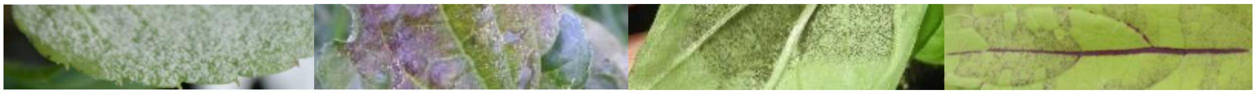

IR-4 Ornamental Horticulture Program: Downy Mildew Workshop

Identifying Knowledge Gaps and Novel Management Strategies for Downy Mildews Impacting Environmental Horticulture Crops

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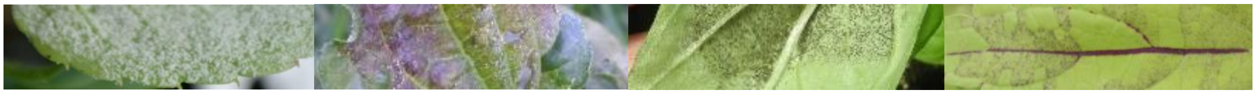


Introduction

The IR-4 Ornamental Horticulture Program hosted this Downy Mildew Workshop, funded by USDA-NIFA-SCRI (Award# 2016-51181-25407), on November 15-16, 2016 in Philadelphia, PA. Scientists, extension personnel, growers, trade association representatives, and registrants gather to discuss what is currently known about downy mildew diseases (DMs) of environmental (aka ornamental) horticulture crops (EHCs) and propose research and outreach objectives. The following white paper summarizes presentations covering the basic problem of EHC DMs, what growers may know about these diseases, their economic impact, their biology and epidemiology, current status of genomics, management options, how to assess barriers for adopting the latest management techniques, operational risk assessments and opportunities for improved outreach and communication of research findings. In addition to the presentations, a grower panel and multiple discussion sessions fostered conversations on these topics enabling the group to effectively brainstorm research and outreach questions to develop into objectives.

Presentation Summaries

Characterizing the Problem (Daughtrey/Ali/Shishkoff)

Nina Shishkoff

Many cases of downy mildew diseases have been prevalent in the news

Downy mildews are obligate parasites that must be on a living host to grow. They belong to the same group of organisms as root rots and potato rots which are represented by Pythium and Phytophthora. Pythium can persist is soils for many years and travel short distances. Phytophthora species can persist in soils for many years and can travel moderate distances through water splashed and wind dispersed spores. In contrast, downy mildews can travel very far via wind dispersed spores; they can also overwinter on plant debris. As an example, cucurbit downy mildew, *Pseudoperonospora cubensis*, travels from host to host up the east coast via wind dispersal.

World trade contributes to spread of pathovars, races and mating types around the world.

Downy mildews produce many, many sporangiospores and thus random mutations occurring during reproduction of these organisms can rapidly lead to development of resistance to fungicides as well as to changes in host range.

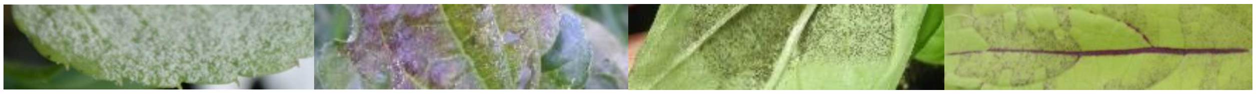
The impact of climate change is unknown. We cannot anticipate which diseases will become prevalent or re-emerge.

Margery Daughtrey

Some notes on old & new downy mildews:

Rose – Hybrid tea roses have long been known to defoliate when attacked by downy mildew. This same symptom can be seen with today's Knockouts and other roses for the landscape, and with miniature roses grown in the greenhouse. High humidity conditions at the optimum temperature produce serious symptoms: both leaves and canes are infected.

Snapdragon – The downy mildew found on snapdragon is a systemic disease, and is possibly seedborne. There may be local and systemic infection on the same plant—individual leaves may show yellow



blotches, and the growing point and the top of the plant can be stunted and chlorotic. Infected seedlings can be severely stunted. This disease is not seen as often as it used to be, as snapdragon production for cut flowers is not as common in US greenhouses as it once was.

Argyranthemum (Marguerite daisy) – This is both a cut flower crop and a bedding plant. *Peronospora radii* infects petals, a unique feature of this downy mildew. It is prevalent in foggy zones in CA and has also been seen on the East Coast. Like other downy mildews, it is hard to identify unless you use a microscope to identify the sporulation: making agar cultures will not allow the pathogen to grow out, as it is an obligate parasite. A diagnostician must think of the possibility of a downy mildew in order to make a correct diagnosis. This DM was found in Europe in the 1960s, then spread to Israel, north Africa, and Mexico, but it was not in US until 2004. We are left with two key questions: how did it spread, and where did it come from originally?

Coleus – DM symptoms are leaf spots or flecks, and sometimes leaf twisting. DM spots and lesions look different from cultivar to cultivar. Leaf drop also occurs. Symptoms are especially severe and conspicuous on light green cultivars. Dark grey sporulation may be seen on the leaf undersurfaces.

Agastache – This crop is grown in perennial nurseries and by the bedding plant industry; it is affected by the same pathogen as on coleus. The downy mildew affecting coleus, a *Peronospora* sp., was first reported from the US, after observations in NY and LA. Growers have indicated that it was in FL early on as well.

Impatiens – This plant shows extremely rapid destruction by downy mildew disease (*Plasmopara obducens*). On mature plants, the flowers deteriorate first;, then the leaves become chlorotic and fall from the plant. Although established on native *Impatiens* species as early as the 1800s, this disease was not seen in the US in 2003, when it first appeared in the UK. In 2004, however, it appeared for the first time in NY, TN and CA.

Rudbeckia – The very popular 'Goldsturm' has been the primary cultivar with this problem. Downy mildew on rudbeckia is caused by *Plasmopara halstedii*: plants show purpling or chlorosis on the upper surface of the younger leaves, and white sporulation on undersurface of the discolored leaves. Different species and cultivars of rudbeckia have varying resistance/susceptibility; Chuan Hong saw no symptoms on cultivars or species other than 'Goldsturm' in a nursery outbreak in Virginia.

Ornamental kale & cabbage – Downy mildew is really problematic on ornamental cole crops because every leaf counts in the ornamentals industry. Growers of cabbage for food production, in contrast, can simply strip off lower leaves that show yellowing and lesions before sale.

Alyssum – With this bedding plant, one might question whether downy mildew is in the seed because disease is often seen in plug trays, early in the life of the plants. The leaves are tiny, making the disease harder to identify than others.

Lamium – This ground cover perennial frequently has downy mildew, but growers and gardeners are apparently not noticing the problem or are simply not concerned with the level of chlorotic and necrotic plant spotting that results. Plants are not killed by the downy mildew.

Geranium species – On hardy perennial geraniums, downy mildew disease is characterized by yellow patches between veins, matched by sporulation on the undersurface.

Geum – This perennial flower in the Rose family almost always has downy mildew. Foliar spotting (yellow or brown) could be mistaken for nutrient deficiency.

Bacopa – this crop (now generally considered to be a *Sutera*) has shown downy mildew sporulation on upper and lower leaf surfaces. This is a potential sleeper for a major epidemic, but the pathogen has not yet been identified. DM has not been a "big deal" in the industry to date, but perhaps will be emergent when the right climate/cultivar combination occurs in the future.

Buddleia – In this shrub, often downy mildew symptoms are misidentified as feeding injury of foliar nematodes.

Osteospermum – Downy mildew on this host causes purple leaves/chlorosis.
Viburnum – Downy mildew has been striking in the landscape on *V. odoratissimum* 'Awabuki' (mirror-leaf) and more recently on *V. suspensum*. Several other species show less dramatic infection. Many people in FL replaced *Ficus* hedges with viburnum because of various pests. Now the new viburnum hedges are becoming defoliated due to downy mildew caused by *Plasmopara viburni*. This DM has been problematic since 2009 in Florida. DM has also been seen in New York a few times, but not recently. In Florida, high humidity periods will cause the pathogen to multiply rapidly and cause a lot of damage. With either *V. odoratissimum* "Awabuki" or *V. suspensum* 'Suspensum' there is a lot of hedge defoliation in the urban landscape.

Foxglove (*Digitalis* and hybrids of *Digitalis* and *Isoplexis* = *Digiplexis*) – These are hot new crops due to horticulturally desirable cultivars. Downy mildew is new to this crop in the ornamental trade: the symptoms of reddening and browning of leaves could go unrecognized by all but the most curious grower. *Peronospora digitalidis* is the pathogen; it was reported most recently from CA, but has been long known in Europe

Ice plant (*Aptenia cordyfolia*) – the cultivar 'Red Apple' recently was wiped out in landscapes after being a high-performance groundcover for 30 years. A widespread outbreak in December 2015 made the newspapers. The pathogen has been in the US, but was not reported before on this particular plant, which is a popular though invasive ground-cover in CA.

Pansy – Downy mildew has appeared occasionally with this crop, most often in the Pacific Northwest where environmental conditions are humid and temperatures are conducive to the disease. This also could be one of the downy mildews that might explode in the future.
From observing the patterns of what has happened in the ornamentals industry over the past several decades, it appears that today's downy mildew disease curiosities may be tomorrow's serious problems.

Nina Shishkoff
Some lessons from edible crop systems:
Lettuce – Downy mildew is caused by *Bremia lactucae*. In the US, lettuce is growing in some region of the country at all times, because there are overlapping growing seasons and extension of growing seasons with the use of high tunnels. It is typically grown as a monoculture crop and subsequently there is heavy use of fungicides. Like other downy mildews, *B. lactuacae* often develops resistance to fungicides, so resistant lettuce cultivars have been developed over many years. While 27 major resistance genes have been catalogued in lettuce, this pathogen can overcome these genes. This is the most well-studied gene-for-gene system amongst downy mildew pathogens. New molecular

technologies are being explored such as sequencing of resistance genes and comparison to R genes in other hosts and estimating the mutation rate for new R genes in pathogen to provide an estimate how long potentially before resistance breaks down. Gene silencing is being used to determine role of host and pathogen.

Cucurbit – Cucumbers are the 4^{th} most grown vegetable crop. Historically, downy mildew was controlled with host resistance. However, in 2004, this pathogen overcame host resistance. *Pseudoperonospora cubensis* caused major epidemics after 60 years of successful management. Possible host factors for this shift include large acreage, monoculture, multiple growing seasons, new cultivars, and climate change. Pathogen factors include new pathovars, races, and mating types along with the development of fungicide resistance. With new races arising, there was a new possibility for sexual recombination and oospore development along with the further development of new races arising from the genetic mixing. This could lead to overwintering in colder climates; historically, it survived in FL during winter months and in northern greenhouse operations prior to infections with sporangiospores.
There is currently some evidence of possible overwintering on wild hosts in addition to the typical survival in semi-tropical areas and in greenhouses.

Grape - Downy mildew of grape is caused by *Plasmopara viticola*. This North American native pathogen spread to Europe in the 19^{th} century, which devastated the European grape industry. Many attempts have been made to accurately model disease epidemiology to reduce the number of fungicide sprays.

Sunflower - Sunflower is widely grown for oil production, and downy mildew, caused by *P. halsteadii*, is the most damaging disease. This pathogen can be seedborne, causes systemic infections, can persist in the soil and has wild hosts. It is homothallic, but is constantly changing genetically. It continues to develop resistance to fungicides and host defenses.

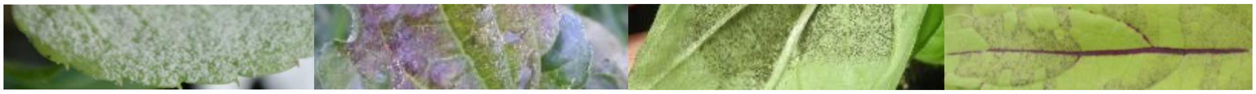
These edible crops have several characteristics in common. They are major crops in developed countries, grown over a wide region and primarily grown as monocultures. Because of their large acreages, they are ideal for major downy mildew outbreaks.

Discussion

Floriculture- instead of thinking about floriculture as many individual crops, think of it as a major block.
Some potential questions:
What characteristics do our ornamental plant hosts share?
What cultural controls applicable to most ornamentals
Should pesticide research concentrate on protectants or can there be threshold-based control after a DM shows up?
What are some controls suitable for organic growing? Downy mildew is a big problem in basil production.
Are disease free seeds/cuttings needed? If so, how can they be produced? How can we sanitize seed?
It is important to get fungicides labeled for DMs of small crops and greenhouses

Grower Survey Results (Baysal-Gurel)

The grower survey started October 25^{th}. Though Nov 9^{th}, there were 28 responses, and most were from growers. The questions were divided between demographics, current impacts from downy mildews, and future needs for information.



Section 1: Demographics

The majority of participants were growers, then suppliers, then extension educator, and IPM Manager. Most produced in greenhouses (over 80%) followed by field container, shade house, hoop house, high tunnel, and open field in ground. Most producers had less than 2 acres with 3-5 acres also being prevalent.

Section 2: Impact of Downy Mildew

Downy and powdery mildews had a major impact while Phytophthora, Pythium, Rhizoctonia and bacterial diseases were categorized more as having somewhat of an impact. Rusts and viral diseases were viewed as having less of an impact with the majority of respondents indicating no impact of salability. However, many noted that this ranking can change from year to year.
When asked about what crops have been impacted by downy mildew in the last 5 years, respondents mentioned impatiens, roses, basil, coleus, rudbeckia, buddleia, agastache, veronica, snapdragons and many other crops including vegetables. There may be a disconnect in being able to distinguish between downy and powdery mildew because some of the crops (i.e. poinsettia, petunia, zinnia, dogwood) are infected by powdery mildew, not downy mildew.

Section 3: Knowledge of Downy Mildew Diseases

In order to start outlining potential critical control points, a series of questions were asked to determine the extent of knowledge related to sources of downy mildew inocula and how to manage these diseases. Respondents tended to agree that plugs/liners/cuttings, seed, other crops, weeds, plant debris, reused pots, irrigation water, tools, and clothing could be a source of inoculum. And most disagreed that fertilizers were not a source.

All respondents thought that resistant cultivars would limit or prevent downy mildew during plant production. Most tended to agree with pesticide/chemical application, discard damage plants, crop-free period, isolate or quarantine incoming plants, provide handwashing stations/training can limit or prevent downy mildew during production but there were some confounding answers for some of the other options. For example, while most thought the use of chemical pesticides (about 83%) were useful, a large percentage (about 68%) thought biopesticides could limit or prevent downy mildew; currently, there aren't any biopesticides with efficacy for downy mildew.

Personal experience was by far the number 1 selected option for determining if one had a downy mildew disease problem, followed by examining the tissue with a hand lens or microscope, sending the sample to a diagnostic lab and searching the internet for pictures of a similar problem.

When needing assistance with downy mildew problems, growers would prefer to turn to county or regional extension personnel and university extension practitioners.

Those that wanted more information related to downy mildew management (65%) preferred more training on preventative methods, disease management products, disease sources, and disease identification. Some requested specific topics including a more comprehensive list of susceptible crops, how genus/family plays a role, and disease forecasting.

The preferred pathways for receiving information are grower meetings (100%), factsheets (80%), training sessions (70%), email (65%), field days (60%), internet websites (55%), and magazines (55%).

Webinars, conversations, social media, and other avenues were selected by less than 50% of the respondents.

Discussion

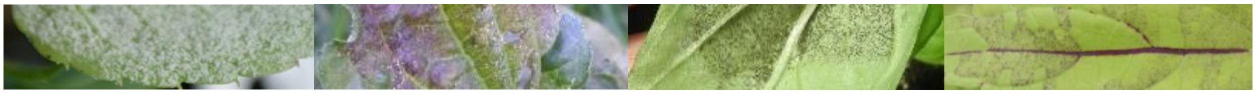
- There is a concern about ID of downy mildew versus powdery mildew
- Is there a correlation between growers that said they go to their extension agents when they have disease and growers who preferred getting information through field days?
- We need more participants for the survey! Survey is still open and will remain open
- The whole survey should be revised to include grower's state and-more pointed questions.
- Individual researchers sent out email and Jill sent out through American Hort Society email blast
- Will the focus be on the host groups or the downy groups?
- Some practices will work for all downy mildews as well as other diseases
- Some practices will be host/pathogen specifics

Economic Impact of DMs (Khachatryan)

The main economic factors for the ornamental horticulture industry are production costs, market demand and competition. Production costs are the cost of all the inputs related to producing that crop. They can be fixed (e.g., greenhouse, irrigation systems or other infrastructure related costs) or variable (e.g., labor, fertilizers, utilities, and other inputs). There are also costs for technical innovation. Many growers will design and build systems optimized to their operation. Optimizing efficiency in this way has short term costs but may over the long term provide production costs savings and gains in output (i.e., increased yield or decreased shrinkage rates). Another factor to modify production costs is the economies of scale. An operation with more output of plants will have lesser input costs on a per pot basis. The cost of goods tends to be lower as one purchases larger quantities. Growing more types of plants (i.e., economics of scope) to be more competitive will also influence production costs. Market demand impacts the types of plants produced and how those crops may be grown. Consumer preferences for color, care requirements or other attributes guide grower choices. Marketing campaigns or ads, on the other hand, influence consumer behavior. Unfortunately, unlike the food sector, the green industry is among the least proactive sectors of the agricultural economy to communicate these essential benefits to end consumers. Demand is also influenced by socio-demographic characteristics such as consumer income as well as by grower responsiveness to price changes in the marketplace. Competition (local, regional, domestic, international) alters the market. Local competitors may differentiate by price, but they may also differentiate with product choice or customer service.

During 2015, an economic impact study of the environmental horticulture industry in Florida was conducted. The researchers (Hodges, Khachatryan, Rahmani, and Court) contacted 10,440 firms by email and USPS from lists of FDACS-DPI certified nurseries and stock dealers and the member list of the Florida Nursery, Growers, and Landscape Association (FNGLA). They garnered 1,546 respondents or a response rate of 14.8%. There were very few large operations with annual sales of more than $5M; most operations had annual sales of less than $250K.

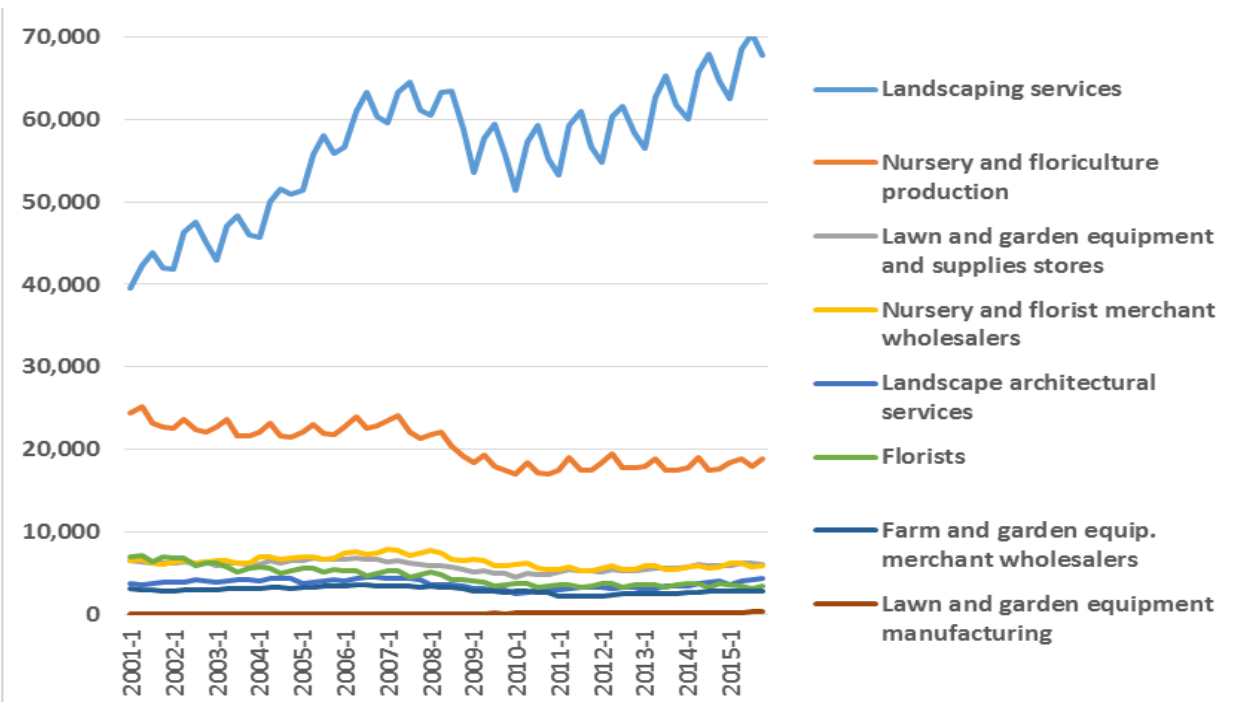
There was an employment trend (based on a surveys conducted from 2001 through 2015) for increased landscape services and decreased employment for production/manufacturing. The recession in 2008-2009 impacted this industry with most sectors not fully recovering in terms of employment (Figure 1). The sector that did recover and grow was landscaping services.



In examining the detailed employment contributions, the environmental horticulture industry was divided into three groups: 1) nursery production and allied manufacturing, 2) landscape design, installation, and maintenance service, and 3) wholesale and retail trade in horticulture products. Across these three, with the landscape design, installation and maintenance services being the largest, the environmental horticulture industry contributed 232,648 full- and part-time jobs in Florida. It had direct, indirect, and induced revenue of $21B and a gross domestic product (GDP) contribution of $13B.

When comparing employment and GDP changes from 2010 to 2015, overall there was a reduction in employment (4.7%) and an increase in GDP (22.5%). Across the industry, we do more with less employees. However, this is due to increases in the wholesale/retail distribution and landscape services areas. The production/manufacturing sector has a loss of approximately 60% in employment and 45% in GDP. The production sector is employing labor more efficiently but the contribution to GDP is a little less than in 2000. Even miniscule improvements in production will lead to lots of savings/gains in the big picture.

Figure 1. Employment trends in the green industry.



However, weak interest in horticulture among younger generations, combined with relatively slow recovery after the economic recession of 2007-09 affected the industry's financial performance. With slowing demand, firms are facing an increasingly competitive market, forcing producers to invest more resources in to higher quality production practices and marketing/promotion campaigns in order to remain profitable. As the green industry aims to stimulate demand, research and outreach efforts are needed to investigate consumer preferences and uncover factors that influence individual choice behavior, which is useful to promote sustainable production. Better understanding of the relationship between product attributes, socio-demographic characteristics, and consumers' purchase intentions will help producers and retailers to adapt by marketing products that are relevant and have traits that are highly valued by the end-users.

An innovative way to assess consumer preferences and their willingness to pay (WTP) is to utilize eye-tacking technologies in conjunction with conjoint analysis to determine what influences consumer attention. Between signage and pictures of the plants, this software records where consumers' eyes are focused. This is a way to assess product attributes such as organic, GM, locally grown, pollinator friendly, etc. Consumers typically do not have the option of buying a product that is best in every attribute. Attributes fall into three categories: search, experience, and credence. Search attributes are those that can be determined prior to purchase such as color, flowering, fragrance. Experience attributes are those that are not determined prior to purchase but consumers become familiar with over time such as longevity, fragrance of flowers not blooming prior to purchase. Credence attributes are those characteristics a consumer cannot verify or verify easily, such as various productions practices (organic, locally-grown, pollinator safe).

Hayk's research interests are examining the product- and consumer-specific factors that influence preference and demand for ornamental horticulture plants. Currently, he is looking at landscape design and the preferences between the percentage of turf and vegetative plantings.

Discussion

- Why are there differences between National Surveys and these studies?
- Different methodologies. Only 15 states surveyed with extrapolation techniques vs. all 50 states.
- Some growers don't want to give real numbers to government, but they will provide accurate information to trusted researchers.
- Impact of timing of when growers are surveyed-time of year/production
- If people know the survey questions ahead of time, they can better keep track of variables as the growing season goes along instead of mining for data all at the end of the year
- Woody ornamentals, floriculture, and turf industries/crops separately vs. combined data.
- Growers who have 2 acres or less will respond even more differently depending on the time of year when they are given the survey.
- It is critical to design surveys carefully to get accurate answers

Identification & Diagnostics (Rivera)

What We Know:

Species complexes complicate diagnostics due to misidentifications, new species, and new genera in some cases. The true boundaries of some species and their host range may not be known. Some examples of species complexes include *Plasmopara halstedii*, *P. sparsa*, *Plasmopara viticola*, and *Peronospora lamii*. These species complexes can complicate diagnostics due to misidentifications. High host specificity is key to high genetic diversity.

<u>Example 1</u>: *Peronospora sparsa* with hosts in the genera Rosa, Rubus, Fragaria and Agrimonia. For common agrimony, *P. sparsa*, *P. agimoniae*, and *P. potentiallae* were all attributed to cause downy mildew on this perennial used for medicinal purposes. Researchers using morphological and molecular characterizations identified all the infections in the Czech Republic and Germany as caused solely by *P. agrimoniae*.

Example 2: *Plasmopara halstedii* infecting sunflower and most Asteracea. This is one of the downy mildew pathogens with perhaps the broadest host range infecting 35 different genera within the Asteraceae. In a study by Choi et al, they looked at Plasmopara halstedii in different hosts, mainly common ragweed and Xanthium. After morphological and molecular analysis they concluded that these are all different species delimited by the host plant. Basically, what infects sunflower is different from what is infecting ragweed and cocklebur. This is an interesting case because you can also see in the literature that cross inoculations between *P. halstedii* on sunflower and other hosts are possible. However, when natural infections are sampled, they still seemed to be genetically different.

Example 3: *Novotelnova scorzonerae* infecting viper's grass (*Scorzonera humulis*). This is a pathogen initially identified as *Plasmopara megasperma* but under recent morphological and genetic examination it was determined that it was not even in the *Plasmopara* genus.

Example 4: *Plasmopara muralis* on ornamental grape ivy (*Parthenocissus tricuspidata*). This pathogen was initially grouped with *Plasmopara viticola* which causes downy mildew on grape and a number of other hosts. With closer morphological assessments and molecular analyses, it now known to be adifferent species.

Example 5. For *Peronospora lamii*, it was thought to have several hosts in the Lamiaceae family in 7 genera (Mentha, Salvia, Ocimum, Plectranthus). In 2002, downy mildew on basil was called *P. lamii*, but then researchers were not certain, so they started calling it *Peronospora sp*. It is seed transmitted, so before we could name it properly, it had spread through Europe, US, West Asia, etc. The uncertainty in diagnosis caused quarantine issues. It was finally determined that *Peronospora belbahrii* on basil is separate species.

Modern Epidemics are distinct.
There is lots of genetic diversity within each group. For example, in *Plasmopara halstedii*, the samples from sunflower appear to be a different species than the samples collected from rudbeckia. For *Plasmopara obducens*, the pre-epidemic and post-epidemic populations are mostly separate genetically. Historic populations are unique relative to the new epidemics.

Molecular Barcodes for Oomycetes may assist in identification.
Downy mildews should be identified with two molecular barcodes, one from the rDNA and one which is either cox1 or cox2 gene. Failing to do so puts us at risk of missing the diversity in the species complexes and thus assigning the wrong name to a pathogen that may need quarantine actions.

Diagnostic tools must match the needs of the grower while identifying an obligate pathogen.

Currently available diagnostic tools include observations, immunoassays, and molecular assays. Observations, in the case of obligate pathogens, include symptoms and signs such as sporangia and sporangiophores. Immunoassays such as ELISA can narrow down to specific pathogens or identify a pathogen group. Molecular assays include PCR, isothermal amplification, sequencing, Next Generation sequencing, and real time sequencing. The two groups of molecular assays include: screening and confirmatory. Screening test typically yield faster results and are field deployable with simple requirements for equipment and expertise. These tend to identify to a group of related species. The confirmatory tests yield relatively fast results but are performed in a specialize laboratory and may have complex requirements for equipment and training. These tend to be highly specific to pathogen species or even subspecies.

What We Know We Don't Know

We don't know the true host range for many DM pathogens. Cross inoculations in controlled settings may not be representative of what happens in nature and the diversity of the pathogens that infect the different hosts.

We don't know how many of the downy mildew pathogens are species complexes. For those that we do know are complexes, we don't truly understand how many species are present.

We also don't know which downy mildew organisms are seed-borne. This has implications for management as well as for implementing regulations.

We also don't know which pathogen will cause the next disease epidemics. There are a number of downy mildew species with uncertain taxonomy and phylogenetic placement.

The next epidemic may be right around the corner so there are steps we can take to prepare for the unknown. First, downy mildew pathogens need to be tracked prior to epidemics by obtaining official reports of downy mildews in each state and any appearing on new hosts. Voucher specimens should be sent to Beltsville and these should be linked to the first report of a new downy mildew or a new report of an identified downy mildew on a new host. Because downy mildews are obligate pathogens, voucher specimens deposited in herbarium help us when we need to go back and look at morphology and genetics. To help prepare, we need to start untangling the species complexes for downy mildews on horticulture crops. It will help to know the true name of a downy mildew species and to know the real host range.

We need to become better at identification (use of taxonomy and systematics to classify a pathogen) to be able to be better at diagnosis (correct identification and detection of a pathogen). They go hand in hand. We need good identification to achieve better diagnostics. One of the biggest challenges in downy mildew diseases.

Discussion

- People really need comprehensive instructions for creating a voucher specimen and perhaps adding a requirement for linking voucher specimen to 'First Reports' in *Plant Disease* –some issues with international publications.
- A study for the economic impact of diagnostics
- Tracking fungicide resistance genes
- Creating a digital image library for recognition of diseases

Biology and Epidemiology (Shishkoff)

Impatiens downy mildew (*Plasmopara obducens*) has been in the US since the 1800s on wild impatiens, but became devastating on commercial impatiens recently. Key questions about this disease include why and how it arose, does it overwinter and how does it spread. So far, we have discovered this pathogen is homothallic and takes about 1 month to form oospores. These germinate and form megasporangia, which in turn germinate and release zoospores. This Impatiens downy mildew (*Plasmopara obducens*) has been in the US since the 1800s on wild impatiens, but became devastating on commercial impatiens recently.

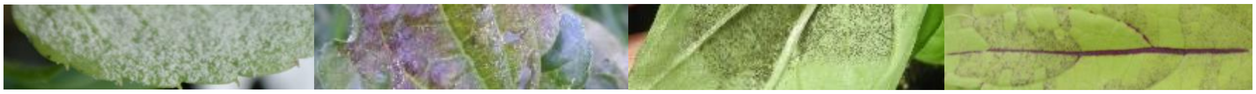


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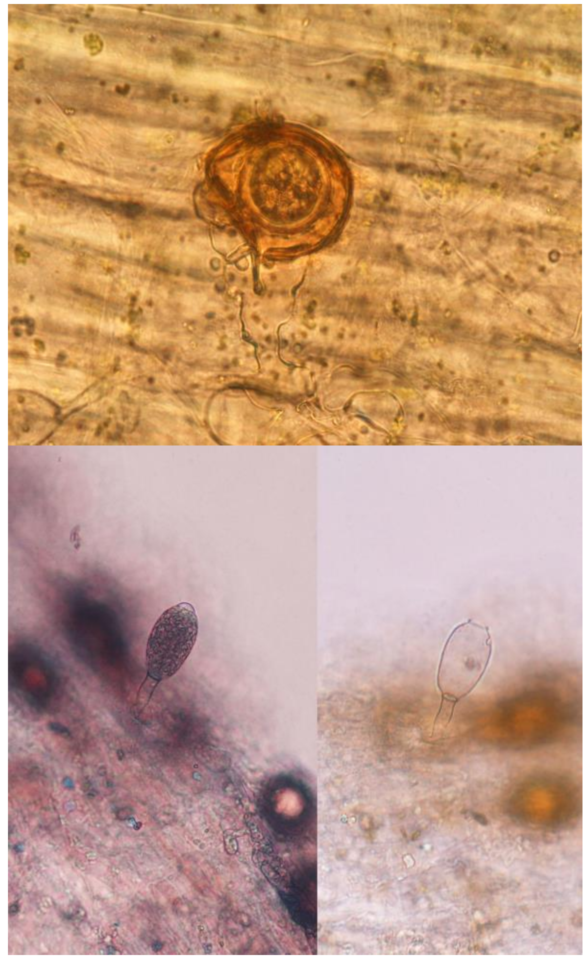
Figure 2. *Plasmopara obducens* oospore and megasporangia



Downy Mildew Strategy #1: Survival on tropical hosts.

For some downy mildew pathosystems (*Peronospora tabacina* (blue mold) on tobacco and *Pseudoperonospora cubensis* on cucurbits), the pathogens persist on tropical or sub-tropical hosts and reinfect to temperate regions annually. Oospores are not important or may be unknown in life cycle. They may also survive in protected greenhouses or on perennial hosts. Movement is via wind-dispersed conidia and infected transplants. Spore traps are used in some areas for forecasting disease development.

Downy Mildew Strategy #2: Overwintering mycelium.

One notable downy mildew that overwinters as mycelium is hops downy mildew (*Pseudoperonospora humuli*). While not truly systemic, the overwintering mycelium ramifies patchily to infect growing spoikes. Growers prune out these infected branches or spikes because they are sources of secondary inoculum. It is too expensive to remove whole infected hop plants and lose the production from the healthy portions so pruning infected shoots prior to sporulation has been an effective control method. Ensuring good air circulation through plant structure has been a useful cultural control tool. The goal isn't eradication; the goal is economical disease suppression and management.

Downy Mildew Strategy #3: Overwintering oospores.

Examples of downy mildews that overwinter as oospores include those that infect grape and sunflower (*Plasmopara viticola* and *Plasmopara halstedii*, respectively). The grape downy mildew pathogen is heterothallic with oospores formed in infected host tissues toward the end of the season. These oospores overwinter in leaf tissues or in soil as the leaves decay. Oospores germinate throughout the season making disease management challenging since different genotypes appear at different times. Oospores produce zoosporangia which then produce zoospores. Zoosporangia and zoospores are carried by rain or wind to the lower leaves where zoospores will encyst, germinate and penetrate through stomata. The sunflower downy mildew survives in soil for up to 10 years as oospores. These oospores produce zoosporangia which subsequently produce zoospores which can infect seedling roots. Oospores are formed in roots soon after infection. Secondary infections occur from windblown zoospores but secondary infection does not cause downward systemic infection. This diseasecan be seed borne

To understand how quickly these organisms can develop new phenotypes, it is important to know whether they are homothallic or heterothallic. Monitoring mating types contributes to this understanding also because frequency of mating types varies geographically, with some regions dominated by sexual clonal populations that don't overwinter as oospores. For example, cucurbit downy mildew is heterothallic, and we are seeing increased prevalence of oospores in other countries.

Is it possible to select a small set of models that would cover various environmental horticulture crops?

Grape Downy Mildew Model
A model doesn't have to be complicated to be effective: There was a "3-10 rule" in grape vineyards:. If air temperature is greater than 10C, if there are 10 cm-length vine shoots and at least 10 mm rain in the last 24 – 48 hours, the model says to spray! It isn't a very good model, however. Another simple model that is more effective is the rosebush-in-the-vineyard model:if there are downy mildew symptoms on roses, spray for downy mildew in grapes. These two diseases are not caused by the same pathogen, but appearance of symptoms on one shows environmental conditions are conducive for disease on both crops. Weather, rainfall, humidity, and oospore maturation can all be variables in a model.

Figure 3. Roses planted next to grapes in vineyard

For greenhouse crops, it is possible to predict conditions leading to leaf wetness. Environmental conditions in greenhouses can be altered more readily than those in fields. It is possible to adjust temperature, humidity, irrigation, and light quality (which affects pathogen sporulation). Fungicide intervention is possible if environmental manipulation is insufficient.

Cultural Practices depending on the crop and when downy mildew appears these can include adjusting: 1) the planting dates to avoid infection period, 2) number of harvests per year, 3) fertilizer rate to harvest earlier, 4) canopy density/trellising to improve airflow, 5) row alignment, and 6) irrigation method/drainage such as moving to drip irrigation to avoid leaf wetness. Another way to manipulate nutrition is to reduce or increase certain plant nutrients. Well-fertilized plants are more prone to disease issues. In plants that take up silicon, this element can reduce disease.

Biological Control practices, theoretically, can include the reduction of oospore levels in soil or reduction of zoospore viability and the use of seed treatments as direct antagonists or to induce resistance in host.

Clean Propagation is a way to prevent downy mildews from entering the production system as seed or young plants. Detection of seed borne inoculum is a critical step followed by developing seed treatments. Quarantine measures may need to be in place for stock plants to prevent initial infection and to eliminate accidental introduction into mother blocks. Sanitize methods for propagation sites will prevent and eliminate contaminants.

This is a question we need to address: How do we organize and perform research on downy mildews?
-By type of pathogen life cycle
-By type of host (annual vs perennial vs. woody)
-By environment (outdoors vs greenhouse)
-By forecasting method (monitor plots, spore traps or models)

Genetics and Genomic (Crouch)
Nothing in downy mildew genes and genomes makes sense except in light of how we can leverage it against what we know about related organisms.

Peronosporales, the plant pathogenic group of oomycetes, contains three main groups: Pythium species, Phytophthora species, and downy mildews. Phylogenetically, they diverged after the origin of their respective hosts in other words after major angiosperm plant lineages diverges in the mid-Cretaceous period. Each of these main groups have different lifestyles. Pythium species are necrotrophs; Phytophthora species are hemi-biotrophs; and downy mildew pathogens are biotrophs. This allows us to examine and compare genes contributing to each of these lifestyles.

The model system for studying downy mildews is *Hyaloperonospora arabidopsidis* on Arabidopsis.

Plants defend against pathogens-via two interconnected lines of defense: basal defense and effector triggered immunity (ETI). Basal Defense is the first line of defense- if the pathogen can't get past this, it is not a pathogen on this host. Transmembrane pattern recognition receptors (PRRs) on plant surface are activated when they perceive pathogen-associated molecular patterns (PAMPs), resulting in PAMP-triggered immunity (PTI). Basal defense is typically induced by very broadly conserved elicitor molecules such as bacterial flagellins, fungal chitins, etc. Specific oomycete elicitors include cell wall components, elicitins, necrosis and ethylene inducing peptide 1-like proteins, and lectins.

Effector-triggered immunity, on the other hand, is much more host-pathogen specific (Figure 4). It is based on the interaction between pathogen effector molecules and plant host resistance genes. Different races and pathotypes can emerge suddenly stemming from the repertoire of effectors. They act on multiple different functions of the plant and evolve very rapidly. Majority of oomycete effectors carry N-terminal signal peptide that mediates secretion from the microbe. Apoplastic effectors act in the apoplast surrounding plant/pathogen cells, while cytoplasmic effectors enter the plant cell, cross the plant cell wall and the plant plasma membrane or the DM extrahaustorial matrix/extrahaustorial membrane (RXLR, Crinkler, CHXC motifs) to suppress immunity. They are delivered through the infection structure (haustoria or hyphae).

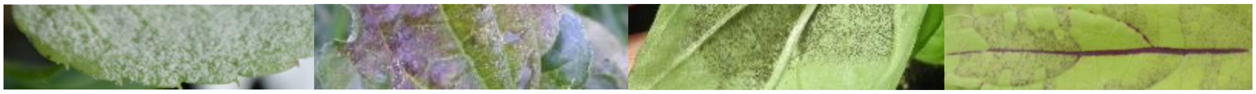
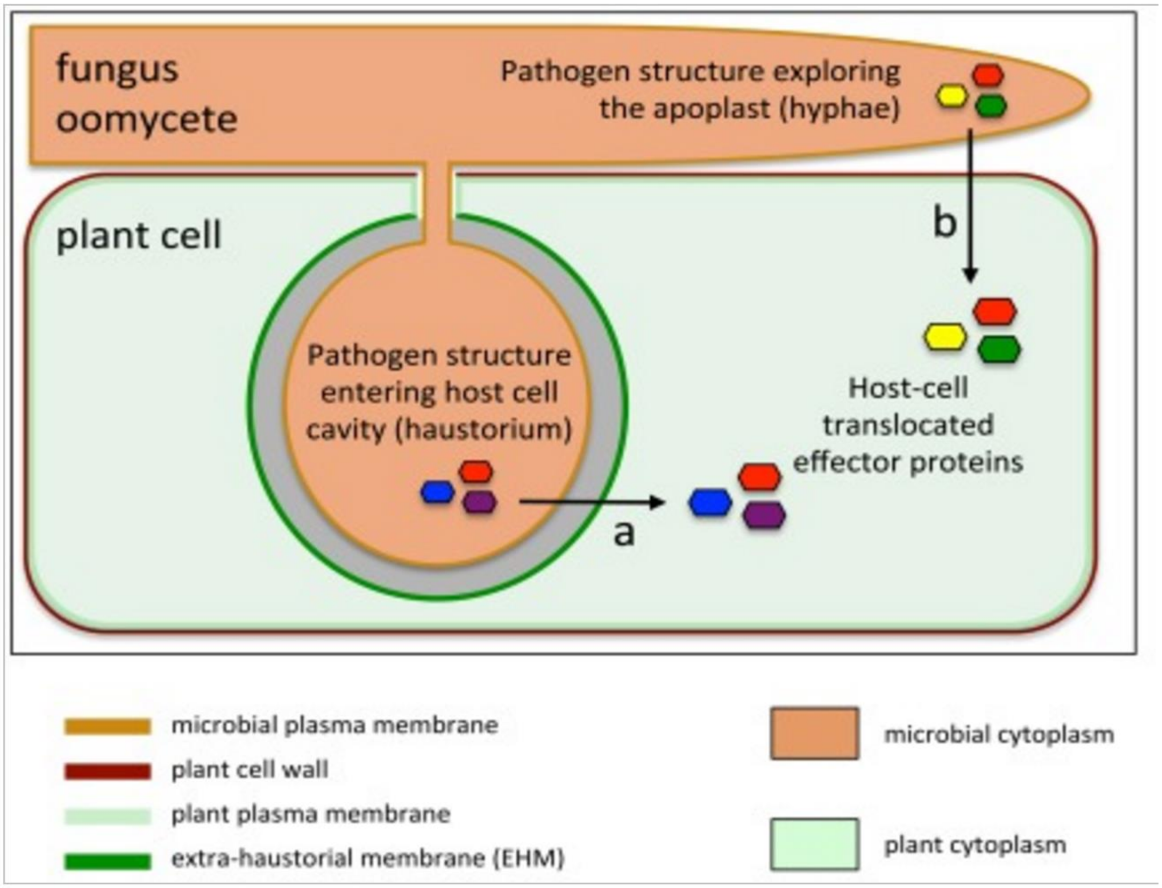


Figure 4. Effector triggered immunity.



PLoS Biol. 2014 Feb; 12(2): e1001801.

Of the top 30 oomycetes, 12 cause disease on environmental horticulture species and 10 are downy mildew pathogens of various edible and non-edible crops (Table 1).

Hyaloperonospora arabidopsidis was the first organism to be used to study plant immune system. In 1994, it was used as a physiological probe to define resistance genes. This led to map-based cloning of R genes in studying resistance. In 2004, the first avirulence effoctor was discovered followed by the RXLR effector family of genes. In 2011, genome sequencing revealed genetic signatures of obligate biotrophy. Slowly, genomes from other downy mildew pathogens are being sequenced and published for comparative analysis.

For those sequenced so far, it is possible to compare genomes relative to *Phytophthora* hemibiotrophs. What has been observed so far includes: 1) reduced cohort of degradative and hydrolytic enzymes, 2)-reduced number of PAMPs/elicitins, 3) reduced number of RXLR effectors (134 vs 350-550), 4) no apoplastic effectors, 4) RLXRs highly heterozygous, under strong selection pressure to diverge/change, 6) no polyamine oxidases and arachidonic acid (role in hypersensitive response cell death), and 7) no large-scale reduction in metabolic capacity as most metabolic pathways predicted to be functional.

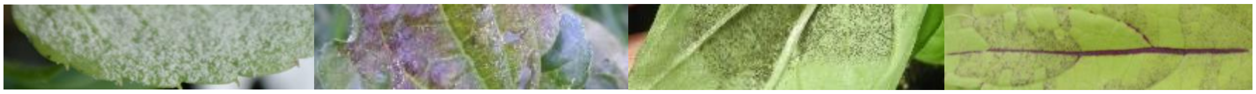


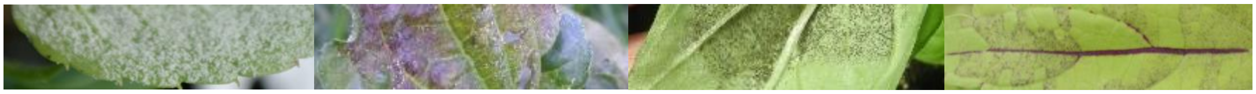
Table 1. Top 10 oomycete parasites ranked on # of papers and scientific community

Rank	Pathogen	Disease	Number of published papers
1	*Phytophthora infestans*	Late blight of potato, tomato	1230
2	***Hyaloperonospora arabidopsis***	**Downy mildew of Arabidopsis**	**137**
2 *	*Phytophthora ramorum*	Ramorum blight	378
4	*Phytophthora sojae*	Stem and root rot of ???	276
5 *	*Phytophthora capsici*	Blight, stem and fruit rot	541
6	***Plasmopara viticola***	**Downy mildew of grape**	**326**
7 *	*Phytophthora cinnamomi*	Root rot, dieback	315
8 *	*Phytophthora parasitica*	Root and stem rot	142
9 *	*Pythium ultimum*	Damping off	319
10	*Albugo candida*	White rust	65
11	*Aphanomyces euteiches*		
12	*Albugo laibachii*		
13	***Bremia lactuacea***	**Downy mildew of lettuce**	
14 *	*Phytophthora palmivora*		
15	***Psedoperonospora cubensis***	**Downy mildew of cucurbits**	
16 *	***Plasmopara halstedii***	**Downy mildew of asteraceae**	
17	*Peronophythora litchi*		
18	***Peronosclerospora sorghi***	**Downy mildew of ???**	
19 *	***Peronospora belbahrii***	**Downy mildew of basil**	
20	*Phytophthora alni*		
21	*Phytophthora brassicae*		
22 *	*Phytophthora cactorum*		
23	*Phytophthora meadii*		
24	*Phytophthora phaseoli*		
25 *	*Phytophthora plurivora*		
26 *	***Plasmopara obducens***	**Downy mildew of impatiens**	
27 *	*Pythium aphanidermatum*		
28	*Pythium oligandrum*		
29	***Sclerophthora raysiae***	**Downy mildew of ???**	
30	***Hyaloperonospora brassicae***	**Downy mildew of ???**	

** Asterisk indicates these pathogens impact environmental horticulture plants, while bold listings are downy mildew pathogens.*

Other obligate pathogens such as rusts and powdery mildews are missing key genes for inorganic nitrogen and sulfur assimilation, but they also secrete large complements of effector proteins, which may suppress host immunity, and their genomes contain large fractions of repetitive elements, which may indicate a plastic genome critical for rapid evolution.

Because these organisms cannot be cultured on artificial media limits experimental manipulation and accumulation of resources. It is difficult to build and share collections of living biological resources. Development of cumulative knowledge is hindered. There are challenges to pathogenesis using conventional genetics and molecular biology, and there are technical challenges to study gene function because obligate pathogens must be maintained on their plant host.



While there are at least 800 downy mildew species, current knowledge is based on just a few downy mildew pathogen genomes. Hence, these current datasets don't capture breadth of diversity of downy mildew species. This contributes to poor taxonomic knowledge and pathogen diversity characterization

Understanding the molecular knowledge of genomes, elicitors, effectors, and R proteins have several practical uses. Commercial and public plant breeding programs use molecular markers for R gene alleles to guide progeny selection instead of using more expensive and time-consuming biological screening tests. It is also possible to stack multiple R genes that act against a single pathogen species to improve faster incorporation of disease resistance which may be more stable over time.

Genome information can aid in design of DNA- or serology-based assays that can be used for classification, diagnosis and epidemiology. Assays constructed based on known resistance gene markers can track the development of resistance to fungicides. Genome fractions common to downy mildew pathogens can be used to develop serological assays to detect the presence of and downy mildew prior to large scale symptom development or to narrow the potential pathogens for diseases of similar symptom expression.

Genome information of downy mildews can also aid in development of RNA interference gene silencing tools. Host-induced gene silencing (HIGS) is an RNA interference-based approach in which small interfering RNAs (siRNAs) are produced in a transgenic host plant and subsequently move into the pathogen to silence pathogen genes. There is demonstrated efficacy for lettuce downy mildew (*Bremia lactucae*) among other pathogen-host systems. Because this is target-specific, development requires knowledge of pathogen genes and sequences.

A question that should be answered is whether we can utilize surrogate oomycetes effector delivery systems (bacterial secretion systems) to study EHC downy mildews. This will overcome challenges working with obligate, genetically intractable pathogens and provide a rapid quantitative method to screen Arabidopsis accessions with known Hpa effectors.

Another activity could be to demonstrate using effector gene polymorphism as as screen to characterize race/pathotype diversity, where extensive phenotypic methods have been used previously.

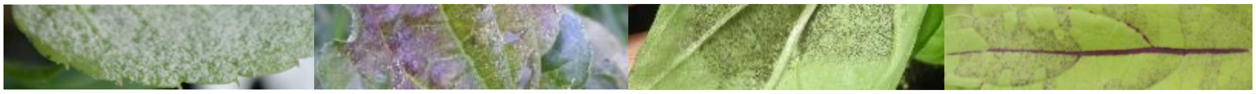
Discussion

- Identify core set of effectors among all downy mildew pathogens and clone and screen germplams
- Use this repertoire to monitor strains that overcome host resistance, develop fungicide resistance
- How do we define these "core set" of effectors? Depends on what species we want to examine

Management Options 1: Biopesticides, Designer Chemistry, Induced Resistance, Environment (Palmateer, Both, Li)

Biopesticides/Designer Chemistry/Induced Resistance: Palmateer

Downy mildew diseases are notoriously difficult to control. Chemical control is the most effective and economic measure currently used for protection. Downy mildews impact many horticultural crops including impatiens, coleus, snapdragon, sunflower, viburnum, and roses.



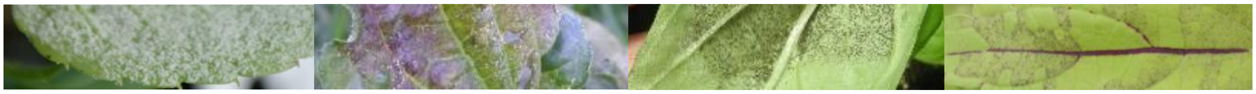
There are several questions regarding disease mitigation. First, it is important to continue to screen new fungicides and test rotations because these pathogens have a propensity to develop resistance quickly and product performance for new and emerging downy mildews may be unknown. While we are beginning to understand the length of residual control for impatiens downy mildew, it is unknown whether other production systems would have similar characteristics. The impact of host cultivar on disease severity or appearance is unknown along with whether there are any cultivar-fungicide interactions. We also have limited options for managing downy mildews with biological control tools either in production or in the landscape. Currently, about 17-20% of the fungicide market goes to DM control.

Historically, mefenoxam has been a mainstay in managing DMs. However, it functions with a single mode of action, so pathogens can develop resistance fairly quickly. Currently, there are 19 different products for managing downy mildews (Table 2). However, many are ranked as high to medium for developing resistance depending on their modes of action. It was noted that Affirm's mode of action target chitin synthase. Chitin is found in fungal pathogens, but chitin is not in the cell walls of oomycetes including

Table 2. Products currently available for managing downy mildews on ornamental horticulture plants

FRAC Code	Product	Active Ingredient	Activity	REI (hrs)	Risk of resistance
4	Subdue Maxx	mefenoxam	Systemic	0, 48	High
11	Compass	trifloxystrobin	Translaminar	12	High
11	Fenstop	fenamidone	Systemic	12	High
11	Flame	fluoxastrobin	Systemic	12	High
11	Heritage	azoxystrobin	Systemic	4	High
11 + 7	Mural	azoxystrobin + benzovindiflupyr	Translaminar + Systemic	12	Med-high
11 + 7	Orkestra	pyraclostrobin + fluxapyroxad	Translaminar + Systemic	12	Med-high
11 + 7	Pageant	pyraclostrobin + boscalid	Translaminar + Systemic	12	Med-high
19	Affirm	polyoxin D	Systemic	4	Medium
21	Segway	cyazofamid	Translaminar	12	Med-high
33	Aliette	fosetyl-Al	Systemic	12	Low
33	Alude	potassium salts of phosphorous acid	Systemic	4	Low
40	Micora	mandipropamid	Translaminar	4	Low-med
40	Stature SC	dimethomorph	Translaminar	12	Low-med
43	Adorn	fluopicolide	Systemic	12	High
45+40	Orvego	ametoctradin + dimethomorph	Systemic + Translaminar	12	Med
M1	Numerous	copper(s)	Contact	24	Low
M3	Protect DF	mancozeb	Contact	24	Low
U15	Segovis	oxathiapiprolin	Systemic	4	Med-high

Fungicides or biofungicides are organized into three categories based on when they have optimal impact on the pathogen: preventative, curative, and eradicative. A preventative compound must be applied before pathogen appears to be efficacious. Curative compounds are active between penetration and appearance of the first symptoms. Eradicative compounds have activity after symptoms appear and can stop further disease development.



Where resistance has not developed in the local populations with some of these products (mefenoxam, fluopicolide), they work very well. To improve mitigation strategies, we must screen new fungicides not yet labeled for EHCs and develop fungicide rotations for greenhouse and nursery settings. Growers should use different programs arranged by FRAC codes. Some new fungicides have been very effective for IDM (Table 3). For example, after 12 weeks, no sporulation has been observed on treatments with Segovis. Orkestra and Catamaran (chlorothaloni + phosphonate) have also been effective at reducing sporulation. Drenches may work better than foliar sprays.

Reduced sensitivity has appeared quickly for IDM with mefenoxam (FL, IL, NJ), fluopicolide (NJ, IL, FL), and pyraclostrobin + boscalid (FL). Not all populations have exhibited reduced sensitivity.

Table 3. Summary of downy mildew efficacy trials

Product	Rate/100 gal	Results	Notes
Adorn	1-2 oz	Good-excellent	Resistance issues; phytotoxicity
Affirm	0.5 lb	Poor	No control of IDM with sprays
Aliette	2 lb	Good-excellent	Control with application both pre- and post0 inoculation
Alude	1.25-2.5 quarts	Good-excellent	Control with application both pre- and post- inoculation
Fenstop	14 fl oz	Good-excellent	Best used preventatively
Heritage	2-4 oz	Good-excellent	Effective with preventative sprays only
Micora	4-8 fl oz	Good-excellent	Best used preventatively
Mural	4-7 oz	Good-excellent	Very good on rose downy mildew
Orkestra	8-10 fl oz	Good-excellent	10 oz rate excellent control of IDM
Orvego	11-14 fl oz	Good-excellent	Best used preventatively
Pageant	12-18 oz	Good-excellent	Effective with preventative sprays only
Protect	1-2 lb	Good-excellent	Effective with preventative sprays only
Segovis	0.6-2.4 fl oz	Excellent	Better as a drench than foliar spray
Segway	2.1-3.5 fl oz	Good-excellent	14-21 day residual
Stature	6.12-12.25 fl oz	Good-excellent	Best used preventatively
Subdue	1-2 fl oz	Good-excellent	Better as drench than foliar spray; resistance issues
Vital	1.25-4 pints	Good-excellent	Control with application borth pre- and post-inoculation; better as drench
Zerotol	50 fl oz	Poor	Application timing is critical

Biopesticides may be an option. In published literature, there a few reported studies that have examined biologicals to manage DM diseases. Not many promising results. Regalia has had some control, as have Cease, Milstop, and Triathlon for basil downy mildew, but environmental variables play a role in efficacy. There may be more possibilities with managing the soil-borne phase(s).
It is possible that some biopesticide may be effective through induced resistance mechanisms. Phosphonates instill induced tolerance: the plants hold up, but there is sporulation. Benzothiadiazole (Bioin 50WG)-induced resistance to *Plasmopara halstedii* on sunflower reduces sporulation, leaf chlorosis and damping off. Timing is important in that pretreatment of seedlings was more effective than treatment after inoculation. Reduced growth of plants occurred commonly possibly due to allocation of energy for induced resistance.

Research Objectives

- Bioassay for pesticide resistance
- Diagnostic tool for detection
- Which part of life cycle is affected by systemic fungicides for systemic disease?
- Impact of fungicides on oospore formation
- Practical biological or biorational fungicides
- Disease forecast models

Environment: Both & Li

Examining the effects of environmental and nutritional variables for disease management will yield a more sustainable approach. Some of the factors to manage in the plant growing environment for downy mildew control include: 1) reducing leaf wetness, 2) altering humidity, 3) manipulating light, 4) altering nutrition, and 5) improving sanitation.

Lowering humidity and leaf wetness in the greenhouse can be achieved by fostering dry air (vapor pressure control, heat and vent strategy), dry floors (controlled irrigation dosing, proper drainage), and dry plant tissue (maintain the temperature above the dew point, avoid overhead irrigation). It is also beneficial to maintain sufficient air movement by horizontal and vertical air flow fans or inflated polytubes above and below the plants.

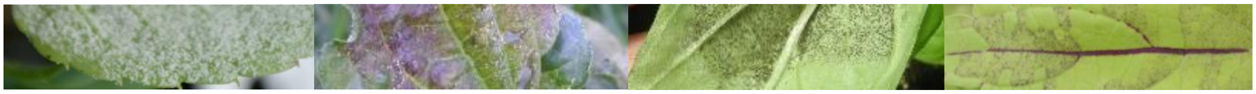
Manipulating the light conditions has an impact on both the plant and pathogen. The addition of red light reduces sporulation. Changing the day/night periods can interfere with the basil downy mildew pathogen. Ultraviolet radiation can also affect pathogen and/or host. Altering the nutrition can have an impact on the pathogen/host interactions.

Highly fertilized plants are more prone to pathogen problems but soluble silicon can reduce development of disease for those plants sensitive to it. The addition of soluble silicon to hydroponic nutrient solutions increases the thickness of cuticles and cell walls. Increased Silicon uptake can also increase the yield of basil plants.

Improving sanitation reduces initial inoculum. In addition to standard sanitation practices, the inclusion of measures surrounding ingress and egress for people and ventilation is valuable. For entryways for people, installing double door entry ways with air showers can reduce aerial spores. Mandatory showers for workers going into production areas with mother plants or other growing areas will reduce spread of pathogens hitchhiking on shoes, clothes or skin. For ventilation, installing electrostatic cleaners can remove pathogens from the outside air needed for temperature control.

Research Objectives

- Manipulate environmental parameters to reduce the presence and/or spread of mildew
- Develop sanitation protocols to reduce/eliminate the entry of mildew spores
- Incorporate Si into the plant nutrient solution to boost plant defenses against mildew



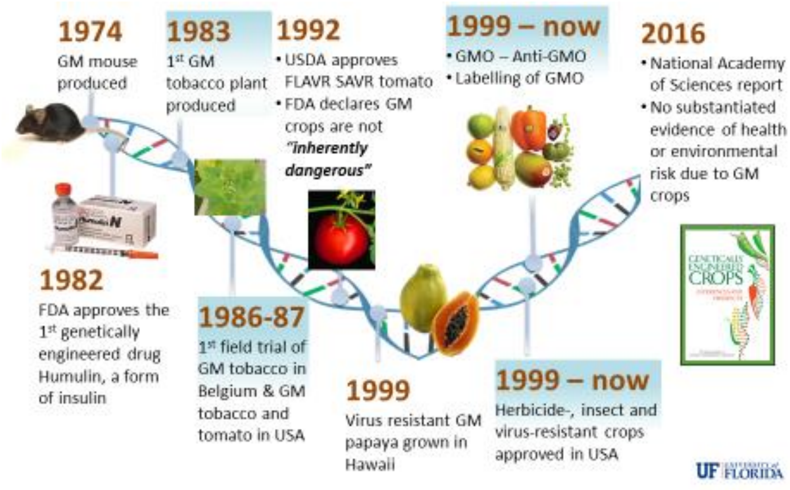
Management Options 2: Host Resistance, RNAi/Genome Editing (Ali, Deng)

Genome Editing: Ali

Approaches for Genome Editing-based Precision Breeding

Traditional transgenic approaches include transformation of crops with dominant resistance and defense genes using *Agrobacterium* and gene gun. In addition to dominant resistance genes, the so called susceptibility "S" genes such as the barley MLO gene have also been used successfully in many crops. S genes can be knocked down using the RNAi approach. However, several recent developments in knocking out genes have provided researchers with tremendous power to precisely manipulate genes associated with defense.

Figure 5. Evolution of genome editing



There are currently three major methods that are utilized for precisely editing DNA seqeunces: zinc finger nuclease, TAL effectors and CRISPR/cas9. Among these methods, the CRISPR/cas9 system is by far well advanced and more efficient. In the CRISPR/cas9 system, a 20-nucleotide-long RNA guides the cas9 nuclease precisely to the target DNA sequence, where it creates a dsDNA breakage. Endogenous DNA repair mechanisms repair the DNA breaks, but these mechanisms are error-prone leading to the introduction of mutations in the target DNA. Similarly, non-functional resistance genes can be repaired or replaced with useful resistance genes taking advantage of the endogenous homologous recombination repair mechanism ubiquitously present in plants. In summary using the CRISPR/cas9 system, susceptibility genes can be knocked out or resistance genes can be knocked in a plant variety very efficiently.

Compared to traditional genetic engineering the CRISPR/cas9 system has several advantages. First, it is very simple consisting of only two components, a nuclease and a target specific guide RNA. Second, it is highly versatile, and any genomic locus can be targeted for mutation. Third, it is very cheap and due to its simplicity is easily accessible to researchers with minimal equipment and resources. Fourth, it is currently not considered or regulated as a genetically modified organism as it is comparable to mutations introduced by chemicals or UV radiation. Similarly, the National Academy of Sciences in a recent comprehensive study showed no substantiated evidence of health or environmental risk due to GM crops

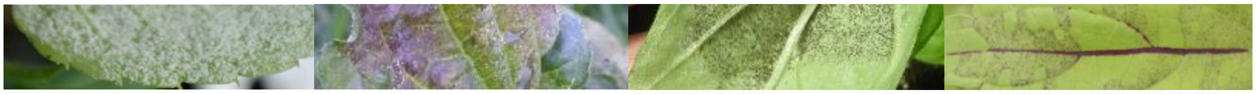
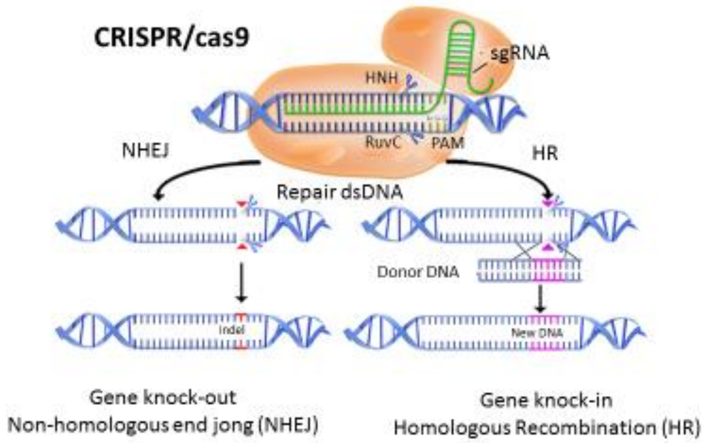


Figure 6. CRISPR/Cas9 mediated mutagenesis



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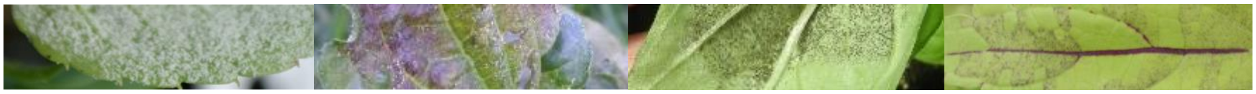
Potential Downy Mildew Disease Resistance Genes that could be targeted for the CRISPR/cas9-based precision breeding

There are two types of potential resistance genes: dominant resistance (*R*) genes and susceptibility (*S*) genes. *R* genes depend on availability and discovery in crops, and they are usually not functional outside their taxonomic family. Once identified, then they can be transferred to intended crops or varieties using the CRISPR/cas9 knock-in approach very efficiently. The *S* genes are discovered through induced mutation using chemicals or radiation. There are a few well characterized *S* genes against downy mildews known in the model plant Arabidopsis. But it is worth the effort to discover new S genes and then target them in crops using the CRISPR/cas9 system. For example, in a screen of mutagenized Arabidopsis populations, researchers have discovered a mutation in the Homoserine kinase gene, which is involved in the synthesis of specific group of amino acids. Another example is the *DMR6* gene, which encodes a putative 2OG-Fe(II) oxygenase that is defense-associated but required for susceptibility to downy mildew. It is possible and relatively easy to identify homologs of these genes in other crops and then manipulate them using CRISPR/cas9.

Since transformation protocols are in place for most crops that are affected by downy mildews, we are set to utilize the CRISPR/cas9 technology for producing downy mildew resistance varieties. Additional research avenues for resistance gene discovery and genomics (transcriptomics) of the impatiens downy mildew include 1) Identification of differentially expressed genes in a downy mildew resistant and a susceptible cultivar, which could lead to shortlisting potential downy mildew resistance and/or susceptibility genes for potential deployment. 2) Utilize traditional breeding methodologies alone or in combination with CRISPR/cas9 based genome editing to develop downy mildew resistant cultivars.

Breeding: Deng

Improving DM Resistance in Impatiens has been examined so far by studying current resistance levels through observing 76 cultivars in field trials with natural inoculum levels, artificially inoculating 32 cultivars. Ten tetraploid impatiens lines have been examined along with 10 lines of transgenic impatiens. Tetraploid impatiens and transgenic impatiens overexpressing the Arabidopsis NPR1 gene tend to have less sporangia production. So far 15 unigenes have been identified that expressed at much higher levels in resistant New Guinea impatiens than in susceptible garden impatiens.



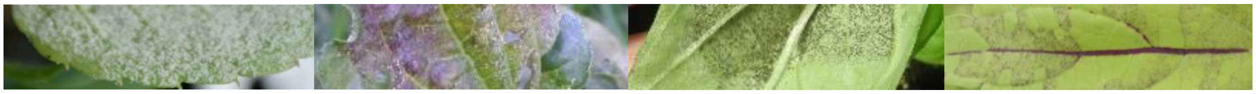
There are several success stories of using traditional breeding to develop DM resistance in ornamental horticulture crops. The F1 Sunrise Orange sunflower exhibits resistance to multiple strains of *Plasmopara halstedii.* There are several sources of downy mildew resistance in wild rose species which can be incorporated into commercial rose cultivars. For coleus, there are cultivars (Fairway Red Velvet, Fairway Salmon Rose, Fairway Rose) with less susceptibility. In addition, IDM has not yet been observed on the NG impatiens and SunPatiens hybrids.

For edible crops breeding, traditional breeding for downy mildew has been very successful for several crops. Three examples include cucumber, lettuce, and grape.

In cucumber, before 1940 there were severe losses due to *Pseudoperonospora cubensis*. In the 1940's there were three sources of moderate resistance and breeding programs began. In the 1960's a source for a higher level of resistance was identified (PI197087) resulting in fairly good resistant cultivars so that between 1961 and 2003 yield losses were about 2.9% with no use of fungicides. In 2004, a new race emerged causing between 40 and 100% yield loss. By 2014 a new breeding line emerges with 2 new cultivars and 2 sources of moderate resistance (DMR-NY264). R genes have been cloned in melon (At1 & At2). Lesson learned from this system include 1) we need sources of resistance; 2) time is needed to produce resistant cultivar with acceptable yield and quality; 3) the resulting cultivar must be effective and cost-effective; 4) there is a risk for new pathogen strains and races; 5) must continuously explore new sources for resistance and breeding; and 6) new technologies can assist breeding efforts.

For lettuce, there are three U.S. cultivars – 'Calmar', 'Salinas', and 'Vanguard' – that are the progenitors of many contemporary cultivars. There are three primary sources of resistance (Dm5/8 from Russian wild lettuce, Dm13 from PI lines, and Dm7) in the United States. In the 1930s in Europe, four cultivars had been released possessing complete resistance. By the 1960's resistance gene Dm7 was in every US cultivar. Dm2, Dm3, and Dm7 were introduced, and researchers discovered Dm11 in L. serriola accession. During the 1970's, these four resistance genes were used in various combinations to manage 4 new DM pathogypes. In the late 1970s to early 1980s, metalaxyl resistance appeared and two new resistance genes were discovered Dm6 and Dm16. By mid 1980s, Dm11 was no longer effective and Dm6, Dm16, or R18 provided effective control. Since the 1960s, resistance from traditional breeding programs has been fairly effective. The lessons learned from this system include 1) DM can be managed by race-specific dominant R genes; 2) wild type germplasm has been a good source for these genes with 51 resistance genes identified out of thousands of accessions; 3) these have been gene-for-gene relationships primarily and require the presence of proper Avr genes in the pathogen strain; 4) there is a rich source of resistance genes in lettuce germplasm (at least 28 Dm genes in at least 5 clusters); 5) these genes differ in level of resistance and none provide durable resistance; and 6) there is interest in looking at quantitative resistance with the potential for older cultivars with partial resistance being a place to start.

In grapes, European grape cultivars belonging to *Vitis vinifera* are highly susceptible, while Muscadine grapes, several American *Vitis* species and some Asian *Vitis* species have variable levels of resistance. The approach had been to cross the European cultivars with American or Asian species. The early DM resistant cultivars disappears after effective fungicides became available. Since the late 1980 when fungicide resistance began developing, discovering and releasing downy mildew resistant cultivars became a priority. In 1996 a new cultivar 'Regent' was released and became the most important new resistant cultivar. This cultivar had resistance due to three quantitative gene loci (QTL). Another cultivar



‘Bianca’ possesses only one resistance gene and had begun to show susceptibility in some geographies. Breeders are developing other resistant cultivars. In this cropping system, both strong host resistance and high quality wine production traits are key characteristics for the development of new cultivars.

Generally, there are several sources of genetic resistance regardless of edible or orn hort crop or annual or perennial. These include:

- Currently grown cultivars
- Breeding lines and genetic stocks
- Obsolete cultivars
- Plant introductions
- Landraces
- Wild forms of the same species (How does this differ from land races??)
- Related species
- Unrelated species

To summarize, resistance varies on a continuum from complete/immunity, to partial resistance to field level resistance to susceptibility. Resistance can be non-specific broad spectrum or be race-specific. Temperature or development stage may impact resistance expression. Resistance can be characterized and qualitative or quantitative, horizontal or vertical. Resistance may be monogenic (single gene), oligogenic (multiple related genes), polygenic (multiple unrelated genes). It may be dominant, partially dominant, recessive or complementary. It is constant warfare to remain ahead of resistance. New pathogen strains overcome host resistance.

Adoption & Adaptation: Barriers for new methodologies (Khachatryan)

Understanding Determinants of Demand

The typical understanding of supply and price versus demand is that as price increases demand decreases (Figure 7). Similarly as quantity increases, price decreases to adjust for lower demand

However, this model doesn’t take into consideration consumer shift in demand due to other factors (Figure 8).
Consumers alter demand by changes in tastes and preferences, income, population or demographic changes, consumer expectations, as well as price and availability of substitute goods.

The demand for environmental horticulture plants is elastic because consumers are highly price-sensitive. Every 10% increase in price leads to a decrease in quantity demand for plants. For example, this decrease is 25% for impatiens, while other crops such as begonia and daylily are typically between 11 and 13%.

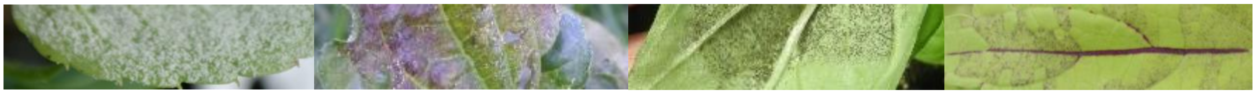


Figure 7. Consumer demand curve

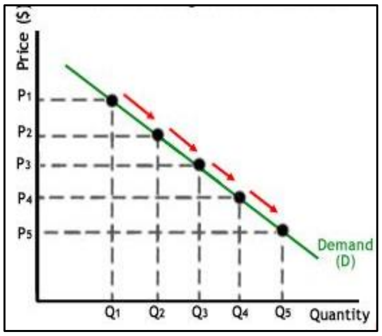


Figure 8. Consumer shift in demand

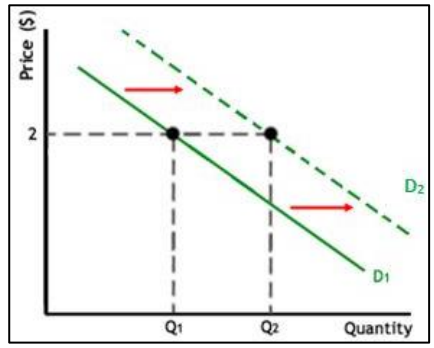


Figure 9. Product attributes, consumer preferences, and demand

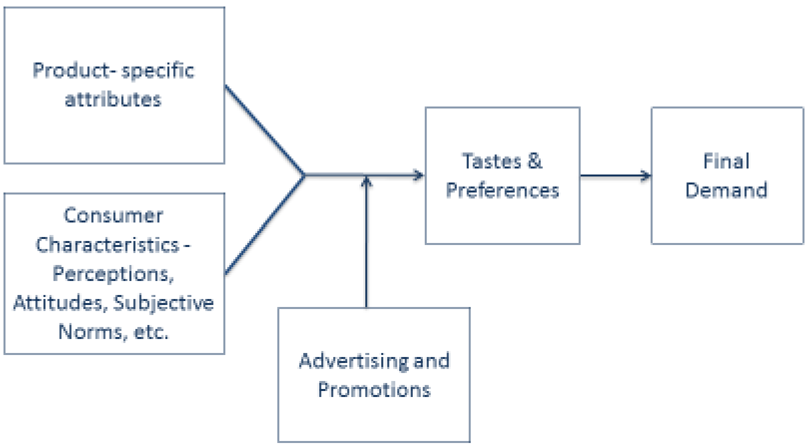
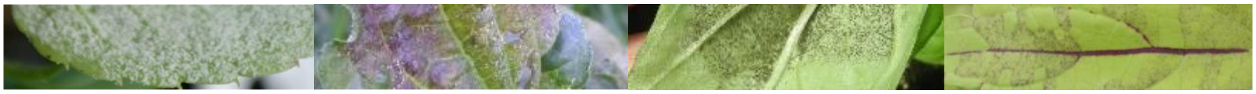


Figure 9 graphically demonstrates the interactions between product specific attributes consumers may need or want and the consumer characteristics (perceptions, attitudes). These basic consumer characteristics can be modified through advertising and promotions to influence specific tastes and preferences to impact final demand.

Consumers do not typically have option of buying the product that is best in every attribute, so they need to weigh those attributes based on their personal tastes and preferences. The product attribute types include Search which can be determined prior to purchase, Experience which cannot be determined prior to purchase unless that consumer has had prior experience with the exact product previously, and Credence which cannot be verified easily. Search attributes include plant color, whether it produces fruit, flower fragrance if flowers are present, etc. Experience attributes include longevity, fruit taste, flower fragrance if flowers are not present, etc. Credence attributes for plant products are typically those related to how those plants were produced by the grower.

As an example of this type of research, the effects of 'Sustainable' attributes were assessed for consumer preferences and willingness to pay (WTP). They compared different options for production methods (conventional, sustainable, energy-saving, water-saving), container types (plastic, compostable, plantable, recyclable) and origin of production (local, domestic, imported). Of the production methods,



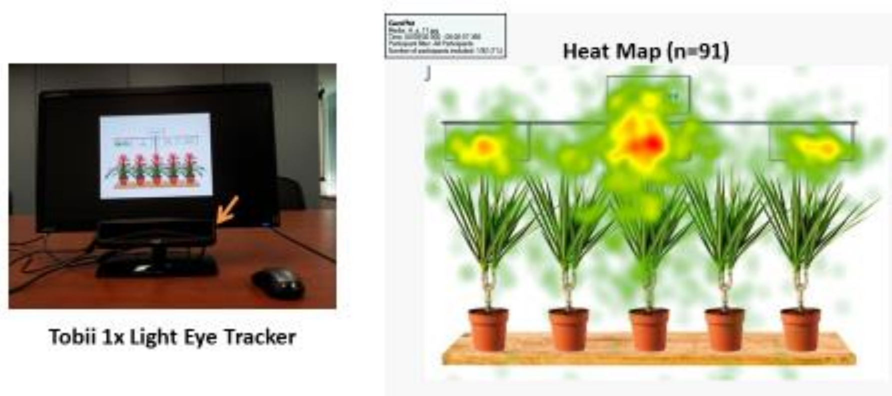
energy-saving increased WTP. Consumers were willing to pay more for the other container types over plastic. Locally produced was highly favored over imported and domestically produced plants.

People tend to underestimate and/or give less importance to future consequences. It is hard for people to see the benefit of something done today which will have consequences far in the future. For example, dieting, exercising, saving, and recycling tend to have little to moderate immediate benefits, but all these activities have positive impacts in the future.

As another example, “Pollinator Friendly” plant labeling has become highly desirable. Although many factors negatively impact pollinator health (nutrition, habitat, parasites, pathogens, genetics, biology, breeding, pesticides), the public has focused heavily on pesticides. There have been limited studies on consumer perceptions. The increased urbanization has decreased and fragmented pollinator habitats. There is a potential for influencing consumers’ plant selection with in-store education and marketing. In this research, there were three objectives: 1) determine the impact of “pollinator-friendly” attribute on consumer-preferences and WTP; 2) investigate consumer perceptions and effects on preferences; and 3) investigate visual attention to these labels and purchase likelihood.

The experimental design was based on multiple factors within each attribute: plant type (petunia, pentas, hibiscus), price ($10.98, $12.98, $14.98), pollinator (pollinator-friendly, no designation), production method (certified organic, organic production, conventional), and origin (in-state, domestic, imported). People participating in this study observed displays with randomized labels for each attribute (Figure 10), and in addition to their willingness to purchase this particular combination their eye movement patterns were recorded and converted to heat maps.

Figure 10. Computer generated willingness to pay study with eye tracking software



In this study, the location of production had the highest results with in-state and domestic production increasing the likelihood of purchase more than other attributes. The type of plant and production system had more impact than whether or not there was a pollinator label present. Price had a negative impact on WTP.

<u>Research Objectives</u>

- Economic feasibility study: profitability of early detection diagnostic tools for downy mildew using enterprise budgeting and benefit/cost
- Investigate factors that determine growers’ adoption of alternative pest management strategies and new tools and what makes people concerned about or aware of different issues. Study the

relationship between growers' awareness or uncertainty about long run economic and environmental benefits and their acceptance of alternative pest management strategies

- Assessment of consumers' perceptions knowledge, preference, willingness to pay using conjoint analysis to determine market potential of genetically altered crops susceptible to downy mildews. This will complement the other strategies for managing this disease.

Operational Risk Management Assessment (Baysal-Gurel)

Common production risks include weather, technical challenges, pest insects, diseases, and input quality and availability. These factors affect the quantity and quality of the final plant product. Risk can be analyzed by identifying potential risks that lower profits, evaluating the operation's risk management strategies, and identifying the cost effectiveness of various risk management alternatives on overall and targeted risk reduction.

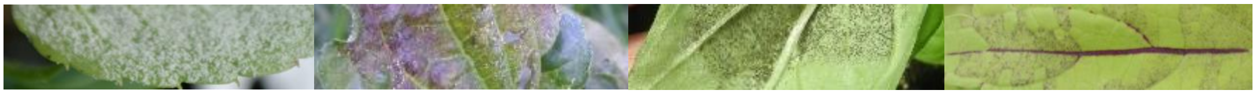
Growers have several strategies they can employ to mitigate risk such as crop insurance, crop diversification, contract production, input use, and new technologies or improved best management practices. Common strategies for risk reduction related to pest and disease management have common components to integrated pest management. Preventing pest/pathogen introduction, monitoring and scouting routinely for issues, accurately diagnosis the problem, and addressing those problems with appropriate solutions are common to risk reduction and IPM. Documenting and auditing should also occur in both to help mitigate repetitions of the same problem.

Risk assessment of an operation will help to identify critical control points which will aid in the creation of localized best management practices (BMPs) for disease and pest issues. Part of these activities is to document current practices which can determine the feasibility of additional practices. Elements of a good BPM are exclusion, detection, mitigation, traceability, recordkeeping, and training. Researcher/Extension prepared BMPs can aid growers in developing customized solutions for their operations.

Outreach & Communication (Santamaria)

Considering that approximately 81% of the agricultural workforce primarily speaks Spanish (U.S. Department of Labor, National Agricultural workers survey https://www.doleta.gov/agworker/report9/chapter3.cfm), there is a need to develop resources to help this diverse audience, especially with material that is relevant to nursery and greenhouse workers. They are at the front lines for growing plants and are more likely to observe problems than the managers. A well-trained workforce will improve productivity and efficacy. The Spanish-speaking workers learn about plants primarily on the job (79%, needs assessment for plant health education in OR nurseries, Santamaria L. data not published). The literacy rate is low; these workers have many years of experience working with plants but they don't know technical words and basic plant biology knowledge. Many thirst for knowledge and want to advance.

There are several key actions for effective communication within an operation. First, training people involved in every step of production is important! Not just people who are applying chemicals. While workers may not want to admit to having disease problems in a production area due to their cultural background, reporting those problems is key to effective production. Training about causes and sources of plant pathogens and microorganisms will help. Second, educational materials must be developed with



bilingual communication in mind. Posters and other materials must be visual with short, simple language in both English and Spanish.-Third, many workers while not well-versed in how to use computers can and do use phones for information. QR codes are helpful in directing workers to specific online resources. Fourth, scouting field books are used frequently.

In Oregon, they implemented modern teaching methods for Oregon's Agricultural Workers by creating a hybrid class where there is a mix of in-person and on-line educational opportunities. This class trains workers for pesticide license testing. This class is a priority for nursery owners. Historically, there has been a low passing rate for multiple reasons. The translation for labels is very poor with the tests conducted with the English version of labels and many labels are not available in both English and Spanish. While engrained in our culture, workers are not used to taking tests or using computers (computer-generated tests), and they may not have access to internet except possibly with cell phones. This class was very popular and workers want to take more because it can be accessed on their own schedule and has built-in quizzes which helps train them on multiple levels.

Most workers say the easiest and most important way to access material is face-to face.-PDF on smartphone and flipbook with side-by-side English-Spanish translations will improve learning. It is also not just translating information into Spanish. We really need to have different resources all together because workers are a different audience.

Grower Panel: Q&A

What is the most important issue facing your business?

Kube-Pak

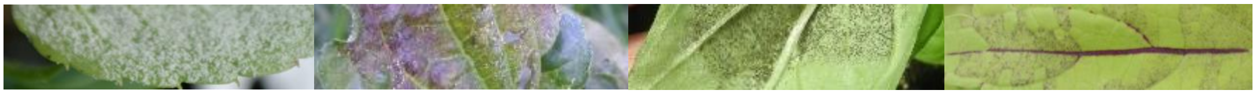
The "most important issue" is constantly changing
4 years ago, thrips was the biggest problem from a grower's perspective, now they know how to control it
in the future, labor will be a big problem. In NJ cost of living is very high; you can barely hire anyone for less than $15/hour

Making sure logistics are in place for every single crop/every single job
100 million plugs per year
~5000 different plant types
100 full time employees
250 employees in the spring

Labor availability and cost with minimum wage increase and water cost availability. More costs with increased regulatory issues. Pest damage thrips (virus), snails, powdery mildews on limoniums, snap dragons, ranunculus

Iwasaki Bros.

Agrees the problems change from year to year
18 acres Green House



How do you handle risk management for pathogens?

KubePak

learn from experience
sometimes it's the genetics of the plant/pathogen that they have little control over, but they have to figure out if that's the case, or if it's something they are doing wrong
prevention is key
Clean plants is crucial regardless of source, assume new stuff is dirty and treat it right away
unrooted cuttings gets a dip; most effective pest and pathogen management
root shield on everything
what's best for employees also drives a lot of their management strategies.
Most fungicides are done through fogs, which means no one is present when the application is done
they would be happy not to have to apply chemicals

Iwasaki

Wait until you get burned, then you're proactive next year. Things become a problem all at once. Sometimes a spray is not enough. You have to do a drench for coverage and control

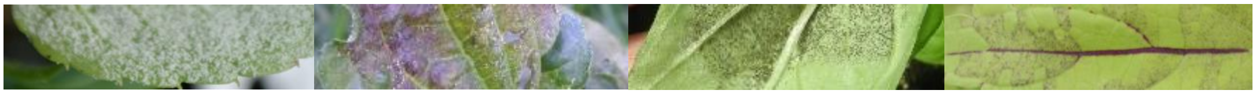
What crops have you seen downy mildew on?

KubePak

Basil, coleus, impatiens, ornamental cabbage and kale. They have protocols in place for these crops, and they have success with using chemicals with foggers. Staying on top of it and rotating chemicals works for them

Iwasaki

Many ornamentals are impacted



Where does downy mildew come into your operation?

Kubepak
Basil DM comes in on seed and there's nothing they can do about it; would like some sort of heat treatment for basil seeds
Upfront, they disinfect floors, surfaces, etc.
Sweep and weed
Doing a heat and chlorine treatment on seeds have had a major success in reducing disease, but they are charged for this service
They are making their own seed-treating pipeline
Needs to make sure costs are reasonable, effective, and streamlined
How can it be eliminated from the seed? This is very important to us.

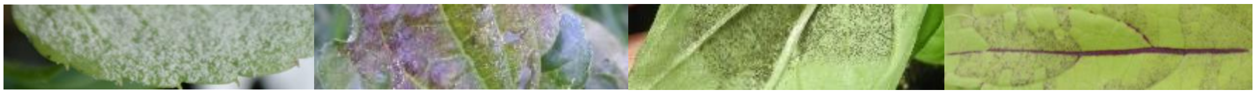
Iwasaki
they don't get treated seeds, but they think that is how it is coming in –or in cuttings
having an early diagnostic test to detect disease in nonsymptomatic transplants

Seed growers know that it's there, but at such a low level, so no practical way to eliminate it. No economic reason for them to chase it, they don't know where it's going to be planted. No Symptoms seen on their end.
Coleus downy mildew wiped out their whole green house.

How have you confirmed that you have downy mildew when suspected, especially on unexpected crops?

KubePak
visual by staff, then the plants are immediately removed from GH and the area is treated
they are hesitant to send out disease samples without getting burned by the government

Iwasaki
they send a lot of samples to OSU plant disease clinic; have connections with Melodie Putnam and Margery Daughtrey



How much impact do downy mildew diseases have on your business?

<u>KubePak</u>

a low consistent cost because they do continuous prevention

they spend weekly input to prevent a catastrophic outbreak. They are constantly preventing it.

an outbreak can be devastating

prior to IDM, 90,000 flats per year at $10/flat. After IDM, they only sold 3,000 flats a year

The biggest problem is they don’t know what crop will get hit next. In one year they lost their number one bedding crop. Switched to sunpatiens, but when will this one be hit?

*seem to be in a period of rapidly evolving problems for downy mildew and what happens when mutations become a problem

<u>Iwasaki</u>

Have a big problem with Brassicola, as it goes on all the different plants

*Having constant reporting of problematic cases of downy mildew outbreaks on all the hosts will be helpful. If we wait until it’s too late and you’ve lost an entire crop and have no resources. An anonymous system that allows tracking but is confidential would be useful

How do DM diseases rank vs. other diseases?

<u>KubePak</u>

Ranks high with botrytis –both have to be prevented continuously

because there are protocols that have been put in place, it is not currently a major problem, but 4 years ago it was very devastating.

to know the economic impact, you would have to stop the treatments(foggers, drenches, sprays and all the sanitation and temp/humidity control) and then find out the cost

<u>Iwasaki</u>

Number 1, plus botrytis and powdery mildew

How would you prioritize the potential research and extension activities?

<u>Kubepak</u>

knowing about seeds: is it coming in on seeds and if it is, how can we treat the seeds

biocontrol means of control

They’ve seen biological work for insect control, similar solution for downy mildews?

they’ve seen Calcium chloride works well for controlling botrytis similar solution for downy mildews?

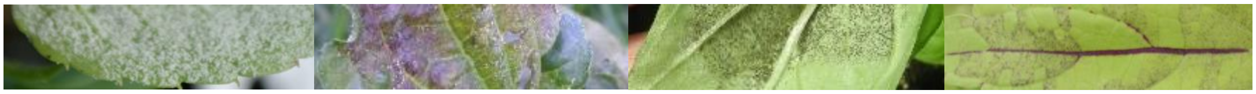
<u>Iwasaki</u>

wants practical information

is what we’re doing effective? What sanitizers, fungicides work?

do certain products/techniques work better for one downy mildew vs. an other? Or is there broadspectrum efficacy?

Could suppliers test their seed lots? Their stocks might not be as clean as they want/expect it to be.



Summary of Table Group Discussions

Over both days of the workshop, participants divided into 4 table groups to discuss research and extension questions for the above presentations. Below are the compiled questions and comments raised during both days organized by general research or outreach area.

Economic Impact of DMs

Questions

When an epidemic takes effect and eliminates a previously profitable crop, how does the industry or a specific grower transition? How can the industry quickly and easily identify replacement crop(s) and become efficient at growing them? How does a grower survive until the consumers start purchasing replacement plants?
What are the costs of education, information dissemination, and training? Are these costs different for smaller producers? How can we effectively reach and train smaller producers?
Many plant species are susceptible to downy mildew pathogens (ie impatiens, sunflower, viburnum, roses). How do we decide which to research? Which are most economically important?
How can we collect the information to really know the economic impact of downy mildews, both from plant and pathogen perspectives?
Can we determine value of diagnosing specific downy mildews?
What is the cost of prevention, vigilance vs cost of 95% loss of impatiens crop
Can we capture the % of crops/crop value impacted by DMs —> losses, required inputs, etc.

Comments

We need to focus economic impact question on single or very few downy mildews.
We should determining the cost of each practice versus return, including multiple practices such as fungicide treatment, sanitation, etc.
We also need to consider the benefit of treatment versus costs.
The focus should be trying to find the real economic impact of the DM and then develop strategies of identification and diagnosis, then control methods.
Industry could provide some numbers
Compare to 95% loss of impatiens crop in one year
20% of chemistry purchased last year was for DM (fungicide or all management tools?)
We must have cost/benefit analysis
What the consumer says matters (perception is reality)
Impatiens and rose can be case studies of economic impact
We can compare control methodologies and assess their economics
Modeling factors that influence adoption of new technologies, how successful would these new products be in current consumer markets. Cost analysis, need to focus on specific crops. Tough to get specific cost information from growers, which makes cost analysis difficult.
IDM still an economic problem. At Kube-Pak: used to grow 90,000 impatiens flats a year, went down to 3,000 flats, but now up to 6,000 flats. The market has adjusted- they have substituted other plants. They got rid of a very inexpensive plant to produce and replaced it with a plant that is expensive to produce---but it took 4 years to get to that point. Be conservative---people want a plant that people know how to grow- and they perform well. Landscapers took the greatest hit because impatiens was used so extensively. Same with impatiens breeders.

Identification & Diagnostics

Questions

How can we foster propagation companies to embed the use of diagnostic tools within their standard operating procedures to reduce spread of quiescent infections?

What kinds of diagnostic tools can we develop to identify systemic, asymptomatic infections?

Can we develop rapid, easy-to-use, cost effective diagnostic tools for downy mildews?

Can these tools be used on different plant matrices (seed, stems, leaves, etc)?

Can what has been learned in the cucurbit downy mildew system be applied to EHC DMs namely sampling for spores via spore traps and development of quick diagnostic tests?

Comments

Diagnostic tools may stop the surprise of downy mildew epidemics

We need quick diagnostic tests for growers.

Serology would be very useful. Developing an Agdia test strip would be beneficial to growers.

Objectives

Develop spore trap to accurately monitor spikes in DM populations

Develop serological diagnostic tools for grower-level diagnoses

Develop tools that growers can implement to show that they have clean stock

Biology & Epidemiology

Questions

What are the basic life cycles for model downy mildews of ornamental horticulture crops? How do they differ from the standard, well-studied systems? Do these differences impact recommendations for disease management? Can we group EHC DMs with those that are more well studied for predicting conditions suitable for infection?

Where is the disease pressure coming from? Is it all coming from infected seed/cuttings? Is it surviving somewhere in the greenhouse vs. coming in from outside? Is there late season pressure?

What are the inoculum sources?

Are oospores a problem for producers? (if so, this can change the management strategies)

What forms of the pathogen are on/in the seed for seed-borne DMs? How does infection start if you know you have clean seed? Does it start?

What are the unique characteristics of basil seeds that make this so highly seed borne? Do any other EHC DMs have the same characteristics?

Do different DM races of the same pathogen have different pathogenicities?

How does temperature and other environmental parameter impact seed-borne inocula?

Can we develop improved knowledge of environmental thresholds for more accurate epidemiological models to target optimal timings for interventions?

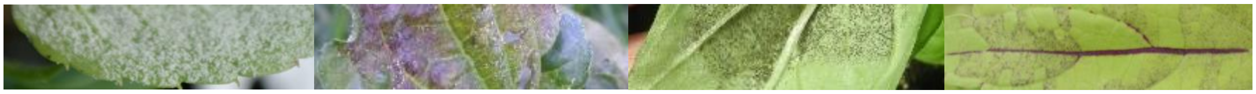
What are the temperature and environmental requirements of various DMs?

Do temperature and light impact pathogen infection and development (ability to form sporangia, oospores)?

Can we group DMs by certain environmental parameters, so that a single model will be predictive for all DMs in that group?

What agriculture engineering solutions can be developed to manipulate temperature, relative humidity, water, gases, light etc to reduce favorable environmental conditions?

What are key plant attributes that can be manipulated during production to improve innate resistance/tolerance to DMs?



How does the plant microbiome impact disease development or pathogen susceptibility/tolerance?
What is the role of nutrition (silicon, calcium, calcium chloride) in reducing disease development?
What is the "right" balance of nutrients? Is the balance different for different host/pathogen systems?
How do phosphonate fungicides work to ameliorate disease (in particular, IDM)? What are their modes of action?
Can UV or infrared lighting be used effectively to reduce sporulation and aggressiveness for several downy mildew pathogens? Will it work in multiple host/pathogen systems?
Is it possible to develop "near remote sensing" technology based on chlorophyll changes to pinpoint early infections?

Comments
It is important to know as much as possible about life cycle because this can be applied to production system to minimize disease development
Establish the seed transmission question for each system
Si accumulators may have reduced DM
We can study the impact of light on DMs using model systems (IDM, Rose, Sunflower, Viburnum, Basil?)
Four genera in orn hort industry: *Peronospora*, *Plasmopara*, *Bremia*, *Basidiophora* (this ignores *Hyalopersonospora*)
Crops impacted by DM: roses (*Peronospra sparsa*), basil (*P. belbahr*ii), rudbeckia (*Plasmopara halstedii*), coleus (*P. belbahrii*), impatiens (*Plasmopara obducens*), alyssum (*Hyaloperonospora lobulariae*), and more

Objectives
Study life cycles for key model EHC DMs and determine inoculum source(s)
Determine role of environmental factors (temperature, light, and plant nutrition) on pathogen infection and development
Develop solutions to manipulate environmental factors to reduce disease incidence and/or severity
Examine host range & susceptibility for distinct DM races across crop cultivars and related species.

Genetics & Genomics
Questions
What are the phylogenetic relationships among geographical/temporal isolates?
Are there cryptic species within model ornamental horticulture downy mildews?
Within a pathogen species, can we identify populations that are no longer sensitive to fungicides?
Do the fungicide-resistance markers from other systems apply to ornamental horticulture downy mildews? Can we use Kale as a baseline model?
Are there other genes for resistance?
What are the effectors in ornamental horticulture downy mildews? Can we identify enough to start building a catalogue?
Are specific DMs homothallic or heterothallic?

Comments
The groupings/relationships can be helpful for understanding management options, the risk of oospores development, and potential dispersion pathways.

Objectives
Determine whether known resistance genes from other systems are present in identified resistant populations of EHC DMs

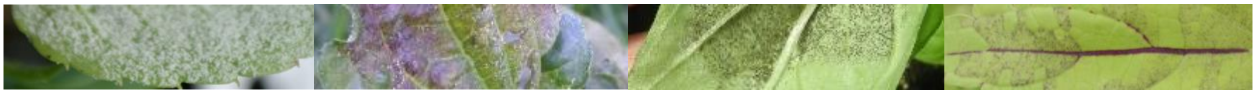
Identify effectors for specific EHC DMs
Determine phylogenetic relationships of spatial and temporal isolates of specific EHC DMs

Management Options 1: Biopesticides, Designer Chemistry, Induced Resistance, Environment
<u>*Questions*</u>
For seed-borne DMs, can chlorine be used to reduce initial inocula? Can the use of heat treatment of seed be expanded to ornamental horticulture crops?
Can we develop spore trap system to monitor spikes in the population so as to alter management strategies as soon as possible? Part of this research would establish baseline levels to determine how many spores is cause for intervention (*overlaps with biology questions*).
What are preventative treatment(s) that work(s) across floriculture, herbs, woody ornamentals, different chemistry to cover all downy mildew pathogens? Are there any?
Can we identify biological controls for DMs?
Are there any endophytes with anti-DM properties that can be the basis for new biocontrol tools?
Can *Trichoderma* isolates negatively impact oospores?
If/when they become available, what are the IPM strategies to incorporate biopesticides?
Can any SAR inducers provide efficacy the life of the crop?
Are there differences in application technology that can be used for optimal efficacy at different growth stages?
Can we develop BMPs to cover all DMs? Can we develop BMPs that can cover subsets of DMs based on similarities?

<u>*Comments*</u>
We need to develop best management practices for rotations
Management of resistance — nutritional interactions (silicon)
We need to consider some alternative treatments for seed borne DMs - heat, chlorine, ozone, reactive oxygen species in general, electrostatic zappers.
In the short term, there are several potentials for management: chemicals, biopesticides, options for- effective land treatments (esp. useful for landscape customers), further exploration of phosphonates (slow release formulation?), nanoparticles (Ag, Cu), and anaerobic soil disinfestation (ASI) for activity against oospores.
Light treatment, perhaps at night
Screening for production and landscape
In the long term, additional research could be conduction on biologicals, biorationals, systemic resistance products. This will provide an integrated method to management.
Depending of the area where the ornamentals are located, and which ornamentals are grown where, specific strategies can be developed that address the local issues.
A good, sound best management practices. That includes information about the pathogen, such as a life cycle. Knowledge about the pathogen is key about this best management practices.

<u>*Objectives*</u>
Develop best management practices for DM
Develop strategies and tools that propagators can use to maintain clean stock
Examine use of molecules or biologicals to stimulate systemic resistance
Screen and develop biopesticide solutions
Screen and develop chemical solutions

Management Options 2: Host Resistance, RNAi/Genome Editing
<u>*Questions*</u>



What level of innate host resistance exists with crop cultivars or related species?
Is resistance in *I. walleriana* against IDM plant based or microbial?
Is genome editing risky?

Comments
There is promise for using CRISPR/cas9 to alter genomes for disease resistance. There appears to be less public backlash as previous genetic technologies.
Host genetics – breeding superior resistant varieties
Screening for traits
Develop resistant plants. There are many groups actively working to find or develop ornamental plant varieties that are resistant to DM. Combination of conventional breeding and genetic modification. Find resistance in the germplasms available. Getting a resistant cultivar is a long process
Good candidates for genetic breeding: Rudbeckia, impatiens. Based on time required to find resistance, at least 4 years for those species displayed.

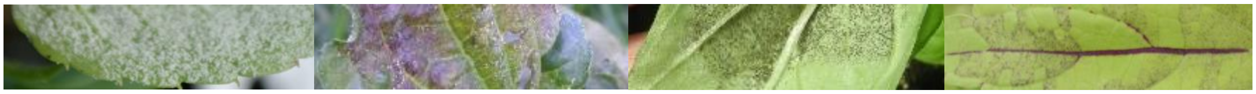
Objectives
Development of host resistance through CRISPR/Cas9 technology
Breed hosts for resistance or tolerance

Operational Risk Management
Questions
What materials can we develop to train/educate growers on how to assess operational risk associated with plant pathogens, DMs in particular? Can we develop handout? Standard form or checklist?
What are the critical control points in a greenhouse/nursery/landscape related to DM management?

Comments
Risk assessment – within a nursery what percent of crops affected by DM
Tracing the critical control points: contaminations, HACCP analysis
Young plants -> export -> potting -> finish -> customer

Outreach & Communication
Questions
How can we facilitate changes to cultural practices to reduce disease development?
What type of systems/tools can we provide for extension? Ie lists of environmental horticulture plants that are prone to downy mildew.
Can we provide a website/tweets/emails with local or weather updates when conditions are favorable for disease development?
What is current level of DM knowledge among growers, landscapers?

Comments
Training workers, educating smaller productions, people new to the business
Information and solutions translated to home owners.
Develop a website for everything DM (pictures, fact sheets, Q&A, training resources, guides, regional outbreaks)
Make sure there is education/outreach for landscaping industry
Improved worker training PM vs DM and then assess with surveys before and after outreach efforts
We need to write explanation for why CRISPR isn't GMO
Develop a way to track infections visually (pictures, info, indication of regional location), simple calendar



Compiling one big resource: host/pathogens, seed architecture, how affects seed, overwintering, homothallic/heterothallic
Reporting as for public gardens only. Not producers. DMs, photos, dates, conditions, way to confirm, way to get data regionally up to confidence level. Need buy-in from NPGA and possibly NPDN.
App — back pocket grower model?
Research activities immediate short term: audit methodologies, practical application of knowledge we have
Hybrid webinar (interactive)

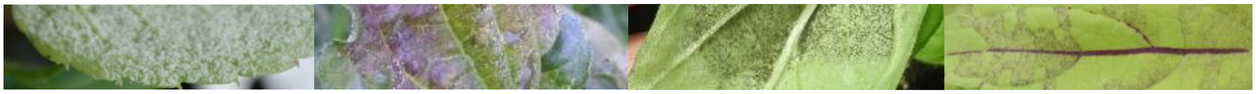
- Basic – for workers (simple, visual, Spanish & English)
- Advanced – for supervisors (life cycle, hosts, etc, minutiae)

Education/outreach to landscape industry – they are very different from the greenhouse industry
Ensure sanitation principles are included in training

Objectives
Determine baseline DM knowledge of workers, supervisors (greenhouse, nursery, landscape) and assess change in knowledge after use of new learning tools.
Develop comprehensive DM website (pictures, fact sheets, Q&A, training resources, guides, regional outbreaks, etc) with areas for workers (basic, simple, visual, Spanish), supervisors (advanced, more in-depth, english), others?
Develop apps geared toward specific communities (public gardens,
Develop webinars or other online learning tools geared to target communities (workers, supervisors, etc)

Table 4. Summary of table group discussions

Area	Key Questions / Activities	Research/ Outreach Objective	Short/ Medium/ Long Term
Biology/ Epidemiology	Further characterize seed transmission for key DMs	Research	Short/Medium
Biology/ Epidemiology	Elucidate the role of nutrition including phosphonates and silicon in managing DMs	Research	Short/Medium
Biology/ Epidemiology	Elucidate the role of plant microbiome in reducing DM infections	Research	Short/Medium
Biology/ Epidemiology	Determine the role of light in limiting DM infection and development	Research	Medium
Economics	Determine impact of epidemic on growers and costs associated with transitioning to new EHCs	Research	Medium ?
Economics	Determine costs associated with education, information dissemination, and training	Research	Short
Genomics	Determine whether known resistance genes from other systems are present in identified resistant populations of EHC DMs	Research	Short
Genomics	Identify effectors for specific EHC DMs	Research	Medium/Long
Genomics	Determine phylogenetic relationships of spatial and temporal isolates of specific EHC DMs	Research	Long
ID/Monitoring	Develop spore trap to accurately monitor spikes in DM populations	Research	Short
Genomics/ Management	Development of host resistance through CRISPR/Cas9 technology	Research	Long
ID/Monitoring	Develop spore trap to accurately monitor spikes in DM populations	Research	Short
Management	Breed hosts for resistance or tolerance	Research	Long
Management	Develop best management practices for DM	Research/ Outreach	Short/Medium
Management	Develop strategies and tools that propagators can use to maintain clean stock	Research	Short/Medium
Management	Examine use of molecules or biologicals to stimulate systemic resistance	Research	Medium
Management	Screen and develop biopesticide solutions	Research	Medium/Long
Management	Screen and develop chemical solutions	Research	Medium/Long
Outreach	Create website as clearinghouse for DM information, possibly inclusion of interactive QA	Outreach	Short
Outreach	Develop training materials for workers	Outreach	Short/Medium
Outreach	Develop/distribute information about CRISPR and how it differs from GMO	Outreach	Short
Outreach	Translate ideas to homeowners	Outreach	Medium



Objectives/Approaches

Short Term

(1 – 2 years from start of grant)

1. BMPs based on current knowledge, constantly update based on research activities. Nursery vs. greenhouse production
2. Website for everything DM (pictures, information, app – back pocket grower model, include training!; focused information for growers & workers)
3. Images of DMs
4. App for ID of DM's with photos, dates, environmental conditions for public gardens. This is a way to get epidemiological data regionally without opening growers up to confidentiality issues. Need buy in from NPGA and NPDN
5. Develop fungicide rotation options for DMs not yet screened based on best available info from similar systems
6. System for growers to confidentially enter information and discreetly retrieve information: disease, fungicide effects, environmental conditions when appeared, general geography (county? state?).
7. Incentivize DM sample ID
8. Consumer survey for factors that impact adoption of new technology
9. Cost analysis for current crops impacted by DMs – cost of maintenance to prevent DM
10. Screening for resistant cultivars or species
11. Collections of the pathogen from different hosts and locations, and screening for general id => sequencing and ID upon receipt, phylogenetic tree; purchase of DM samples???
12. Sanitation protocols, research (screening) for products to kill oospores/sporangiospores, in plants & hard surfaces
13. Role of nutrition in disease development (Si, Ca, PO3, etc)
14. Role of environmental triggers (light, moisture, temperature) in pathogen biology and development

Medium Term

(3 – 5 years from start of grant)

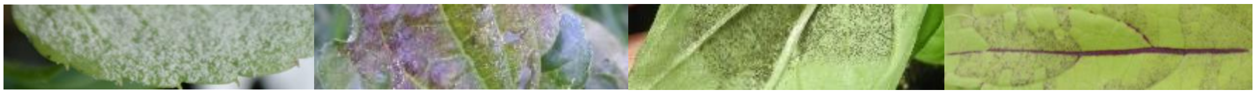
1. Screening for new biologicals
2. Screen SAR/ISD materials
    a. Combos of 1 & 2 above
3. Environmental controls inside greenhouse: light, temperature, humidity
4. Diagnostics
    a. Serological assay to detect general DM infections (by genera? Or all)
    b. Molecular assay
5. ID of fungicide resistance, especially fungicide resistance diagnostic tools
6. Development of Spore traps & immunofluorescent assay
    a. Help to track start of infections
7. Seed transmission & treatment
8. Impact of BMPs
    a. Economic analysis – need to make sure we capture all current practices
9. Customer survey for WTP using visible threshold levels (realistic portrayal of reduced quality with certain management strategies)

10. HACCP
    a. Systems approach to determine points at which infection occur
    b. Need surveys and in person visits to determine critical control points and site BMPs.
    c. Also need long term validation of approach

Long Term

(5 years and beyond from start of grant)

1. Host-pathogen interaction, effecter genes
2. Genome editing (growers largely focus on producing very good crops, but retailers and consumers could be less amenable) and traditional breeding
3. Environmental Controls
    a. Out of short/medium term research
4. Validation of systems



Appendix 1: Plants We Have Seen With DM in the Flower/Nursery Industry

Prepared by M. Daughtrey and A. R. Chase, 11/13/16

Adoxaceae
Viburnum

Aizoaceae
Aptenia (ice plant)

Asteraceae
Argyranthemum
Aster
Coreopsis
Felicia
Gaillardia
Helianthus (sunflower)
Helichrysum
Osteospermum
Rudbeckia
Solidago

Balsaminaceae
Impatiens, Balsam impatiens

Brassicaceae
Alyssum
Iberis
Matthiola (stock)
Ornamental cabbage and kale

Cannabaceae
Hops

Capparaceae
Cleome

Dipsacaceae
Scabiosa

Gentianaceae
Lisianthus

Geraniaceae
Geranium

Lamiaceae
Agastache
Basil
Coleus
Lamium
Rosemary
Salvia

Lumbaginaceae
Limonium (statice)

Plantaginaceae
Digitalis (foxglove) and Digiplexis
Snapdragon
Sutera
Veronica

Rosaceae
Geum
Potentilla
Rubus - Creeping raspberry, blackberry
Rose

Rubiaceae
Galium

Scrophulariaceae
Buddleia

Violaceae
Pansy