate induced unexpectedly strong SAR responses, generating the highest amount of lignin and callose formation, and also displayed above average topical fungicide properties with statistically no difference compared to the commercial control. Monopotassium phosphate did little to prevent powdery mildew infection, allowing the highest conidial germination and leading to no significant formation of lignin or callose in the tissue. The combination of the two compounds caused unexpected conidial germination, higher than the other treatments on the last day, but also encouraged a strong SAR response. Though potassium bicarbonate controlled infection to the same degree as the commercial standard, the combination of biorationals performed significantly worse than the standard and would not be recommended for field use.

Species of *Pythium* present in Long Island greenhouses

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Long Island flower crop greenhouses were sampled for *Pythium* spp. repeatedly from spring 2007 to spring 2008. Samples (5 ml) of container mix from pots and debris from floor surfaces were collected and placed in plastic bags; floor areas with no debris were wiped with wet filter paper. Filter paper was plated on PARP-CMA, a medium selective for *Pythium* spp., for 24 h at 26°C. Samples of debris and mix were baited by adding 50 ml of tap water and two 1-cm long strips of peeled potato to bags containing 5 compiled samples. Potato pieces were rinsed after 24 h and placed on PARP-CMA; single hyphae from resulting colonies were transferred to CMA. Isolates were identified based on morphology on ryegrass in water and ITS-DNA sequence analysis. *P. irregulare* was found in container mix from healthy plants and on floors. The cryptic species *P. cryptoirregulare* was found in all greenhouses sampled and often was insensitive to 100 ppm of mefenoxam. Other species detected were *P. aphanidermatum*, *P. orthogonon*, *P. rostratum*, *P. rostratifingens*, *P. segnitium*, and *P. ultimum*.

Characterization of silicon absorption by *Equisetum arvense*

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Silicon (Si) is the second most abundant element in the Earth's crust and soil, and is accumulated by plants in similar amounts to several macronutrients, yet its role in plant development remains obscure. In general, it is reported for its beneficial effects against biotic and abiotic stresses, such as fungal diseases, mineral toxicities or excess salinity. Plants vary greatly in their ability to absorb Si, ranging from 0.1 to 10% in top dry weight. However, absorption of Si in non-enriched soil is seldom sufficient to optimally protect plants from diseases. In this report, our objectives were to characterize the mechanisms of Si absorption in horsetail (*Equisetum arvense*) in an effort to transfer the acquired information to agricultural plants and thus optimize the prophylactic role of Si. Horsetail was selected because it is known to accumulate a very high concentration of Si and it has no known diseases or insect pests. Si absorption in horsetail was studied in plants grown in pots supplied with a solution of 1.7 mM silicate potassium. Si deposition in aerial parts was dosed using Inductively Coupled Plasma (ICP) and X-rays. Si deposition was characterized by a specific pattern of dense accumulation in silicaphile cells. Horsetail plants were also grown in hydroponic solution with or without added Si. Plants without added Si showed necrosis of aerial parts and ultimately died, confirming the essentiality of this element for horsetail. These results will shed light on the differential affinity of plants for Si, and should thus help optimize the use of this element in agriculture.

The proteomic analysis of flocculosin production by *Pseudozyma flocculosa*

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The fungus *Pseudozyma flocculosa* is known for its biocontrol activity against many powdery mildew species. This activity is conferred, at least in part, by the release of an antifungal glycolipid, flocculosin. While the factors and conditions that affect production of flocculosin have been studied, the molecular basis of the synthesis and production of flocculosin is not well understood. In this work, we conducted a proteomic analysis of flocculosin production by *P. flocculosa* by comparing the proteome map of *P. flocculosa* grown under conditions conducive or repressive for flocculosin synthesis. In total, more than 830 proteins were revealed. Spots of interest were excised from polyacrylamide gels after 2-D electrophoresis for peptide fingerprint analysis by serial mass spectrometry (LC-MS/MS). An LC-MS/MS ion search was performed using the Mascot search engine and a database containing all

available nucleotide sequences order to find protein homologues. We identified several activities with increasing expression level in the inductive medium, which correlated to the carbon and fatty acid metabolism such as the thiamine biosynthesis protein, the transaldolase and the electron transfer flavoprotein.

Applications of gibberellic acid for control of *Botrytis* and other bunch rots in wine grapes

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Gibberellic acid was applied to *Vitis* interspecific hybrid 'Vignoles' and *Vitis vinifera* 'Chardonnay' at 5, 10, and 25 parts per million, either two weeks pre bloom or at full bloom. Applications were aimed at reducing cluster compactness, a precondition for the development of *Botrytis* (*cinerea*) and other bunch rots (*Rhizopus* spp., *Penicillium* spp., *Aspergillus* spp.), either by increasing cluster length (pre bloom application) or reducing fruit set (bloom application). On Chardonnay, *Botrytis* was responsible for nearly all harvest rot, and was significantly reduced in 2006 by bloom applications at 5 and 25 parts per million. In 2007, a pre bloom application at 25 parts per million and all bloom applications, significantly reduced *Botrytis* on Chardonnay. On Vignoles, other bunch rot organisms accounted for 8 to 58% of the total rot, being lowest in treatments receiving bloom applications of gibberellic acid and highest in treatments relying solely on fungicides. This is significant because these organisms are not generally controlled by grape fungicides and their presence in fruit can have devastating effects on juice and wine quality. On Vignoles, total rot was significantly reduced in 2006 by pre bloom applications at 5 and 25 parts per million and by all bloom applications. In 2007, total rot was significantly reduced on Vignoles by pre bloom or bloom applications at 25 parts per million. There were no negative 'year after' effects of gibberellic acid on return clusters per shoot in Chardonnay or Vignoles.

In vitro inoculations with *Phytophthora ramorum*: Foliage susceptibility of six eastern Canadian forest species

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In North America and Europe, *Phytophthora ramorum* (Pr) causes a complex disease named sudden oak death, ramorum leaf blight or ramorum shoot dieback. The pathogen infects more than 120 hosts, several of which are present in Canadian forested and urban areas. Although Pr is absent in the wild in eastern North America, there is concern regarding its possible introduction and spread into this area. In order to assess this risk, detached leaves/needles of six eastern Canadian forest species were inoculated with Pr and the amount of necrosis and sporulation was evaluated. Inoculation was also performed by plant dipping. *Abies balsamea* (Ab), *Acer saccharum* (As), *Betula alleghaniensis* (Ba), *Fraxinus americana* (Fa), *Larix laricina* (Ll), and *Quercus rubra* (Qr) were the species tested whereas *Rhododendron* 'Nova Zembla' (Rh) served as a positive control. On detached leaves, the amount of necrosis on Ba and Fa was higher compared with that on As and Qr, whereas preliminary results of the plant dip experiment showed that Fa was the most susceptible. On detached leaves, reisolation assays and real-time PCR analyses revealed that there were no differences between Ba, Fa and Rh as well as between As and Qr. For the coniferous species, the necrosis on needles of Ab was higher than that on Ll but the real-time PCR analyses were very similar for both species. While sporulation was negligible on detached leaves/needles, it was noticeable for the plant dip assays. Preliminary results revealed that sporulation was much higher on leaves of Ba, Fa and Rh than on those of As and Qr, while for the conifers it was higher on needles of Ab than on those of Ll. Overall, among the susceptible species, Fa appears to be particularly susceptible to Pr and it could also serve as a source of inoculum.

Measurement of *Bacillus subtilis* antibiotics in the rhizosphere

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Biological control agents like *Bacillus subtilis* offer an alternative and supplement to synthetic pesticides. Unfortunately, this alternative is not consistently effective, as biocontrol agents vary greatly in pathogen suppressiveness. Antibiotic production by biocontrol strains of *Bacillus subtilis* can play a major role in plant disease suppression. Our current understanding of *Bacillus subtilis* antibiosis comes from culture media measurements of antibiotic production and in vitro suppression of pathogens. An analytical method based on high-performance liquid chromatography (HPLC) and mass spectroscopy (MS) has been developed to quantify antibiotics produced by *Bacillus subtilis* growing on plant roots. Cucumber (*Cucumis sativus*) was grown in composted soil and potting media inoculated with *Bacillus subtilis* strain QST 713 (AgraQuest, USA). Two important *Bacillus* antibiotics, surfactin and iturin A, were extracted from root and rhizosphere soil using acidified organic solvents followed by cleaning and concentration using solid-phase extraction (SPE). HPLC and HPLC-MS were used to measure surfactin and iturin A. Rhizosphere concentrations of both antibiotics increased with plant age. For plants grown in peat-based potting media, surfactin concentrations increased from 9 $\mu g\ g^{-1}$ fresh root (FR) at 15 days to 30 $\mu g\ g^{-1}$ FR at 43 days. Iturin concentrations were 7 $\mu g\ g^{-1}$ FR at 15 days and 180 $\mu g\ g^{-1}$ FR at 43 days. In an initial field trial in a composted fine sandy loam, we have demonstrated rhizosphere production of surfactin and iturin under competition and predation by the myriad macro- and microfauna existing in a fertile organic soil, with mature *Bacillus subtilis*-inoculated cucumber roots yielding 33 $\mu g\ g^{-1}$ FR surfactin and 630 $\mu g\ g^{-1}$ FR iturin at 78 days. Quantifying the antibiotic metabolite chemistry of *Bacillus subtilis* biofilms growing on root surfaces will ultimately lead to more consistent efficacy of this versatile biocontrol agent.

Inhibition of *Monilinia fructicola* sporulation on peach blossom blight cankers by QoI fungicides

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The QoI fungicides azoxystrobin (Abound), trifloxystrobin (Flint), and pyraclostrobin + boscalid (Pristine) were examined for their anti-sporulant activity on established peach blossom blight cankers. During summer 2008, shoots having one or more cankers were selected and tagged on trees in an experimental 'Autumnglo' peach orchard. Cankers were then washed with water using a pressurized hand sprayer to remove any conidia already formed. Immediately after drying, azoxystrobin at 0.150 g/L, trifloxystrobin at 0.074 g/L, and pyraclostrobin + boscalid at 0.069 g/L + 0.136 g/L were applied until run-off to the shoots using hand atomizers; control shoots received no treatment. After 7-days of field exposure, tagged cankers were cut from the trees and incubated at 22°C and RH>95% in cover trays. After 24 h, tray covers were removed and incubation continued for another 24 h at 22°C and ambient RH (67–95%). The incidence of canker sporulation was assessed via stereoscopic examination; conidia production was assessed by using a hemacytometer to count spores harvested from the twigs with an atomizer. The experiment was repeated once. Analysis of incidence data revealed that Flint and Pristine significantly reduced the percentage of sporulating cankers from 54.5% (control) to 37.6% and 41.2%, respectively. Furthermore, all three fungicides significantly inhibited conidia formation. Flint, Abound, and Pristine reduced the number of spores/canker by 73.3%, 60.7%, and 54.7%, respectively. These results demonstrated that application of QoI fungicides to established blossom blight cankers can help reduce the availability of inoculum for subsequent brown rot epidemics on ripening fruit.

Resistance to blue mold in Connecticut shade tobacco

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A pedigree breeding program for resistance to blue mold, caused by *Peronospora tabacina*, was established for shade-grown wrapper tobacco in Windsor, CT. Sources of resistance were two non-adapted breeding lines with high (#292-343) and moderate (#509) levels of resistance to the disease. Crosses were made between the two resistant lines and two commercially grown Connecticut shade lines (8212 and male-sterile #37). Selection for blue mold resistance was made in the F2 and a cross between the two selections was used to create a male sterile resistant line. Recurrent selection in the greenhouse and field was used to select for resistance to blue mold, tobacco cyst nematode and *Tobacco mosaic virus*. Resistant lines were evaluated in 2006 and 2007 for blue mold development under field conditions and compared to a standard susceptible line (8212) that were either sprayed with a best management practices blue mold fungicide program or not sprayed. Resistant lines had greater numbers of healthy leaves harvested after the appearance of blue mold than 8212 with fungicide (nearly 2 ×) or without fungicide (nearly 200 ×). Blue mold lesion area on resistant leaves averaged 45% of susceptible 8212 and the number of sporangia per cm^3 of leaf was less than 10% of unsprayed 8212. Resistance to blue mold in adapted lines can

increase marketable yields under field conditions and impact the rate of epidemic development.

Sensitivity to fungicides of the cucurbit powdery mildew fungus in NY and PA in 2007 and 2008

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A seedling fungicide sensitivity bioassay was used to assess fungicide sensitivity of *Podosphaera xanthii* in commercial plantings of squash and pumpkin (*Cucurbita pepo*). Seedlings were sprayed with various fungicides and concentrations, placed for at least 4 hours in fields where powdery mildew was developing, then kept in a greenhouse until symptoms developed. Severity on treated seedlings was compared to non-treated ones to estimate frequency of the pathogen population able to tolerate each fungicide concentration tested. In spring squash assayed early in disease development during July 2007, pathogen strains resistant to QoI fungicides (FRAC Group 11) were detected in 4 of 5 NY fields (estimated frequency 1% to 100%) and both fields in eastern PA (16% to 30%), strains tolerating 120 ppm myclobutanil (Group 3) were found in 3 NY fields (2% to 91%) and the PA fields (7% to 32%), strains tolerating 175 ppm boscalid (Group 7) were in all fields (1% to 22% and 1% to 2%, respectively), and strains tolerating 5 ppm of quinoxyfen (Group 13) were at a very low level (0% to 2%). Strains tolerating these concentrations were detected in bioassays done in NY pumpkin fields during Aug to Oct. There appeared to be an increase in sensitivity to Group 3 fungicides. Strains tolerating the same fungicide concentrations were found in 2008. Resistance to thiophanate-methyl (Group 1) also was checked and found.

Evidence of reduced suppression of powdery mildew (*Podosphaera xanthii*) provided by resistant squash (*Cucurbita pepo*) cultivars in NY

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Phytopathology 99:S194

Squash cultivars were evaluated in replicated experiments conducted under field conditions in 2006 to 2008. The main goal was to obtain information for growers on disease suppression and yield of powdery mildew resistant (PMR) cultivars, especially new ones, relative to standard cultivars lacking resistance. Fungicides were not applied for powdery mildew. Average suppression on both leaf surfaces for the cultivars tested in 2006 and 2007 based on severity on 9 Aug in both years was 88% (67% to 100%) in 2006 and 62% (47% to 91%) in 2007 for yellow squash cultivars while it was 86% (68% to 100%) in 2006 and 35% (0% to 95%) in 2007 for green zucchini cultivars. For acorn squash cultivars tested both years control was 57% to 92% in 2006 and 11% to 72% in 2007. There is one common major PMR gene in commercial cultivars of squash (*Cucurbita pepo*). Most cultivars are heterozygous. The zucchini, acorn, and yellow squash cultivars tested with resistance from both parents exhibited excellent suppression in both years. Based on preliminary results from 2008, PMR cultivars are continuing to suppress powdery mildew on leaf blades as well as on petioles and stems, although not at the high level observed in 2006. Butternut squash also was tested in 2008. There is concern *Podosphaera xanthii* could evolve to overcome the major PMR gene; therefore, chemical and genetic control should be used together for managing powdery mildew.

A new tobamovirus isolated from waters draining forest stands in New Zealand

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Asymptomatic and endophytic plant viruses have been previously proposed as ideal gene vector candidates for genetic improvement of forest trees. Water samples were collected from Pohangina River near Palmerston North, New Zealand to assay for infectious plant viruses that may be systemic, but asymptomatic in eucalyptus and radiata pine predominant in adjoining forest stands. Twenty liter water samples were prefiltered and virions adsorbed to electropositive Zeta Plus 50S membranes. The eluates were examined for virions by transmission electron microscopy. Rod shaped particles with a modal length of 300 nm and width of 18 nm with characteristic axial canal were observed. Three distinct viruses were isolated based on local lesions in *Chenopodium quinoa* and *Phaseolus vulgaris.* An $A_{260/280}$ ratio of 1.33 and a buoyant density of 1.32 in CsCl supported the conclusion that all three isolates were tobamoviruses. Maximum Parsimony trees generated from a partial replicase, and complete capsid protein gene sequence showed maximum similarity of the Pohangina River Virus (PRV) with TMV variant 1 (V01408), and TMV crucifer isolate (Z29370) respectively. On the basis of a less than 90% sequence similarity established as the species demarcation criterion by ICTV, PRV was determined to be a new species of the tobamovirus genus. The remaining two isolates were determined to be new strains of TMV and ToMV respectively on the basis of phylogenetic reconstruction based on nucleotide sequences obtained from RT-PCR amplification of the replicase, movement protein and capsid protein regions. The isolates were designated TMV-NZ and ToMV-NZ respectively. Distinctive host range and symptomatology, especially in hosts in the family Fabaceae, Solanaceae, Chenopodiaceae, Cruciferae, and Cucurbitaceae, combined with sequence differences with well-described tobamoviruses, and serological differentiation based on Ouchterlony tests, suggests that one new tobamvirus species and two new isolates of TMV and ToMV are present in water sampled from the Pohangina River. The presence of these infectious tobamoviruses in waters draining forest stands in New Zealand creates a possibility of employing them as gene vectors in future tree improvement programs, as well as in studies involving host gene and protein expression, induction of pathogen resistance, novel protein production and gene silencing.

Challenges in managing diseases caused by *Rhizoctonia solani* and *Rhizoctonia*-like fungi on vegetables in New York

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Root and foliar diseases caused by *Rhizoctonia solani* (telemorph: *Thanatephorus cucumeris*) and *Rhizoctonia*-like fungi on vegetables including table beets, carrots, beans and cabbage have become more prevalent and damaging. Difficulty in managing these fungi is in part due to their soilborne habitat, wide host range, genetic complexity and the difficulty in the timing and application of effective fungicides. In New York, the inclusion of grain crops like corn in vegetable rotations has been an effective management strategy. However several isolates collected from naturally infected vegetables were recently characterized as being able to infect corn. A greenhouse trial was conducted to further characterize the pathogenicity of three isolates (R39, AG2-2; R43, AG4; and R62, CAG2) with variable virulence on corn on several grain crops (corn, oats, rye, sudangrass, wheat, and buckwheat). After six weeks all isolates caused light to moderately severe symptoms on the rye and sudangrass roots. Additionally, isolate R39 was able to infect corn, wheat and buckwheat; R43 infected oats and buckwheat and R63 was able to infect wheat. Infectivity of the isolates 30 days after incorporating the grain crops as green manures was bioassayed with snap beans. Isolate R39 caused the most severe symptoms on snap bean roots while isolate R62 was least virulent regardless of the grain crop treatment. The potential role of these grain crops as hosts to these fungi warrants further study.

The effect of rate and spray interval of demethylation inhibitor fungicides on an insensitive population of *Sclerotinia homoeocarpa* on a golf course fairway

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Dollar spot, caused by *Sclerotinia homoeocarpa,* is the most rampant and economically important turfgrass disease in the North America. The disease is primarily controlled by preventive or curative fungicide applications. Sterol demethylation inhibitor (DMI) fungicides are among the most effective and widely used in the United States. Fungicide resistance to the demethylation inhibitor chemical class has been confirmed in dollar spot and exhibits a gradual population shift towards insensitivity to DMI fungicides. Previous research has yet to pinpoint the duration of DMI field efficacy on insensitive dollar spot populations. This project aims to correlate DMI field efficacy to *in-vitro* fungicide sensitivity values, while examining the effect of fungicide rate and spray interval. Treatments were arranged in a randomized complete block design with three replications on an *Agrostis stoloniferous* and *Poa annua* fairway maintained at 0.50 inch mowing height. Treatments of two DMIs, propiconazole and triticonazole were applied every 14, 21 and 28 days at rates of 1.0/2.0 oz/1000 ft^2 and 0.5/1.0 oz/1000 ft^2, respectively. Chlorothalonil was applied every 14 days at a 5.5 oz/1000 ft^2 rate in order to exemplify acceptable control. 396 samples were taken prior to DMI application this summer and revealed an average of 52.25% ± 14.29 relative mycelial growth on potato dextrose agar amended with a single discriminatory concentration (0.1 µg a.i./ml) of propiconazole. All DMI treatments showed a reduction in number of dollar spot infection centers 7 days after application, but infection center totals increased 14 days after application. A dose-rate

effect was also observed in all DMI treatments, increased active ingredient did show a reduction in dollar spot infection centers for both DMIs. The AUDPC of infection centers over 8 ratings from plots treated with 2.0 oz/1000 ft^2 of propiconazole on 14 and 21 day intervals were statistically similar to plots treated with chlorothalonil.

Influence of irrigation quantity on anthracnose severity of annual bluegrass putting greens

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Irrigation can influence both vigor and playability of golf course putting greens. Anthracnose (*Colletotrichum cereale* Manns) disease is more severe on stressed turf. The objective of this field trial was to evaluate the effects of irrigation quantity on anthracnose severity of annual bluegrass [*Poa annua* L. f. *reptans* (Hausskn) T. Koyama]. This 3-yr study was initiated in 2006 on a 5-yr old annual bluegrass turf mowed daily at 3.2-mm and used a randomized complete block design with four replications. Irrigation was applied daily to 2.4 by 2.4-m plots at 100, 80, 60 and 40% of reference evapotranspiration (ET_o), based on the Penman-Monteith equation. Individual plots were syringed with no more than 2.5-mm of water when wilt stress was visible. Anthracnose severity was assessed from mid-June through mid-August. Drought stress (40% ET_o) increased anthracnose in all three years; anthracnose was less severe under 60% ET_o irrigation, and irrigating at 80% ET_o reduced severity compared to 60% ET_o. Anthracnose severity was initially lower under irrigation at 100% ET_o than 40% ET_o; however, 100% ET_o resulted in similar disease severity by the end of 2006 and 2008. While not due to anthracnose, 100% ET_o irrigation also reduced turf quality late in 2007. Thus, deficit irrigation that induced wilt stress intensified anthracnose severity and irrigation at 80% ET_o often resulted in the least disease and best turf quality.

Using phosphite fungicides to control sooty blotch and flyspeck on apples

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Phytopathology 99:S195

Phosphite fungicides were applied to control sooty blotch and flyspeck (SBFS) in four field trials during the 2006–07 growing seasons. In one trial, ProPhyt (54.5% potassium phosphite), LI-700 (a surfactant and acidifier), and Tactic (a synthetic latex and organosilicone sticker, surfactant, and deposition agent) were applied alone, with captan, or in three-way combinations with thiophanate-methyl (TM) plus captan. Treatments were applied to 'Golden Delicious' trees on 1 Sept. using a handgun sprayer. Fruit were evaluated 33 days later and 96% of unsprayed fruit had flyspeck. ProPhyt applied alone suppressed flyspeck by 50% whereas LI-700 and Tactic did not. Fruit sprayed with TM+captan+Tactic had the least flyspeck (14%), but ProPhyt+captan provided statistically equivalent control with 31% of fruit affected. Captan with LI-700 or Tactic was less effective. Thus, ProPhyt activity against SBFS involves more than spray acidification or surfactant activity. In another trial with 'Honeycrisp', 'Royal Court' and 'Cameo' apples, ProPhyt was applied at two rates either alone or in mixtures with captan, TM, or Pristine (pyraclostrobin plus boscalid). In 10 disease-control data sets from this trial, ProPhyt consistently boosted activity of captan but only occasionally improved activity of TM or Pristine. However, ProPhyt+captan had less residual activity than TM and Pristine and was less effective than TM and Pristine for controlling summer fruit rots. Thus, ProPhyt+captan can be used to control SBFS, but other fungicides may be needed to control fruit rots caused by *Botryosphaeria* species.

Oil sprays control Fabraea leaf spot on pears

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In southeastern New York and Connecticut, *Fabraea maculata* causes fruit blemishes and premature defoliation of pears. Field trials were conducted to determine if petroleum-based horticultural oils would control Fabraea. In 2007, a highly refined petroleum oil (1% solution) was applied to Bosc pear trees on 22 May, 6, 26 June, and 13 July. Compared to trees sprayed with mancozeb on 1 May, 6, 26 June, the oil-treated trees had more infected leaves on 16 August (34% vs. 16%), similar levels of defoliation in mid-September, but fewer infected fruit (22% vs. 52%). In 2008, trees receiving oil sprays (1% solution) applied seven times between petal fall and 8 August were compared with trees receiving four applications of mancozeb followed by two applications of kresoxim-methyl during that same period. On 22 August, trees treated only with oil had a higher incidence of leaf infection (88% vs. 53%) but similar levels of defoliation (ca. 9%). Leaf disks (4-mm diam.) containing Fabraea lesions were removed from oil-sprayed and from unsprayed leaves on 7 August, 14 days after the preceding oil spray, were suspended in distilled water, and were vortexed for 30 sec to remove mature spores from lesion surfaces. Hemacytometer counts of the resulting spore suspensions showed that spore release from oil-treated leaves was reduced by 94% compared to control leaves. Spore germination as assessed 24 hr after spores were streaked on potato dextrose agar was also 63% lower for spores from oil-treated leaves. This is the first report that oil alone can provide commercial control of Fabraea leaf spot.

New developments in epidemiology of *Ailanthus* wilt

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Verticillium albo-atrum and *V. dahliae* have both been identified as causing wilt and mortality of the invasive tree species *Ailanthus altissima* (Chapter I). *Verticillium albo-atrum* is more virulent and aggressive to *A. altissima* than *V. dahliae*, under both greenhouse and field conditions. Overwintering (survival) occurs within infected *A. altissima* trees, on fallen *A. altissima* leaves, and in soil. Overwintering may also occur within striped maple trees. Inoculation likely takes place in the spring at time of leaf emergence. Wounding of *A. altissima* seedlings roots increased the rate of symptom development by 4 weeks following inoculation of *V. albo-atrum*, as compared to non-wounded *A. altissima* seedling inoculations. Examination of infected plant tissues by means of histology revealed that phenols were deposited in most outer xylem parenchyma within at the least 1 week following inoculation, indicating that the fungus had began to invade the circumference of seedling initially, and then spread both upward and downward until plant mortality occurred. Extensive fungal colonization was not observed until 4 weeks after inoculation, and the seedling had completely wilted. Dissemination potentially involve wind disseminated leaflets, seeds transmission, or ambrosial beetle transmission. Within 1 year of inoculation *V. albo-atrum* had spread from five initially inoculated trees in two stands of 39 and 95 trees, to 94.9% to 86.3%, respectively, of the *A. altissima* trees within the two stands. Rate of spread is fairly rapid. Between 200 and 2006, the average spread of mortality caused by *V. albo-atrum* downwind is approximately 116.2 m/yr and upwind is 64.6 m/yr.

What have we learned from studying *Grapevine fanleaf virus* from its hypothesized origin?

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Grapevine fanleaf virus (GFLV), the causal agent of grapevine degeneration, is one of the most important diseases of grapevine. GFLV is a *Nepovirus* belonging to the family *Comoviridae*. Its particles are isometric and its genome is composed of two +ssRNAs each coding for a polyprotein. Because it is hypothesized that GFLV has originated in Iran we attempted to examine this hypothesis by molecular characterization of the virus isolates from this country. Accordingly, total RNA extractions from leaves of diseased vines from vineyards in northwest Iran were subjected to RT-PCR assay with primers which were designed according to sequences of GFLV-F13 and -NW. As a result, segments of the virus RNA2 corresponding to the movement and coat proteins were amplified. Cloning and sequencing of the PCR product isolates facilitated designing new primers according to genotypes of local isolates. Subsequently, many GFLV isolates were detected and segments of their genomes were sequenced. Phylogenetic analysis based on the sequence data showed that the local isolates were distinct from previously reported strains and supported the hypothesis.

2009 Southern Division Meeting Abstracts

Abstracts presented at the joint meeting of the APS Southern Division and the Southern Association of Agricultural Scientists (SAAS) in Atlanta, Georgia, February 1–2, 2009. The abstracts are arranged alphabetically, by first author's name.

Recent advances in systematics, taxonomy, and evolution of rust fungi (Pucciniales) and their relatives
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Rust fungi (Basidiomycota, Pucciniomycotina, Pucciniales) consist of >7000 species of obligate plant pathogens that possess the most complex life cycles in the Eumycota. Historically there has been considerable variation in the taxonomic ranks, groupings, and names applied within these fungi and phylogenetic inference has been hampered by a lack of morphological characters and incomplete life cycle and host-specificity data. In this study, several genes (primarily 18S and 28S rDNA) were examined across the breadth of the Pucciniales to resolve systematic conflicts and provide a framework for further study of the group. It is concluded that morphology alone is a poor predictor of rust relationships at most levels. Host selection, on the other hand, has played a significant role in rust evolution. Additional questions regarding rust evolution that are addressed within a phylogenetic context include inference about ancestral rusts and the relative success of heteroecious versus autoecious lineages. Finally, molecular data are examined to make predictions about the life cycles of emergent invasive rusts.

Improving deposition and control of peanut diseases with early morning and evening fungicide sprays
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The effectiveness of a fungicide is determined in part by its concentration at the site of infection. Peanut has a dense canopy that is difficult to penetrate, therefore soilborne pathogens are hard to control with conventional sprays. Four applications of chlorothalonil (1.26 kg/ha a.i.), azoxystrobin (0.88 kg/ha a.i.), pyraclostrobin (0.88 kg/ha a.i.), and prothioconazole plus tebuconazole (0.58 kg/ha a.i.) were sprayed on peanut either i) early morning (3–5 am) when leaves were folded and wet, ii) after daylight (9–11 am) with unfolded and dried leaves, or iii) in the evening (9–10 pm) when leaves were folded but dry, to compare disease control and yield. Two field experiments were conducted in 2008 with cv. Georgia Green in a split-plot design. All three spray timings provided similar control of early leaf spot (*Cercospora arachidicola*), but early morning and evening sprays reduced southern stem rot (*Sclerotium rolfsii*) incidence by 32% and 23% compared to day sprays, respectively. Early morning and evening sprays increased yield by 547 kg/ha and 312 kg/ha, respectively, compared to the day sprays. These results suggest that early morning and evening sprays are effective on foliar diseases and can improve fungicide efficacy on southern stem rot, thus increasing peanut yield.

Efficacy of *Bacillus mycoides* isolate J on pecan scab
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Pecan scab (*Fusicladium effusum*) is a serious disease in the humid southeastern United States and growers usually apply 6–10 applications of fungicide annually. This study evaluated the efficacy of foliar applications of *B. mycoides* isolate J (BMJ) applied at 2–3 week intervals at a rate of 0.29 kg/ha. The induced systemic resistance from BMJ provided significant nut scab control in trials from 2006–2008, but control was less than that from commercial standards. Tank mixes of BMJ with ½ rates of either triphenyltin hydroxide (Super Tin 80WP) or dodine were as effective as the combination of those fungicides, or a full rate of Super Tin (0.53 kg/ha). If successfully registered for use, BMJ would be a valuable option for production of organic pecans. It also offers a unique mode of action that may have value in conventionally-managed orchards facing increasing problems from resistance to other fungicides.

The impact of rust diseases on the ornamental industry
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Relatively recent introductions of quarantine-significant rust fungi severely and negatively affected production of daylily and gladiolus, respectively. The introductions resulted in the implementation of costly quarantine and eradication measures, including crop destruction and little or no ability to ship product. *Puccinia hemerocallidis* was discovered on daylily in 2000 and had spread primarily on infected plants throughout much of the U.S. by 2003. At this time the federal quarantine was lifted. *Uromyces transversalis* was detected in Hawaii on cut gladiolus flowers in 2006 and subsequently found in Florida and California. Eradication measures have proven largely successful however repeat infestations have occurred at one farm in Florida (2007, 2008) and new infestations at homeowner locations in California (2008). Containment of *U. transversalis* to a limited number of sites suggests eradication is still attainable. A rapid response by regulatory agencies and cooperation by commercial growers and homeowners has limited spread of *U. transversalis* and additional damage to the industry.

Pathogenicity evaluations of nematophagous fungi to control the Reniform nematode (*Rotylenchulus reniformis*) under microplot conditions
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Nematophagous fungi were isolated from *Rotylenchulus reniformis* in Alabama. The pathogenicity of these fungi to control *R. reniformis* was evaluated under microplot conditions. Treatments were: 1) Control, 2) Temik 15G, 3) *Arthrobotrys dactyloides*, 4) *Dactylaria brochopaga* 5) *Paecilomyces lilacinus*, 6) *Arthrobotrys dactyloides* + *Dactylaria brochopaga* + *Paecilomyces lilacinus*. The experimental design was a complete randomized design with 5 repetitions, and the entire experiment was repeated twice. Fungi

were grown on barley seed for 60 days, and then applied 50 cm^3 per pot. Pots were filled with Decatur silt loam soil (sand, silt, clay of 24-49-29%) from nematode infested cotton crops. Additionally, each pot was inoculated with 2000 juveniles of *R. reniformis* and cotton cultivar ST 5599 BGRP was planted. Soil samples were taken at mid and late season days after planting (DAP), and nematodes extraction from 150 cm^3 of soil by the sucrose centrifugation-flotation method. Data were analyzed with SAS version 9.1.3 software using GLM procedure, and means compared using Fisher's protected least significant difference test. Plant growth was not affected by any fungi treatments during the season thus phytotoxicity is not a problem. Nematode numbers show that at mid season, all the treatments were similar to the control. However by late season, the Temik 15G treatment did reduced the number of nematodes compared to the control. Nematohpagous fungi treatments all produced similar numbers of nematodes as the control. When nematodes were counted using a compound microscope at 40x no colonized nematodes was observed. Previously, a reduction in numbers of *R. reniformis* was observed with the same fungi in autoclaved soil under greenhouse conditions. The present study suggests that there is a need to explore alternate formulations of these fungi, to provide an advantage over other micro-organisms that inhabit the soil, and achieve a successful control of *R. reniformis*.

Characterization of developmental mutants of *Fusarium graminearum*

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Phytopathology 99:S197

Fusarium graminearum is an important fungal pathogen of small grains and maize cultivated throughout the world. Losses are due to reduction in overall yield, seed quality, and contamination of infected grain with mycotoxins that make the harvest less suitable for human consumption. To better understand fungal development and its relationship with pathogenicity, we initiated a forward genetics approach to identify random-insertional mutants of *F. graminearum* wild-type strain (PH-1) impaired in asexual development. The process of conidiation is important to the survival and dispersal of a wide-range of fungal species that impact humans. Thus, understanding the sensing and signaling mechanisms that respond to environmental cues such as light and nutrient availability that influence fungal development, may reveal potential targets for controlling fungal pathogenesis. We identified two mutants, designated 6A8 and 8B5, that fail to produce macroconidia when cultured under conditions otherwise conducive for macroconidial development by wild-type and control strains. Two additional mutants, 8E8 and 8C2, were identified by screens developed to reveal gain-of-function phenotypes. We developed a culturing system to analyze the expression of differentially regulated genes and metabolite production in the various mutant backgrounds as compared to PH-1. Furthermore, plasmid rescue analysis has revealed the identity of a gene, putatively encoding a phosphatidylinositol transfer protein, SEC14, disrupted in mutant 8B5.

Real-time PCR-based detection and quantification of *Cercospora kikuchii* in soybean plants

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Cercospora kikuchii (Matsumoto & Tomoyasu) is the causal agent of leaf blight and purple seed stain in soybean. Soybean leaf blight, which usually occurs at late reproductive (R5 to R6) stages, has become prevalent in the southern United States, and it has been observed in the Midwest. Significant yield losses have been attributed to this disease. The most obvious symptom is the development of a purple cast on the younger, upper leaves that are exposed to direct sunlight. Once symptoms are apparent, the disease is very difficult to control, and yield loss is assured. Therefore, it is very important to detect the pathogen in soybean well before symptoms appear in order to implement disease management practices. In this research, a set of gene-specific real-time PCR primers and probe were developed based on the NADPH-dependent reductase (CTB6) gene sequence of *Cercospora kikuchii*, which can differentiate *Cercospora kikuchii* from *Cercospora sojina*, the causal agent of soybean frog eye leaf spot disease. The presence of 1 pg of *Cercospora kikuchii* genomic DNA in soybean leaf samples was detected with confidence. *Cercospora kikuchii* DNA was detected in soybean leaves collected as early as 22 days after planting (V3 stage) using this set of primers, and the level of *Cercospora kikuchii* DNA increased from 0.002% of total leaf DNA at early vegetative stage to 0.315% at the late reproductive stage.

Efficacy of brassica amendments for cotton disease management

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Phytopathology 99:S197

Numerous soilborne pathogens reduce stand establishment, plant development, and yields in cotton. Seedling disease pathogens, including *Rhizoctonia solani*, *Pythium* spp., *Thielaviopsis basicola*, and *Fusarium* spp., may reduce stands and early-season growth under favorable environmental conditions, even with the universal use of fungicide seed treatments. *Meloidogyne incognita*, the root-knot nematode, and *Rotylenchulus reniformis*, the reniform nematode, are common in cotton fields in the midsouth and fields are often treated with Telone II (1, 3-Dichloropropene) or Temik (Aldicarb). Brassica green manure amendments were compared with Telone II and winter fallow at two sites over two years for the management of soilborne diseases on cotton. Aboveground biomass of the Indian mustard cultivar Fumus averaged 13,000 kg/ha over sites. No changes in cotton stands were found among the treatments. However, brassica amendments were observed to reduce seedling root and hypocotyl disease symptoms in some years. Brassica amendments were observed to reduce early-season galling from the root-knot nematode in both years. Brassica treatments gave plant height increases similar to Telone II at both the root-knot nematode and reniform nematode locations. Brassica treatments reduced nematode populations compared to winter fallow throughout the growing season in both years. End-of-season cotton mapping indicated the number of bolls and yields for brassica treatments were similar or greater than those found for Telone II. Soil microfloral populations, specifically total bacterial, streptomycete, and fungal populations did not differ among treatments. Brassica winter cover crops appear to be effective in managing a number of diseases in cotton production systems and offer an alternative management strategy to fumigant nematicides.

Modeling acibenzolar-S-methyl field application for TSWV management in tobacco

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A field trial was designed to evaluate the reduction of Tomato Spotted Wilt Virus (TSWV) on tobacco using acibenzolar–S-methyl (Actigard) and Imidicloprid, (Admire). Float house applications of Actigard and Admire, plus field applications of Actigard made at one week increments after transplanting in the field, were evaluated. Admire and Actigard applied in the float house system reduced disease from 20% to 10% and disease was reduced to 3% for treatments receiving Actigard and Admire in the float house plus Actigard applications made at 5 and 6 weeks post transplant. Yields were inversely related to percent TSWV and ranged from 2,445 to 2,953 kg/ha. Numbers of thrips peaked at 5 to 6 weeks post transplant, indicating a direct relationship between thrips numbers and TSWV incidence. ELISA evaluations tended to be higher than percent incidence, suggesting not all infected plants displayed visible symptoms.

Effect of seeding rate on spotted wilt incidence in new peanut cultivars and breeding lines

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Tomato spotted wilt, caused by thrips-vectored tomato spotted wilt virus (TSWV), is a very serious problem in peanut (*Arachis hypogaea* L.) in the southeastern U.S. Establishment of plant density of 13 or more plants/m of row is recommended as part of an integrated management system for minimizing losses to spotted wilt. To achieve that plant density, growers often sow 19 or more seed/m of row. Cultivars with higher levels of field resistance than that of the standard moderately resistant cultivar, Georgia Green, might allow use of lower seeding density with subsequent lower seed cost, without increasing risk of damage by spotted wilt. In one field experiment in 2008, incidence of spotted wilt in new cultivars Florida-07, Georgia-06G, and Tifguard, was 18.3, 16.2, and 15.1%, respectively, at 9.8 seed/m of row and 12.8, 10.6, and 9.6%, respectively, at 19.7 seed/m of row, while incidence in Georgia Green was 53.9% and 40.7% for those same respective seeding rates (LSD = 3.6, P = 0.05). In another experiment in 2008, incidence of spotted wilt in genotypes GA 052524, GA 052527, GA 052529, Georgia-01R, Georgia-02C, and C724-19-25 was 9.1, 10.3, 6.0, 21.8, 12.5, and 18.9%, respectively, at 9.8 seed/m of row and 6.7, 4.1, 2.4, 15.5, 6.7, and 19.6%, respectively, at 19.7 seed/m of row; whereas, incidence in Georgia Green was 48.7% and 40.1% for the 9.8 seed/m and 19.7 seed/m seeding rates, respectively (LSD = 3.8, P = 0.05). These results indicate that levels of field resistance to TSWV in several new cultivars and breeding lines are adequate to allow use of lower seeding rates than with the moderately resistant cultivar Georgia Green without increasing the risk of losses to spotted wilt.

Update on southern corn rust caused by *Puccinia polysora*

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Yield losses to southern corn rust, caused *Puccinia polysora*, of over 45% have been reported. A single dominant gene, *Rpp9*, has been used in North America as a source of resistance to *P. polysora* though this gene has not been effective in other parts of the world due to the presence of virulent races. In July 2008, an *Rpp9*-virulent isolate was confirmed on *Rpp9*-resistant corn grown in Grady Co., GA. In August 2008, isolates of *P. polysora* collected from Macon Co, GA were also identified as *Rpp9* virulent; however, samples from Burke Co, in eastern GA were avirulent against the *Rpp9* gene. We believe this is the first public documentation of *Rpp9*-virulent isolates of *P. polysora* in the continental USA in the past 50 years. Hybrids containing the *Rpp9* gene may still be resistant in most of North America if the old, wild-type race of *P. polysora* is prevalent. If sporulating uredinia are found on hybrids with the *Rpp9* gene, applications of foliar fungicides may be warranted.

Epidemiological relevance of seed detection assay to seedling transmission threshold for bacterial fruit blotch in watermelon

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Bacterial fruit blotch (BFB), caused by *Acidovorax avenae* ssp. *citrulli* (Aac), is one of the most economically important diseases of watermelon worldwide. Contaminated seeds are the primary source of inoculum, and under ideal weather conditions, BFB can cause up to 100% crop loss. Seed testing is a critical component of BFB management. A disease transmission threshold of 1 infested seed per 10,000 is widely recognized as the tolerable inoculum threshold based on work done with black rot of crucifers. In practice, if 1 infested seed in 10,000 is detected, then a seedlot cannot be sold. Ideally, the detection threshold for a seed health assay should be more sensitive than the seedling transmission threshold. The objective of this research was to determine the epidemiological significance of the 1:10,000 inoculum threshold for Aac and ascertain its relevance to BFB seedling transmission. In two independent BFB seedling transmission studies conducted under greenhouse conditions, one seed with 10^7, 10^5, 10^3, and 10^1 (cfu)/seed, resulted in seedling disease in 100, 100, 75, and 15% of attempts, respectively. However, in four independent trials using immunomagnetic separation-polymerase chain reaction for seed health testing, one seed with 10^7, 10^5, 10^3, and 10^1 (cfu)/seed when combined with clean seeds (n = 10,000) separately, could be detected in 100, 100, 75, and 18.7% of attempts. These observations suggest that the 1:10,000 inoculum threshold is relevant when Aac populations are $\geq 10^5$ (cfu)/seed. When Aac populations are $\leq 10^3$ (cfu)/seed seedling transmission is significantly reduced. Hence a zero tolerance strategy is suitable for effective management of seedborne Aac inoculum.

Comparative analysis of copper tolerance testing methods in *Xanthomonas axonopodis* pv. *vesicatoria*

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Copper-based fungicides are widely used to control bacterial spot on peppers caused by *Xanthomonas axonopodis* pv. *vesicatoria* (XAV). Currently field isolates are tested for their tolerance to copper by applying a 10^6 cell suspension on a standard $CuS0_4$ amended media and monitoring the growth till 48 hours. This can be effective but only provides qualitative data. Experiments have shown that (XAV) isolates can have varying ranges of Cu tolerance. Isolates from three predetermined tolerance levels (tolerant, intermediate, and sensitive) were chosen. Treatments included nutrient broth (NB) alone, NB plus 125, 250, and 500 ppm $CuSO_4$. All isolates grown on NB alone grew at the expected rates and were comparable. However, on 250 ppm $CuSO_4$, the amount of bacterial cells present at 15 hours of growth was considerably less than the control. The tolerant isolates showed differences in OD_{600} and slope of the growth curve of 0.199 and 0.3, respectively, intermediate isolates showed differences of 0.136 and 0.34, while sensitive isolates were 0.157 and 0.05. All XAV isolates were completely killed on 500 ppm $CuSO_4$. Results for isolates tested on 125 ppm $CuSO_4$ will be discussed. These results suggest that different concentrations of Cu do have an affect on different isolates with respect to their rate of growth and that Cu tolerance in XAV is quantitative.

Characterization of cucurbit powdery mildew in north Florida

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Powdery mildew is a common and important foliar disease of cucurbit crops in all major vegetable producing regions of the world. In Florida, cucurbit Powdery mildew occurs on cucumber, melon, squash, pumpkin, and increasingly, on watermelon. Recently, the incidence and severity of disease outbreaks in Florida has increased resulting in a rise in crop loss and a growing need for improved cultivar resistance and fungicides (2). Cucurbit Powdery mildew is known to be caused by two obligate ascomycetous fungi, *Podosphaera xanthii* and *Golovinomyces cichoracearum* (1). Multiple physiological races have been defined in both fungi using muskmelon differentials (1,3). Race 1 of *P. xanthii* is the most common cucurbit Powdery mildew pathogen in the eastern U.S. (3). In our 2008 study, the disease response on five muskmelon differentials ('Topmark,' 'Edisto,' PI414723, PMR 45, PMR 5) planted at two north central Florida locations (Live Oak and Citra) did not fit the characterization for any one of the 3 physiological races known to be found in the U.S. It is likely that the Powdery mildew population was composed of mixed races, or that the mix of races may have contained one or more new, or unidentified races. Based on morphological characteristics the predominant pathogen was likely *P. xanthii* (1). Fungicide efficacy trials with 16 treatments applied at first sign of disease were established at Live Oak and Citra with highly susceptible 'Burpee Butterbush' butternut squash. At both locations disease pressure was moderate and fungicides did not provide significant control of Powdery mildew when compared to the untreated controls. At Citra, three of our treatments resulted in yields that were less than our untreated control. Loss of disease control with trifloxystrobin (QoI), thiophanate methyl (MBC), and triflumizole (DMI) suggested that there may be fungicide resistance present in the Powdery mildew pathogen population.

Soil fertility related to *Tomato spotted wilt virus* in tobacco

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Tobacco plots were established in the northernmost field of the Bowen Farm near Tifton, GA along the eastern perimeter and in the center of the field in 2007 and 208, respectively. Mean severity ratings for Tomato spotted wilt virus (TSWV) were recorded for 25 different plots across the field. Soil samples were taken from multiple locations within each plot in both years and combined into a composite sample for each of the 25 sites. In addition, 84 and 170 individual tobacco plants were selected from the center area of each field and rated for TSWV in 2007 and 2008, respectively. Soil samples were taken at the base of each plant and were analyzed individually. All soil samples (composite and individual) were analyzed for macro- and micronutrients. Individual nutrient values (lbs./A) and ratios of the different nutrients were regressed against TSWV severity ratings (0–10). The r-values of two ratios, namely phosphorus/magnesium and iron/copper, were significant at P = 0.05 or better as related to TSWV severity for both composite and individual samples in both years. In addition the ratio of copper/boron had a significant r-value as related to TSWV severity in both sampling methods in 2007 as well as with individual samples but not with composite samples in 2008.

Application of flutriafol and other fungicides via drip irrigation for control of cotton root rot caused by *Phymatotrichopsis omnivora*

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Phytopathology 99:S198

The experiment was done in a cotton field near San Angelo, TX with a history of severe root rot (CRR) caused by *Phymatotrichopsis omnivora*. Commercial formulations of fungicides were injected with a pump into drip tape when plants were at match head square growth stage on 23 June 2008 and again, three weeks later. The drip tape was 30 cm deep under the row, with emitters every 61 cm. The treatments and rates (kg/ha active ingredient) for each application were: propiconazole (2.91), azoxystrobin (1.12), prothioconazole combined with tebuconazole (2.24 of each), tetraconazole (2.24) and flutriafol (2.24). Each treatment was replicated three times. Each replicate was a single row, 198–228 m long. At the time of the initial application, a few plants were wilted. On 2 September, the mean CRR incidence of control rows was 75%. CRR incidence was significantly ($P < 0.05$) less with flutriafol treatment, only 2%. CRR with tetraconazole and propiconazole treatments were also significantly ($P < 0.05$) less, 60% and 53% incidence, respectively. CRR incidences with prothioconazole combined with tebuconazole, and azoxystro-

bin treatments were 74% and 73%, respectively, which was not significantly ($P < 0.05$) different from the control. The data suggests that flutriafol may have efficacy for CRR management, if future experiments demonstrate a high degree of control using lower, economical rates.

The affect of *Pythium* spp. and cold storage on the survival of longleaf pine seedlings after outplanting

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Prior to outplanting, cold storage of pine seedlings is a common practice used by managers of southern forest tree nurseries. Occasionally, bareroot seedling survival tends to be less after storage (>1 wk) for seedlings lifted during November to early December than when seedlings are lifted and stored in January. In contrast, survival of container-grown seedlings is not affected when stored at the same period for longer durations. There is some evidence that *Pythium* spp. could be infecting seedling roots through wounds sustained as they are lifted form nursery beds. The combination of the fungus, wounded roots, and the cool, moist environment in cold storage may encourage fungal growth and subsequent outplanting failure. The objective of this research was to examine if the presence of *Pythium* spp. had any effect on seedling survival and physiology after cold storage. Bareroot and container-grown longleaf pine (*Pinus palustris*) seedlings were inoculated with either *P. dimorphum* or *P. irregulare*. To simulate lifting damage, roots of container-grown seedlings in peat moss were either wounded or not wounded. After 12 weeks of storage, bareroot seedling survival was >20% and container seedling survival >70% for non-treated seedlings. Bareroot seedling survival was <5% and container-grown seedling survival >70% when inoculated with either *Pythium* spp. after 12 weeks of storage. To determine the effects of *Pythium* spp. on root growth potential, bareroot longleaf seedlings were inoculated with either *P. dimorphum* or *P. irregulare*, cold stored for 3 wk, and placed in a hydroponic system for 60 d. Root growth potential was not affected by *Pythium* spp., however, inoculation resulted in a reduction in root collar diameter. These results indicate that presence of *Pythium* spp. during cold storage can negatively affect seedling survival and early diameter growth of bareroot longleaf pine seedlings.

Impact of plant-parasitic nematodes on corn in Georgia

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In 2007 and 2008, corn trials were planted in Seminole Co. and in 2008 in Mitchell Co. Fields were naturally infested with root-knot nematodes (*Meloidogyne incognita*). In 2008 a trial was planted in Tift Co. in a field naturally infested with sting nematodes (*Belonolaimus* spp.). Seeds in each control were treated with Poncho 250 or Cruiser 5FS insecticides. Nematicides were Counter 15G (terbufos, 7.8 kg/ha) with untreated (2007) or insecticide-treated seed (2008) and Telone II (1,3-dichloropropene) plus insecticide-treated seeds. Telone II was applied at 28 and 46.7 L/ha (2007); and at 28 L/ha (2008). In 2007, Telone II reduced nematodes extracted per root system and increased nitrogen content in foliage over the control. Neither rate of Telone II nor Counter 15G increased yield over the control. In the 2008 Tift Co. trial, Telone II and Counter 15G improved plant vigor and yield. Telone II increased nitrogen content in foliage, plant height, and growth stage development. In 2008, Telone II improved yields over the control by 502 kg/ha (Seminole Co.) and 941 kg/ha (Mitchell Co.).

The threat of Ug99 stem rust and efforts towards breeding for resistance in wheat

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Breeding for resistance to stem rust in wheat throughout the world has been effective for over 30 years. This success has contributed to making the farm-to-table food supply chain in the United States the most effective in the world. Nevertheless, in 1999, a new race of stem rust (caused by *Puccinia graminis* f. sp. *tritici*) was detected in Uganda and has since spread east and north into Kenya, Ethiopia, Yemen, and Iran. The new race, designated Ug99, is virulent to the globally-deployed resistance gene, *Sr31*. Systematic screening of U.S. winter and spring wheat cultivars, breeding lines, and experimental germplasm began in 2005 in Njoro, Kenya. To date, nearly 10,000 lines have been screened for stem rust resistance in this program. Of the winter wheat cultivars in the U.S., about 65% of the hard red, 77% of the soft red, 72% of the hard white, and 93% of the soft white can be considered to be susceptible to moderately susceptible to Ug99 and its descendents having virulence to the widely deployed genes *Sr24* and *Sr36*. The U.S. spring wheat cultivars and germplasm have greater vulnerability to Ug99 than do winter wheats. Good levels of resistance can be found in U.S. durum wheat. New sources of seedling and adult-plant resistance are being pyramided into adapted U.S. germplasm.

Determination and compatibility of putatively hypovirulent and virulent isolates of *Cryphonectria parasitica* collected from the Great Smoky Mountains National Park

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A two-year study was conducted to characterize isolates of the chestnut blight fungus (*Cryphonectria parasitica*) from the Great Smoky Mountains National Park (GRSM). Of 339 isolates, 54 had abnormal cultural morphologies and 3 contained dsRNA. Analysis of vegetative compatibility (VC) divided all isolates into 34 groups, 16 of which only contained one isolate. A total of 19 isolates and 3 controls were inoculated onto healthy American chestnut trees in the Nantahala National Forest, North Carolina, and data on canker growth and stromata production were obtained over six months. Results from the field trial indicated that five isolates were potentially hypovirulent. Based on those data, one isolate (236-1C) has the greatest potential for use as a biological control agent for the pathogen in the GRSM, but compatibility is limited to select VC groups. Additional hypovirulent isolates representative of the other VC groups must be identified before large scale biocontrol can succeed.

Optimal timing of preventative fungicide applications for fairy ring caused by *Vascellum pratense* in creeping bentgrass putting greens

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Fairy ring symptoms occur around the outer edge of a subsurface fungal colony, where density of mycelium is greatest. This mycelial mat can result in soil hydrophobicity, resulting in turf loss and making delivery of curative fungicide applications difficult. In 2007 and 2008, single spring applications of the low and high rates of triadimefon and tebuconazole were evaluated for control of fairy ring on 'A-1' creeping bentgrass maintained under putting green conditions. Each treatment was applied when 5-day average soil temperatures (2 inch depth) reached 10°C, 13°C, 16°C, 18°C, 21°C, or 24°C. Treatments were arranged in a split plot design with timing as main plots and fungicides as subplots. Fungicides were applied in 0.08 L H_2O m^{-2} and immediately watered in by hand with 6 mm of irrigation. Surfactants were not tank-mixed with fungicides, but Cascade Plus (Precision Laboratories, Waukegan, IL) was applied on monthly intervals to prevent localized dry spot. Visual and objective disease severity ratings were taken every 7–14 days. Data were subjected to analysis of variance and means were separated with the Waller Duncan k-ratio t-test (k = 100). Fairy ring symptoms were most severe in 2007 due to drought conditions. In 2007, symptoms began to appear prior to the 24°C application timing, rendering these treatments curative in nature. In both years, all preventive fungicide treatments resulted in adequate control of fairy ring in early summer, but suppression failed later in the season. In 2007, plots treated with the low rate of triadimefon had higher disease severity in late summer than those treated with the high rate of triadimefon or either rate of tebuconazole. Area under the disease progress curve (AUDPC) was lowest for fungicides applied at 13°C, 16°C, and 18°C in 2007. In 2008, AUDPC values were highest for the earliest (10°C) and latest (24°C) application timings.

Effects of soil types on the reproduction of *Rotylenchulus reniformis* in cotton

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The reniform nematode, *Rotylenchulus reniformis*, is a leading economic pest in cotton in Alabama. Six soil types common to Alabama were evaluated for their effects on the reproduction of *R. reniformis* under irrigated and non-irrigated conditions. The test was conducted in microplots placed in a factorial within a RCBD replicated five times. The soil types evaluated were a Dothan sandy loam (S-S-C = 57-28-15), a Decatur silt loam (S-S-C = 24-49-28), a Hartsells fine sandy loam (S-S-C = 56-33-11), a Ruston very fine sandy loam (S-S-C = 59-33-8), a Pacolet sandy loam (S-S-C = 75-17-8), and a Vaiden clay (S-S-C = 5-42-53). Significant interactions between soil type and irrigation occurred for nematode reproduction and yield. At harvest, *R. reniformis* populations were significantly higher ($P < 0.10$) in the Decatur silt loam over the Hartsells fine sandy loam, the Ruston very fine sandy loam, and the Pacolet sandy loam. The Decatur silt loam also had higher populations

than the Vaiden clay and the Dothan sandy loam by an average of 3,785 and 5,647 vermiform/150cc soil respectively. Soil types with greater than 45% silt + clay (Decatur silt loam and Vaiden clay) had an average of 83% more *R. reniformis* than those without. *Rotylenchulus reniformis* populations were higher in the irrigated plots by an average of 54% at mid-season and an average of 30% at harvest. The Vaiden clay yielded significantly higher ($P < 0.10$) than all other soil types, while the Ruston very fine sandy loam yielded significantly lower ($P < 0.10$) than all other soil types. Yields were significantly higher ($P < 0.10$) in the non-irrigated plots. The combination of soil type and irrigation is directly related to *R. reniformis* reproduction and can potentially be used with other factors to predict cotton yield loss.

Asian soybean rust four years later: Is the disease a nonstarter or are we still at risk?

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Asian soybean rust (ASR) was discovered in the continental United States on November 6, 2004, in Louisiana. Plans to deal with this threat to the US soybean industry were formulated and enacted prior to the 2005 season. Initiated programs included a multistate fungicide evaluation project, a program to provide Section 18 labels for fungicides, a nationwide sentinel plot program, a spore trapping and reporting network, and a publicly accessible website that continues to provide daily disease updates. While models had been formulated regarding potential damage from ASR, there was still uncertainty about the eventual effects during the 2005 season and beyond. The 2005 and 2006 seasons were unusually dry and ASR remained confined to the South where it had overwintered in Florida. Left unchecked, the disease was destructive in Florida, Georgia, Louisiana and elsewhere. In 2007, ASR was found late in the growing season as far north as Ontario, Canada. We postulate that ASR must move from the Gulf South by mid-June to pose a threat to soybeans grown in Midwestern states. This will require unusually cool spring and early summer conditions in the South with appropriate winds or storm fronts. We must await such conditions before the full effects of ASR in the U.S. can be documented.

First report of tomato yellow leaf curl virus in Kentucky

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Tomato yellow leaf curl virus (TYLCV), occurs in many tomato-producing areas worldwide. In the United States, the virus was first reported in the late-1990's in Florida, and has since been found in another eight states in the southeastern, southern, and western portions of the country. In 2005, greenhouse-grown tomatoes with symptoms suggestive of tomato yellow leaf curl were discovered. Incidence was near 100% and a significant infestation of *Bemisia tabaci*, a known vector of TYLCV, was observed. From symptomatic plants, DNA was extracted and degenerate primers prV324 and prC889 were used to confirm the presence of TYLCV. Additionally, primers TYLCV CP-F (5′ CTATGTCGAAGCCACCAG 3′) and TYLCV CP-R (5′ GTAACAG-AAACTCATGATATA 3′) were used to obtain the complete sequence of the virus coat protein gene. Coat protein gene sequences were BLAST-searched, and were found to share 98–99% similarity with published TYLCV sequences. Additionally, three asymptomatic weeds (*Acalypha*, *Galinsoga*, and *Ipomea*) growing immediately adjacent to the greenhouse were sampled, and *Acalypha* tested positive for TYLCV despite. These results confirm the first reported case tomato yellow leaf curl, caused by TYLCV, of tomato in Kentucky. It appears likely that TYLCV was introduced into Kentucky from imported plant material carrying either the virus, the vector, or both. Although no subsequent outbreaks resulted from the case in 2005, TYLCV was again confirmed in one greenhouse in late summer of 2008. Widespread damage from tomato yellow leaf curl has not yet occurred in the state. However, multiple introductions of TYLCV (2005, 2008) point towards the potential for future problems.

FAME Analysis as an alternative means for distinguishing *Meloidogyne* species and races

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Fatty acid methyl ester (FAME) analysis can be used as a means for differentiating among plant-parasitic nematode genera. Species such as *Rotylenchulus reniformis*, *Heterodera glycines*, and *Meloidogyne incognita* all have significantly different fatty profiles (Mahalanobis distances ≥ 7.22, $P \leq 0.0005$) at concentrations greater than 250 individuals. We hypothesize that it will be possible to further demarcate among plant-parasitic species and races using FAME analysis. Fatty acids were extracted from samples containing 1000 individuals of each *Meloidogyne* species *M. arenaria* (Race 2), *M. hapla*, *M. incognita* (Races 1, 2, and 3), and *M. javanica* and analyzed using the FAME gas chromatography system. The resulting profiles generated by the Sherlock Analysis Software were then analyzed with the STEPDISC and CANDISC procedures of SAS version 9.1.3. All profiles were significantly different among species and races. The four *Meloidogyne* species separate out easily with a minimum Mahalanobis Distance (D^2) between *M. incognita* and *M. arenaria* (16.24, $P < 0.0001$). The first canonical axis defines 66.0% of the difference among species and 23.6% is defined by the second axis for a total of 89.6% defined by the first two axes. When the species are separated by race, the minimum $D^2 = 15.77$ ($P < 0.0001$) between *M. arenaria* Race 2 and *M. incognita* Race 1. D^2 values among *M. incognita* races are all significant at $P < 0.0001$ with a minimum distance between Race 1 and Race 3 of 57.8. A total of 82.5% of the differences among races within species was explained by the first two canonical axes; 57.6% in the first and 24.9% in the second. By incorporating these profiles into a Sherlock Analysis Software library, it is believed that the FAME method can be used to distinguish among *Meloidogyne* species and races and provide an alternative source of identification.

Taxonomy of the pecan scab fungus based on the cytochrome b gene sequence

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Pecan scab is the most devastating disease of pecan trees in the southeastern U.S. The pecan scab fungus was first described by George Winter in 1882 as *Fusicladium effusum*. However, since then, the fungus has been reclassified eight times, and renamed as *Cladosporium effusum*, *C. caryigenum* and *Fusicladosporium effusum* and most recently, as *Fusicladium effusum* based on ITS nrDNA data and conventional taxonomic methods. To better understand the taxonomy of the pecan scab fungus, in this study a conserved region of the mitochondrial cytochrome b gene was amplified and sequenced from three isolates of *Fusicladium effusum* and compared to other fungi. The obtained 195-201 bp sequences from these three isolates had 95% nucleic acid homology with the apple scab fungus, *Venturia inaequalis*. The 65 amino acids of *Fusicladium effusum*, had 100% amino acid homology with the amino acids coding the locus 201-266 on exon 5 of the cytochrome b gene of *V. inaequalis*. Additionally, in a maximum parsimony tree based on nucleotide sequences *Fusicladium effusum* clustered in a clade with *V. inaequalis* with a 92% bootstrap value. These results support the previous work that the pecan scab fungus and *V. inaequalis* are closely related and the fungus should be placed in the family Venturiacea.

Control of forest nursery seedling diseases of *Pinus* spp. with Proline 480 SC

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The availability of fungicides to control specific forest seedling nursery diseases is either nonexistent, limited or faces possible loss of registration. Proline 480 SC (41% prothioconazole) is a broad-spectrum systemic fungicide labeled for the control of ascomycetes, basidiomycetes, and deuteromycetes on numerous field crops. While not registered for forest seedlings; laboratory, greenhouse and field trials have shown Proline to be efficacious against three fungal pathogens that cause significant damage and seedling mortality in forest-tree nurseries. Disease control using Proline has been obtained at a 402 ml/ha application for the control of fusiform rust (*Cronartium quercum* f. sp. *fusiforme*) on loblolly pine (*Pinus taeda*) in the greenhouse and in two nursery field trials. In greenhouse trials, a biweekly application (402 ml/ha) controlled pitch canker (*Fusarium circinatum*) on longleaf pine (*Pinus palustris*) and resulted in an 11% increase in seedling production over non-treated seedlings. In vitro fungal growth studies on media amended with Proline resulted in fungicidal activity against *Fusarium circinatum* at all 3 rates (0.25x, 0.5x and 1x the label) used. A biweekly application of Proline in nursery field tests significantly reduced *Rhizoctonia* foliar blight on loblolly pine when compared to Heritage (50% azoxystrobin) and the non-treated control. In addition to disease control, Proline treated seedlings were significantly larger and appeared much greener than non-treated seedlings.

Detection limits and relative abundance of *Aspergillus flavus* in microbial communities of peanut soils

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Phytopathology 99:S201

Extracting total DNA from field soil samples and utilizing molecular diagnostic techniques may lead to a rapid identification of soil microbial communities that may reflect soil health. This study focuses on the soil microbial community in peanut fields as influenced by cropping sequences. In order to determine the sensitivity of kits used for extraction, the minimum microbial load of aflatoxin producing *Aspergillus flavus* in the soil at which the pathogen can be detected among soil microbial communities was determined. The methodology involved extraction of total soil genomic DNA in different peanut cropping sequences (continuous Peanut; continuous Bahia; Peanut-Cotton; and Peanut-Corn with four replications each) and sampling times with the DNA fingerprinting technique called ARISA (Automated Ribosomal Intergenic Spacer Analysis) using universal fungal specific primers targeting the 18S-28S region. Detection of *A. flavus* population loads in soils was carried out using *A. flavus* specific primers and through quantitative estimation on AFPA (*Aspergillus flavus* and *parasiticus* Agar) medium. The population level of *A. flavus* from the soil samples ranged from zero to 1.2×10^3 cfu g^{-1} soil but was not at detectable limits using *A. flavus* specific primers, FLA1 & FLA2. Investigations on determining the minimum detectable inoculum load at which the pathogen could be amplified from the total soil genomic DNA through artificial inoculation of *A. flavus* spore suspension at different concentrations revealed that a population density of 2.6×10^8 cfu g^{-1} soil is required. Therefore, some microbial population in fields at low levels may not be detected by current techniques. However, as more specificity of primers are developed, these techniques may provide a rapid method for determining the relative population levels of aflatoxin producing fungi as well as determining detection levels need for other microbes in soils.

Genetic structure of *Sclerotinia homoeocarpa* populations from turfgrasses in North America, Asia and Europe

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Phytopathology 99:S201

Sclerotinia homoeocarpa is the fungal pathogen responsible for dollar spot disease on turfgrasses. This pathogen infects all turf species and is found worldwide. *Sclerotinia homoeocarpa* was first described by F.T. Bennett in 1937. Bennett described the fungus as producing both apothecia and microsclerotia, both of which are not seen today. This has resulted in a scientific debate where most believe the fungus belongs to the family *Rutstroemiaceae* due to the production of substratal stromata. Isolates of *S. homoeocarpa* were obtained from turfgrass species collected in the United States, United Kingdom, Italy and Japan. Vegetative compatibility groups (VCGs) were evaluated on PDA amended with red food coloring, and eleven VCGs were identified in the sample population. Four loci (ITS, Beta-tubulin, IGS, and calmodulin) were amplified using PCR and then sequenced via cycle sequencing. Isolates of *S. sclerotiorum*, *Rutstroemia paludosa* and *R. cuniculi* were also included for comparison. All isolates analyzed thus far are distinct from *S. homoeocarpa* type-isolates described by Bennett in 1937. The results obtained to date indicate that genetic diversity among isolates is dependent on host species rather than geographic location, with isolates from warm- and cool-season turfgrasses separating into distinct clades. Host species is clearly a major factor that determines genetic diversity in populations of *S. homoeocarpa* causing dollar spot in turfgrasses. Although 10 VCGs were detected among isolates from cool-season (C3) turfgrasses, all have identical ITS, IGS, calmodulin and Beta-tubulin sequences. Additional methods such as mitochondrial gene analysis or microsatellites are needed to detect genetic variation within this group.

Efficacy of endophytes in the management of leaf blight disease of amaranth, plant growth promotion and in inducing systemic resistance

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Phytopathology 99:S201

Amaranth (*Amaranthus tricolor*), a member of Amaranthaceae, is a highly nutritious, inexpensive, leafy vegetable in the tropics. Among the different diseases of amaranth, leaf blight disease caused by *Rhizoctonia solani* Kuhn is a major production constraint. The pathogen infects more than 90% of plants in the field and causes considerable economic loss owing to reduction in the marketability of the produce. Farmers are reluctant to cultivate amaranth during monsoon seasons because of the susceptibility of this plant to leaf blight. Even though chemical control with Mancozeb is promising, use of chemicals on a regular basis is a serious human health concern. In an effort to find alternatives to chemical management, a study was conducted in Kerala in 2006–2007 to evaluate the efficiency of endophytic microbes on amaranth in the management of Rhizoctonia leaf blight. The methodology involved the isolation of endophytes, *in-vitro* evaluation of isolated endophytes against the pathogen *R. solani*, *in-vivo* pot culture evaluation of selected endophytes in comparison with the recommended chemical (Mancozeb at 0.2%), standard fungal (*Trichoderma viride* and *T. harzianum*) and bacterial (*Pseudomonas fluorescens*) biocontrol agents and estimation of enzymes related to induced systemic resistance. Forty-six bacterial and 17 fungal endophytes were isolated and evaluated against *R. solani* in dual culture studies. The results revealed that one endophytic fungus (EF-2) and six endophytic bacteria (EB-4, EB-20, EB-22, EB-38, EB-43 and EB-45) were antagonistic against pathogen with varying degrees of inhibition. In the pot culture experiment EB-22 and EB-43 were found to be effective in reducing leaf blight severity whereas EB-20, EB-22 and EB-43 were effective in plant growth promotion. EB-20 and EB-22 induced maximum levels of enzymes related to ISR.

Measurement of numbers of starch grains and cambial cells in roots of loblolly pines

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Phytopathology 99:S201

Quantitative histology was used to measure changes in diseased and healthy feeder roots of *Pinus taeda* L. Histological stain schedules were compared for their accuracy in describing root cell organelles. Feeder roots were fixed, cut, and stained for light microscopy. Two root traits were tabulated for their response to three staining schedules. A total of 300 sections of feeder roots were examined using the staining schedules of Papanicolaou, Hematoxylm-eosin and Periodic Schiff. No significant differences were found in the number of starch grains per cortical or cambial root cells when these three stains were compared. Starch averaged 7 grains per cell (range = 2.8 to 13). Cambial root cells averaged 8.6 grains per cell (range = 2.4 to 15). The intensity of these stains is ideal for measurements of cellular starch and cambial organelles in tree tissues affected by a variety of forest diseases.

Uredinia of Asian soybean rust as a unique niche for other fungi

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Phytopathology 99:S201

We examined uredinia of *Phakopsora pachyrhizi*, causal agent of Asian soybean rust, on field-grown soybean leaves as a niche for other fungi. An unidentified fungus was recovered from sporulating uredinia but not from noninfected leaves. While other fungi were recovered from uredinia, they were not unique to this niche. Observations with a scanning electron microscope revealed hyphae of this fungus intertwined with urediniospores within pustules. Inoculation of this fungus onto field-grown rust-infected leaves resulted in a significant reduction in hyaline urediniospore production within 7 days of inoculation. The fungus colonized rust pustules within 10 days, but it failed to establish on noninfected leaf surfaces. Colonized spores turned dark brown and did not germinate. There was no effect on number of urediniospores per pustule, but there was a significantly higher proportion of red-brown pustules on leaves that had been inoculated with the co-inhabitant. Prospects for using this fungus as a biological control agent will be discussed.

Effects of fumigants and in-furrow fungicides on Verticillium wilt development in peanut

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Phytopathology 99:S201

Verticillium wilt (caused by *Verticillium dahliae* Kleb.) is an increasingly important disease of peanut (*Arachis hypogaea* L.) throughout the southern High Plains of Texas. Field trials were conducted to evaluate the effects of the fumigants metam sodium or chloropicrin, as well in-furrow applications of azoxystrobin, prothioconazole, and acibenzolar-S-methyl. Metam sodium rates of 46.8 and 65.5 L/ha reduced soil populations of *V. dahliae* compared to non-treated controls; however, no differences in disease incidence, yield, or quality were observed. Chloropicrin did not impact *V. dahliae* populations. Disease incidence was 7–9% lower in plots treated with chloropicrin; however, this did not translate to any differences in yield or quality. The use of in-furrow fungicides had no effect on disease development or yield. Additional tactics need to be investigated for management of Verticillium wilt, so that peanut producers in the region can maximize yields and profitability.

Molecular and biochemical characterization of two PR10 proteins from *Zea mays*

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Phytopathology 99:S202

Pathogenesis-related protein 10 (PR10) is one of the seventeen pathogensis-related protein families that have been reported to play important roles in plant responses to biotic and abiotic stresses. A novel PR10 (*ZmPR10.1*), which has 89.8% and 85.7% identity to previous *ZmPR10* in nucleotide and amino acid sequence, respectively, was recently isolated from maize. *ZmPR10* and *ZmPR10.1* were highly expressed in root tissues, but low in other vegetative and reproductive organs with the level of *ZmPR10.1* consistently lower than that of *ZmPR10* in all tissues examined. The expressions of both genes were induced by most abiotic stresses including ethephon, SA, $CuCl_2$, H_2O_2, coldness, darkness and wound, and biotic stresses such as *Erwinia stewartii* and *Aspergillus flavus* infection. However, their expressions were up-regulated initially, but down-regulated later when treated with KT, GA_3, MeJA or NaCl. *ZmPR10.1* possessed significantly higher (8-fold) RNase activity *in vitro* than *ZmPR10* with the optimum pH and temperature for both proteins at 6.5 and 55°C, respectively. Their activity was significantly inhibited in the presence of 1.0 mM Cu^{2+}, Ag^{+}, Co^{2+}, SDS, EDTA, or DTT. In addition, *ZmPR10.1* like *ZmPR10* also showed antifungal activity against *A. flavus*, the causal agent of pre- and post-harvest aflatoxin contamination in several major agricultural crops.

2009 Potomac Division Meeting Abstracts

Abstracts presented at the APS Potomac Division meeting in Gettysburg, Pennsylvania, March 25–27, 2009. The abstracts are arranged alphabetically, by first author's name.

Trichoderma isolates from tropical environments induce resistance against _Phytophthora capsici_ in Korean hot pepper

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Phytopathology 99:S203

Isolates of several *Trichoderma* spp. were collected from environments where *Theobroma cacao* is grown as potential biocontrol agents for cacao diseases. The diversity of isolates collected led us to consider if these isolates have biocontrol activity in the *Phytophthora capsici* and Korean hot pepper (*Capsicum annuum*) pathosystem. Six *Trichoderma* isolates were tested for endophytic, mycoparasitic, antimicrobial, and induced resistance capabilities. Isolates DIS 70a, DIS 219b, and DIS 376f were mycoparasites of *P. capsici* in plate culture while isolates DIS 70a, DIS 259j, and DIS 320c produced antimicrobial compounds. Pepper seedlings were grown in soilless mix colonized by *Trichoderma* and analyzed for endophytic growth on roots and stems. All 6 isolates internally colonized pepper roots but not stems. Expression of plant defense related pepper ESTs were evaluated using RNA from leaves and roots of 32 day old *Trichoderma* colonized peppers. Microarray analysis of pepper gene expression 36 to 72 h after inoculation with isolates DIS 259j and DIS 376f identified a large group of highly induced EST putatively involved in plant defense. In bioassays, isolate DIS 376f provided the most consistent protection against *P. capsici*. Using the described screens, it may be possible to identify multiple pathways for protection of Korean hot pepper *Trichoderma* spp. leading to a logical approach of combining isolates to maximize synergistic effects and increase biocontrol efficacy.

Does snoA (suppressor-of-nimO) antagonize a DNA damage checkpoint pathway controlled by Rad9/53BP1 and gamma-H2AX?

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Phytopathology 99:S203

In the fungus *Aspergillus nidulans* the *Dbf4*-dependent kinase (DDK) is composed of regulatory and catalytic subunits encoded by *nimO*^{Dbf4} and *cdc7*, respectively. *nimO*^{Dbf4} associates with cdc7p, activating the kinase and escorting it to origins of replication where it triggers DNA synthesis. A *nimO* mutation, *nimO*18, confers temperature sensitive cell cycle arrest in late G1, and at permissive temperature exhibits profound sensitivity to agents that cause double strand breaks (DSBs), such as Diepoxyoctane (DEO). We discovered a novel suppressor of *nimO*18, called *snoA* (suppressor-of-nimO). Intriguingly, loss of *snoA* substantially alleviates the ts-lethal and DNA damage-sensitivities of *nimO*18, indicating that *snoA* may act normally to inhibit *nimO* function and thereby restrain DNA synthesis in response to DNA damage. In a search for other DNA damage responses that may be inhibited by *snoA*, we discovered that DEO-sensitive defects in gamma-*H2AX* (*H2AX*-S129A) and *Rad9*^{53BP1} (?*Rad9*) are also partially relieved by loss of *snoA*. gamma-*H2AX* and *Rad9*^{53BP1} are components of an ATM-dependent DNA damage response pathway that responds to DSBs. In this study, we are assessing epistasis relationships between gamma-*H2AX*, *Rad9*, *snoA*, and *ATM* with the aim to determine if *snoA* influences gamma-*H2AX* and *Rad9*^{53BP1} in an ATM-dependent or ATM-independent manner. (Supported by grants from Gettysburg College to SWJ and JRB).

Life cycle of _Uromyces salsolae_, a candidate fungal biological control agent for _Salsola tragus_

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Phytopathology 99:S203

Salsola tragus (Russian thistle, Chenopodiaceae) is a major weed pest in the western United States. An isolate of the rust fungus *Uromyces salsolae* from the Yasensky Spit in Russia is currently under evaluation as a candidate for biological control of *S. tragus* in a Biosafety Level 3 (BL-3) containment greenhouse facility. The life cycle of *U. salsolae* has been completed in greenhouse studies, demonstrating that it is macrocyclic and autoecious on Russian thistle. Plant inoculations were made with spores from each stage in the fungus life cycle, demonstrating their viability and role in the life cycle. Data will be included in a risk assessment of *U. salsolae* for biological control of *S. tragus*.

Greenhouse germination and characterization of _Synchytrium solstitiale_ resting spores

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Phytopathology 99:S203

During evaluation of *Synchytrium solstitiale* for biological control of yellow starthisle (YST, *Centaurea solstitialis*), protocol was developed for germination of resting spores. Resting spores mature in large numbers 7 to 10 days after galls develop, and they are distinct morphologically from sori in galls. Resting spores are dark, single-celled, and embedded within leaf and petiole tissues. The protocol involved dried leaves containing resting spores. Leaves were surface sterilized for 10 min in 10% bleach and rinsed (3 ×) for 10 min in sterile distilled water (SDW). Resting spores were scraped out of plant tissue, placed on 2% water agar (WA) and incubated in the dark at 10 (night) and 15 (day) centigrade. After 10–25 days, some resting spores germinated and formed a round, yellow-orange vesicle (that becomes a sorus) on the outside. Further development of the sorus occurred only after individual germinated resting spores were picked off of the WA and placed in SDW with streptomycin (100 ppm) in the well of a hanging-drop slide. Prepared slides were placed in a moist chamber and incubated under conditions mentioned above. Opaque orange sori changed to translucent pink sporangia in 2 to 24 hours, and movement of zoospores was seen after that development. Sporangia eventually ruptured and zoospores were released in a manner similar to that of release from sporangia developing from sori in galls. YST inoculated with germinated resting spores with sori became infected following the same protocol for inoculating YST with galled leaf material. These results prove the viability of resting spores and suggest they are a

functional part of the *S. solstitiale* life cycle. Characterization of resting spore germination should facilitate taxonomic treatment of *S. solstitiale*.

How does loss of snoA (suppressor-of-nimO) protect nimO (never-in-mitosis) mutants from genotoxic stress?
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Phytopathology 99:S204

In *Aspergillus nidulans*, *nimO*^{Dbf4} is the regulatory subunit of *Dbf4* dependent kinase (DDK), which acts to trigger DNA synthesis at origins of replication. The *nimO*18 mutation confers temperature sensitive cell cycle arrest at G1/S, and at permissive temperature exhibits profound sensitivity to agents that cause double strand breaks (DSBs), such as Phleomycin (PHL). We identified a *nimO*18 suppressor, called *snoA* (suppressor-of-nimO), by mutations that rescued *nimO*18 ts-lethality. Intriguingly, loss of *snoA* function also substantially protects *nimO*18 from PHL, restoring near-wild type PHL-resistance. By examining the response of *nimO*18 to acute versus chronic PHL exposure, I was able to determine that the PHL sensitivity of *nimO*18 results from a defect in DNA repair, rather than by disabling a DNA damage checkpoint. I am examining how replication and repair processes may be perturbed by examining the recovery of mutants after induction of the S phase DNA replication checkpoint via hydroxyurea (HU) block-release experiments. Surprisingly, although *nimO*18 grows ~20% more slowly than WT, *nimO*18 mutants recover more quickly from HU-induced S phase arrest, and loss of *snoA* in turn slows this recovery. I am using a similar approach to determine how *nimO*18 and *snoA* mutants recover from DNA damage incurred during S phase. (Supported by grants from Gettysburg College to SWJ and LAG).

Functional characterization of the *HYR1* gene involved in detoxification of reactive oxygen species in the rice blast pathogen
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Phytopathology 99:S204

Rice blast fungus, *Magnaporthe oryzae*, causes a serious disease in cultivated rice worldwide. When compatible interactions happen between plants and pathogens, the plant releases reactive oxygen species (ROS) to attack the pathogen and protect itself. The pathogen has its own mechanism to cleave those ROS. *HYR1* gene (MGG_04476) is one such candidate and encodes a glutathione peroxidase (GSHPx) domain in *M. oryzae*. Its homologue in the yeast, *Saccharomyces cerevisiae*, is Hyr1/YIR037W and was reported to be a glutathione-dependent phospholipid peroxidase (PhGpx) that specifically detoxifies phospholipid peroxide. To characterize it in *M. oryzae*, we have successfully knocked out *HYR1* gene using homologous recombination method. The knockout mutants did not show any abnormal phenotypes in spore germination or pathogenicity assays, but we observe increased levels of growth inhibition when grown in complete media containing 0.5 mM, 1.0 mM and 2.0 mM hydrogen peroxide, respectively. Moreover, in yeast, Hyr1 has been identified to sense and transfer the oxidative signal to the transcription factor Yap1 upon accumulation of H_2O_2. So in order to figure out the pathway of how this gene interacts with other reactive oxygen species cleavage-related genes in *M. oryzae*, we are extracting RNA from the *hyr1* mutants and trying to compare their gene expression levels upon hydrogen peroxide contact. Thus far, we can conclude that the *HYR1* gene is important for growing allowing the fungus to grow in low levels of hydrogen peroxide, but this gene does not seem to play a role in spore germination or pathogenicity.

Chlorosis and necrosis associated with introgression of a barley oxalate oxidase gene into flue-cured tobacco
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Phytopathology 99:S204

Rhizoctonia solani causes damping-off and sore shin on tobacco (*Nicotiana tabacum*) seedlings, while basidiospores of its teleomorph, *Thanatephorus cucumeris*, cause a foliar disease referred to as "Target Spot". Oxalic acid production may be an important factor the initiation of these important tobacco diseases. Field experiments were conducted in 2007 and 2008 to compare target spot severity among 7 transgenic entries of flue-cured tobacco cultivar 'K 326' containing a barley oxalate oxidase gene with disease levels on the non-transformed wild-type. Both experiments included the 7 transformants and the non-transformed wild-type, and were replicated 4 times. The 2007 experiment was arranged in a randomized complete block design, while the 2008 test was arranged in a split-plot design in which main plots were composed of 2 harvest treatments. Plots were either harvested sequentially (typical for flue-cured tobacco) in 2008 or harvested once at the end of the season. The 7 transformants and the non-transformed wild-type were randomized within each harvest method. Target spot severity was extremely low on all entries in both trials, but in both years an unusual and severe foliar chlorosis and necrosis was observed among the transgenic lines, but not in the non-transformed wild-type. Symptoms were first observed near the topping stage of plant development and worsened as leaf was harvested. Chlorosis on 29 Aug 2007 was greater ($P \leq 0.05$) for transgenic entries 15-5, 7-2, and 7-4 compared to 8-4, 5-1, and 5-2. A 1 through 5 rating scale for necrosis was also greater on 11 Sep 2007 for these same three entries versus the transgenic entries 8-4 and 5-1. Similar trends were observed in 2008, and were consistent across the 2 harvest methods evaluated. The causes and impacts of these symptoms are currently unknown and are being investigated.

Mitigating deoxynivalenol contamination in hulless barley and fuel ethanol co-products
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Phytopathology 99:S204

Hulless barley (HLSB) is a new and emerging crop in Virginia, and may be an important source of fuel ethanol in the future. Dried distiller's grains with solubles (DDGS), a co-product of fuel ethanol fermentation, are rapidly becoming one of the main sources of feed for domestic animals. Fuel ethanol production may concentrate mycotoxins such as deoxynivalenol (DON) in DDGS, posing a significant threat to domestic animal health. Our work aims to genetically engineer Virginia HLSB lines with reduced DON potential and thus provide a safe supply of DDGS for animal feed. We determined the DON potential of 20 Virginia HLSB lines, and a number of these lines demonstrated low levels of DON across three years of testing. We generated callus from 17 HLSB lines, and five of the lines were selected for further tissue culturing analyses and genetic transformation. We cloned TRI101, a gene encoding a 3-O-acetyltransferase responsible for the conversion of DON to 3-acetyl-DON, from four different species of Fusarium. We expressed these genes in vitro, and assessed their ability to detoxify DON in a series of feeding studies. We are currently moving TRI101 into five selected HLSB lines, and we plan to monitor decreases in DON in both raw grain and DDGS following fuel ethanol production using our genetically-engineered lines.

Identification and characterization of a MAS3-homolog from the rice blast fungus, which is potentially involved in a novel function in appressorial development
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Phytopathology 99:S204

Magnaporthe oryzae, the causal agent of the most threatening disease of rice, uses a specialized structure called an appressorium to infect its plant hosts. The appressorium further develops a penetration peg to invade the plant cell. Thus far, more than thirty genes have been identified that play a role in appressorial formation and development through targeted deletion studies. In order to identify more genes involved in *M. oryzae* pathogenicity, we undertook a global gene expression experiment using microarrays. Of the genes with interesting expression profiles, we are currently studying one with similarity to a *MAS3* (Magnaporthe appressoria specific) virulence factor gene; this gene showed increased expression 72 hours post-inoculation of barley, and during carbon and nitrogen starvation conditions. Targeted replacement of the MAS3-similar gene resulted in mutants with decreased appressorial formation. Database searches with the sequence of this gene revealed the presence of a CAS (capsule-associated) transmembrane domain from *Cryptococcus neoformans*, a human pathogen. This gene also presented protein homology to two other previously described *M. oryzae* genes, termed *GAS1* and *GAS2*, which were involved in pathogenicity by decreasing the ability of the fungus to invade the plant, however, these mutants showed no appressorial defects. Based on the contrast between the homology of this newly-identified gene as well as its phenotype compared to the *GAS1* and *GAS2* genes, we proposed that this gene is involved in a novel function, which affects appressorial formation and potentially pathogenicity.

Activation of plant defense genes of *Theobroma cacao* using endophytic *Bacillus* spp.
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Phytopathology 99:S204

In cacao, increasing loss to diseases supported by interest in sustainable agriculture has lead to research on biocontrol options for reducing cacao diseases. Endophytic *Bacillus* spp. were isolated from superior cacao trees near Quevedo, Ecuador and screened as potential biological control agents of cacao diseases. One elite *Bacillus* spp. reduced witches' broom disease over

18-months of field evaluations, while a second isolate reduced disease during the dry seasons. It is hypothesized that one mode of action for this disease reduction is induced resistance. The two *Bacillus* spp. were tested for their ability to activate plant defense genes of the susceptible Pound7 genotype. Surface sterilized seeds were treated with log 8.0 CFU/ml bacteria, then planted into a sterile soil mix in double magenta boxes. Boxes were maintained in a growth chamber and opened at onset of leaf flush. At maturation of the initial leaf flush, samples were collected to determine impact of endophytes on gene expression. Additional plants were sprayed with *Phytophthora capsici* zoospores (log 3.0 spores/ml) and harvested 24-hours later to determine impact on priming. Analysis of gene expression, using quantitative-PCR determined that endophytic colonization of cacao plants by native *Bacillus* spp. altered expression of some cacao defense genes. Data will be presented on the effects of endophytic colonization on cacao defenses and priming for plant defenses.

Preliminary evidence for mixed populations of Ca. Liberibacter species in Huanglongbing infections

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Huanglongbing (HLB) is the most serious insect-transmitted disease of citrus in the world. Originally found only in Africa and Asia, it was discovered in Brazil and Florida in 2004 and 2005, respectively. Three Candidatus Liberibacter species, Ca. L. asiaticus (Las), Ca. L. africanus (Laf), and Ca. L. americanus (Lam) have been identified as causal agents. DNA was extracted and the ITS region was cloned from 29 different HLB samples from four continents (11 countries). Up to 50 clones per sample were sequenced. In 84 clones from seven single HLB samples from China, Indonesia, Japan, Philippines, Taiwan, Thailand, and Vietnam only Las was found. A single sample from India, contained 3 Laf and 24 Las clones. All 87 clones of three samples from South Africa were identified as Laf. In 168 clones of 13 samples from Brazil, 124 were identified as Las, 30 were identified as Laf, and 14 were identified as Lam. Single clones from India and Taiwan appeared to be recombinants of Las and Laf. Seventy-one of 72 clones from three samples from Florida were Las, with the lone exception being a Laf clone. These results demonstrate that multiple Liberibacter species can coexist in a single plant.

Detection limit of *Phytophthora ramorum*-infected *Rhododendron* leaves using the Cepheid SmartCycler

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The Sudden Oak Death pathogen, *Phytophthora ramorum*, has killed thousands of trees in the coastal forests of California and has affected numerous other plant species in nurseries by causing a range of symptoms from small lesions to plant death. The devastating impact of this pathogen has prompted quarantines to prevent pathogen spread and increased sampling to identify infected areas. Since lesions vary in their size and number, the goal of this study was to determine the smallest amount of tissue needed to consistently detect *P. ramorum* from *Rhododendron* 'Cunningham White' plants. Using a previously described mitochondrial based real-time PCR assay and a Cepheid SmartCycler®, DNA samples extracted from symptomatic and asymptomatic tissues were tested. Consistently reproducible results were possible with as little as 19.2 mm2 of tissue taken from the margin of a lesion. Increasing the amount of infected tissue for the DNA extraction did not seem to improve detection. Varied results were obtained for DNA samples extracted from asymptomatic leaf tissues.

Sporulation capacity of *Phytophthora ramorum* on northern red oak and chestnut oak

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Branches from six 2 to 3-year old northern red and chestnut oak seedlings were dip-inoculated with ca. 5,000 sporangia/ml of *P. ramorum* isolate Pr-6 and incubated at 100% relative humidity in dew chambers for 6 days. Three plants were then used to assess sporangia production, while the other three plants were used to assess chlamydospore production. Sporangia production was evaluated by incubating infected seedlings in a mist chamber and collecting sporangia produced on four misted leaves per plant suspended over 15µ-diameter nylon mesh screens. Chlamydospore content of leaf disks (6 mm diameter) removed from diseased leaves following a one month incubation in a greenhouse was also determined. Chestnut oak exhibited significantly greater disease incidence and severity compared with northern red oak ($P < 0.01$). However, sporulation levels were observed to be much larger in northern red oak. Total sporangia production per plant was not significantly different between the two species but when adjusted by lesion area, northern red oak produced 2294 sporangia/cm^2 compared with only 259 sporangia/cm^2 for chestnut oak ($P < 0.05$). Mean chlamydospore production per 6 mm-diameter leaf disk also was significantly greater for northern red oak compared with chestnut oak (28 versus 1 chlamydospore per disk). Knowledge of *P. ramorum* sporulation capacity in relation to disease incidence and severity on Eastern U.S. oak species will help determine the potential for epidemic development should the pathogen be introduced.

Susceptibility of sprouted oak acorns to *Phytophthora ramorum* zoospores

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Phytophthora ramorum is a recently emerged pathogen having established in Europe and several western U.S. states, including California and Oregon. It has a wide host range and is a threat to forest ecology and the nursery industry. In California, coast live oak (*Quercus agrifolia*) is a major host in natural settings. Although *P. ramorum* has not established in the eastern U.S., artificial stem and foliar inoculations have demonstrated that native eastern *Quercus* spp. are susceptible when inoculated with sporangia. The purpose of this study was to determine if the primary roots of different *Quercus* spp. native to the eastern U.S. could be infected by *P. ramorum* zoospores, which could be released from sporangia into natural water run-off. Sprouted acorns of *Q. rubra, Q. palustrus, Q. coccinia, Q. alba, Q. michauxii* and *Q. prinus* were exposed to motile zoospores (3000/ml) of *P. ramorum* for 1, 6, or 24 h, rinsed in water to remove any nonattached cysts, and transplanted to potting soil. After 4 weeks, the roots were weighed, surface sterilized, plated on PARPH+V8 selective medium and incubated for 5 to 7 days at 20°C. Developing *P. ramorum* was identified visually based upon colony morphology and characteristic chlamydospores and sporangia. Results showed that the primary roots of all oak species tested were susceptible to *P. ramorum* zoospores, and that infection could occur when exposed for only 1 h to the inoculum. Root weights were not negatively impacted by exposure to *P. ramorum* after 4 weeks, regardless of the oak species ($P = 0.746$).

Efficacy of acibenzolar-S-methyl and fungicides for Fusarium wilt of watermelon

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A preliminary experiment was conducted in the greenhouse in the fall of 2007 to evaluate the efficacy of soil-applied treatments for management of Fusarium wilt in watermelon caused by *Fusarium oxysporum* f. sp. *niveum*. Fifteen treatments [14 fungicides and a systemic acquired resistance (SAR) inducer] or water were applied to potting mixture in pots containing watermelon seedlings. Azoxystrobin (Quadris), acibenzolar-S-methyl (Actigard, the SAR inducer), thiophanate-methyl (Topsin M), prothioconazole (Proline), metconazole, and ipconazole (Vortex) reduced Fusarium wilt incidence and severity. These six chemicals applied once at transplant were also evaluated in naturally infested watermelon fields in Delaware and Maryland in 2008, which had moderate and high levels of *F. oxysporum* f. sp. *niveum*, respectively. In Delaware, acibenzolar-S-methyl, thiophanate-methyl, prothioconazole, and ipconazole significantly reduced wilt at 2½ weeks after transplanting compared to untreated plants. These treatments also had the highest numerical vine length and plot vigor scores although there were no significant differences among treatments ($P > F = 0.0551$ and 0.0516, respectively). In Maryland, all treatments except azoxystrobin reduced wilt incidence 4 and 5 weeks after transplanting but not at 6, 7, or 8 weeks. Marketable fruit yields did not differ among treatments in either state. Metconazole caused phytotoxicity in all experiments.

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