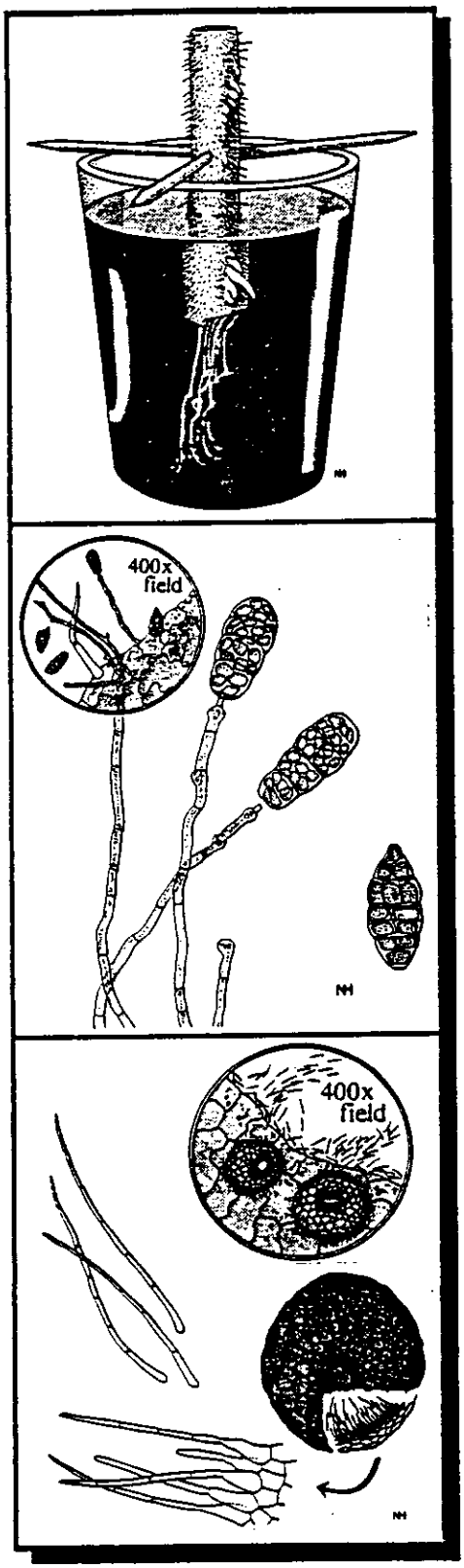

June, 1993
Volume XIV Number 2

PLANT DIAGNOSTICS QUARTERLY

Features

Light Microscopy for Plant Virus Detection:
Part 1. Materials, Methods, and Utility

On the cover: *Pseudomonas solanacearum* streaming from tomato stem.

Stemphylium lycopersici.

Septoria lycopersici.

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June, 1993

PLANT DIAGNOSTICS QUARTERLY

FROM THE EDITOR

We are slowly catching up to a normal issue calendar!

Issuance of PDQ is still off the normal quarterly calendar schedule but we are gaining ground. By the end of 1993, we will be back on the proper timing for issues - assuming we have something to publish! I have started begging some of you individually for "Feature" articles for PDQ future issues. More of you will receive letters and/or phone calls in the future to help carry future issues of this newsletter. I did volunteer to take a turn at editing PDQ, however this does not include writing articles for every issue!

The schedule for the "September" issue is fast approaching behind this one. We need overviews on activities planned for the November APS meetings that focus on diagnostics. I would also appreciate some summary information on the recent Sixth International Congress of Plant Pathology held at Montreal. Don't wait for a formal invitation to submit articles. Call me at (904) 392-1994, FAX topics to me at (904) 392-3438 or use BITNET EXTPPCLINIC@IFASGNV.

G.W. Simone
Editor

REGIONAL REPORTS

Northeast Region

Anne Bird Sindermann

From Connecticut to DC drought stress has been the primary problem reported by plant pathologists in the northeast region. Field crops, especially corn, ornamentals and shade trees are all showing signs of prolonged hot and dry weather.

In Maryland a stunted, variegated lemon balm, *Melissa officinalis* 'Aurea', under suspicion for impatiens necrotic spot virus due to bronzing, vein-reddening and small black spots, tested positive for alfalfa mosaic virus in ELISA. A follow-up visit to the greenhouse revealed that spider mites had caused the bronzing. More testing would be necessary to conclude that the yellow variegation is due to the virus. The grower reports that the variegation is not present under all growing conditions, for example early spring flushes of growth are often not variegated.

Also in Maryland, Ethel Dutky reported *Ditylenchus dipsaci* commonly called stem and bulb nematode, in field-grown *Phlox*. Symptoms included stunted plants with yellow and brown leaves, and poor bloom. Nematodes may be seen in dried leaves if pieces are torn underwater, and will emerge from phlox stems and leaves put in a dish of water for several hours or overnight. They are large nematodes and have small stylets in comparison to many other plant parasitic nematodes.

Virus-like symptoms in dahlias were due to hopper burn in New York. Margery Daughtrey described the symptom as diffuse yellowing from edges of leaves. The sample didn't contain flowers so she cannot say whether flowers were damaged by the leafhoppers. Samples with Dutch Elm Disease were received and bacterial blight continues to cause problems in New York.

Mary Ann Hansen determined herbicide injury to be the cause of a water lily problem in Virginia. Marginal leaf necrosis and chlorosis, slow new growth and foul smelling crowns in nursery grown water lilies was traced to use of the herbicide Aquathol. The grower was treating for potamogeton, a common pond weed, and found the aquatic herbicide is also effective against water lily.

Barley leaf rust that overwintered in Virginia became widespread in fields when temperatures increased in the spring and secondary rust cycles started. Also in Virginia, tobacco blue mold was reported in late June in Charlotte County. The source of inoculum was thought to be infected tobacco transplants.

Pacific Northwest

Ellen M. Bentley

It's said in the Pacific Northwest that "If you don't like the weather, wait five minutes!" As a drysider I always applied this maxim to the wetside of the region. This spring our diagnosticians report to the contrary.

Western Washington experienced severe disease pressure this spring related to the wet weather conditions. Carrie Foss (WSU-Puyallup) reports Coryneum blight, cherry leaf spot (*Coccomyces*), and brown rot on *Prunus* have been submitted in high numbers. Peach leaf curl was common on all peach varieties, including 'Frost' peach. Severe apple scab was observed on susceptible varieties which had few or no fungicide applications. Yellow patch on nutrient-deficient turf has been a problem for golf course superintendents through June. Powdery mildew infections and drought stress last summer combined with winter injury during 1993 were contributing to defoliation of rhododendrons this spring. Christmas tree growers have observed sporulation of both *Pucciniastrum* and *Uredinopsis* rust on grand fir during early July.

Twenty-five veteran Master Gardeners who volunteered in the Puyallup Plant Clinic last summer have returned during 1993. In addition, there are 30 trainees who are volunteering in the Plant Clinic this summer. At present, 1186 disease and insect samples have been processed.

Over the Cascades, Ellen Bentley (WSU-Prosser) has learned her wheat pathology. Both dryland and irrigated production have had problems. It began with snow molds (*Typhula* and *Fusarium*), nutrient deficiencies (cold, wet soils), barley yellow dwarf virus, physiologic leaf spot, strawbreaker (*Pseudocercosporella herpotrichoides*), sharp eyespot (*Rhizoctonia*), take-all (*Gaeumannomyces graminis*), Cephlasporium stripe and now the spring wheat has wheat streak mosaic virus! Topping the list is a region-wide occurrence of severe common dryland root rot, even in drought stressed irrigated wheat. I usually attribute this to *Bipolaris sorokiniana* as much as to *Fusarium culmorum*. This year infections have continued into Fusarium leaf blight and head scab, and isolations have clouded the identity of the guilty *Fusarium* sp. Similar root rot problems have developed on wheatgrass in CRP ground. Stripe smut (*Ustilago striiformis*) is increasing in timothy hay.

A blast of 90+ temperatures (mid-May) nipped the emerging potatoes causing symptoms which resembled virus or herbicide damage. Once again late blight has reared it's ugly head causing concern due to the aggressive, metalaxyl-resistant strains of *Phytophthora infestans*. On a brighter note, the moderate weather has extended the green season for weeds and wheat, delaying leafhopper and curly top virus migration to vegetables.

Cherry harvest survived the capricious rainstorms although many orchards displayed symptoms (shock, shot hole, ugly fruit) of Prunus necrotic ringspot virus after the May heat wave. This interval also kicked off four fire blight infection periods threatening both pears and apples (especially 'Gala/M26', 'Fuji/M26' and 'Braeburn'). Our growers seem to have forgotten that apples are also susceptible and have not been practicing diligent sanitation in the past few years! Scab is also plaguing many orchards.

In the unique category was a submittal from one of our first commercial ginseng growers. Thank goodness he brought his copy of the excellent U Wisconsin Extension Bulletin. If only all of our client's were so thoughtful!

To the south, Phil Hamm (OSU-Hermiston) seconds similar problems in dryland and irrigated wheat (take-all, WSMV, strawbreaker, *Septoria*, common dryland root rot). Late blight is also present in potatoes. Dryland peas suffered from root rot complex (*Fusarium, Pythium, Aphanomyces*). Of note are high counts of stubby root nematode in onions.

Melodie Putnam (OSU-Corvallis) adds that spring was the end of western Oregon's drought of the past several years. Sixteen inches of rain fell in Corvallis between March and May -- an exceptionally damp spring, even for Oregon. Fruit harvests are far below normal (or non-existent, as is the case with cherries) because of poor pollination. Not surprisingly, all kinds of leaf spots and needlecasts were

prevalent. *Septoria* was found on seemingly everything, even loganberry fruits. The needlecast fungus *Hormonena merioides* was active on 'Blue Atlas' cedars (a new host), as was a species of *Sirococcus* (also a new host record). Both fungi were present together on all the samples received. The primary symptom on the tree was a tip dieback, with needles becoming infected first and the fungi then moving into the twigs.

Root rots were also common, especially *Phytophthora* on blueberries and strawberries. Witch hazel growing in containers that were showing dieback symptoms had *Thielaviopsis basicola* beautifully sporulating on the roots. Miscellaneous root rots were abundant on strawberry and mint; the latter crop also came in with a lot of *Phoma* black stem this spring.

The big news is that soil-borne wheat mosaic has been confirmed in wheat from western Oregon. This is believed to be the first incidence of this virus west of Kansas (and if anyone has information otherwise, please let me know). There was also an interesting virus found in daikon radish grown for seed. Symptoms were striking in early spring -- yellowing, stunting, foliar distortion and superficial black lesions on stems and leaves. We are still working on the identify of the virus, which is easily mechanically transmissible and appears to be "spherical" in shape. Stay tuned.

Karen Flint (USU) reports that spring is a capricious thing in Utah--warm and dry one week, deluge and possible frost for the next two. We were still covering our tomato plants at the end of June! It has been especially hard on hay growers because there is never enough predicted dry weather to cut and many growers lost the gamble with the rain. (I got lucky and got eight tons of dry hay in the barn.) Many crops and landscape plants just aren't growing well this spring. The cool rains brought us many diseases which I haven't seen here before as my tenure at USU has been one of drought.

On barley, we had the usual yellow dwarf virus, a little higher in incidence than in the recent past; we also have stripe, spot blotch, scald and common root rot in abundance. Wheat has been less interesting, with just wheat streak mosaic and eyespot (my first one!) Stem nematode has had a delightful holiday in alfalfa. Typically I find it just in low patches, but now whole fields are affected. We had so much snow, the mice were well hidden from predators and destroyed many acres of alfalfa in Cache Valley--in some fields, 95% loss of plants!

Of course, we have rampant fire blight in pears and apples; but I am also seeing apple scab in abundance, which is unusual for Utah. I have seen peach leaf curl here for the first time, and also bacterial canker (*Pseudomonas syringae*) in cherries and possibly chokecherries. Can anyone confirm that chokecherry is a host?

The latest in vegetables is tomato spotted wilt virus (along with abundant curly top) in tomato fields. In both cases where I've identified this, the transplants were brought from Georgia. Another case of pathological sabotage! I am stumped with an asparagus problem: I isolated a *Fusarium* from dying crowns but the symptoms aren't right for the described *Fusarium* diseases.

We have the usual, expected turf problems (snowmolds and melting out) in epidemic proportions. Several times I've looked at seedling turf, obviously dying, but I cannot find lesions or any other apparent signs of pathogens, and don't isolate anything significant either. The characteristic symptom is the purpling of the older leaves and nothing else. The turf workshop, no matter when it is, won't happen soon enough for me! We have a greenhouse operation growing snapdragons for cut flowers. The grower had a serious problem with downy mildew, but remained convinced that he could control it with chemicals and refused to discard the infected plants. So, now all his seedlings have downy mildew. A severe case

of extension education going awry, through no error of our own! Finally, I have diagnosed yet another case of Botrytis rhizome rot in iris, and again the rhizomes came from the Pacific Northwest. Thank you comrades, for keeping my life interesting!

In Idaho, a severe outbreak of fire blight has been observed in apple orchards mainly on 'Johnathan' and 'Rome Beauty' varieties. This was favored by the unusually wet spring weather, with occasional hail storms and gusty winds. In addition to blossom blight, shoot blight was quite common. Many blocks showed blighting of young fruit. All the isolates obtained so far are streptomycin sensitive.

Also favored by the wet spring weather, widespread incidence of shot hole disease (*Wilsonomyces carpophilus*) has been observed on peaches and apricots, especially with severe fruit symptoms. (S. Krishna Mohan, UI-Parma).

Wheat streak mosaic virus is severe in Montana wheat this season. Also reported by Martha Bamford (MSU) is tan spot, *Septoria* leaf spot, common root rot and wind injury. On barley, barley yellow streak mosaic, net blotch and scald. Irrigated alfalfa is suffering from Verticillium wilt, bean stands were reduced by damping off and wirestem is present in cabbage.

Bacterial diseases include fire blight of apple and *Pseudomonas* blight of lilac and aspen. Rainy days are good for ducks--and fungal diseases. Cool, wet weather this spring was ideal for anthracnoses on ash and maple. Juniper-hawthorn rust was also observed on juniper and juneberry.

Wyoming's growing season also began cool and wet according to Colette M-S Beaupre' (UW). Rust and virus diseases have been more common than usual on small grain; as has tan spot. The cool, wet soil conditions have delayed development of corn, with some unexpected herbicide injury. As the weather warms, severity of Rhizoctonia root rot in sugar beet is increasing.

Cytospora canker and fire blight have both been especially aggressive this year. Conifers are showing effects of winter desiccation as well as needle cast disease. Rust diseases in ornamentals appeared early this year, and continue to be a problem.

The volume of samples submitted to the UW Plant Disease Clinic has diminished considerably with the establishment of our fee system ($10 general diagnosis, $5 plant/weed identification, and $15 turf problem diagnosis). The clinic has processed 39 samples since the beginning of 1993. Predictably, none of these were turf problems.

No reports were submitted from Alaska, the Dakotas, or Colorado.

Southeast Region

Jackie Mullen

Early spring started off (March 12-14) with some unusual weather as much of the Southeast experienced an abrupt temperature drop and blizzard-like (that's right - snow!) conditions that produced about 2-10 inches of the white stuff and caused temperatures to drop into the single digits and teens in many parts of the Southeast. The low night temperature for Mobile (in the peak of azalea bloom!) was 20^0F the night of March 13 (J. Olive). The low temperatures came after the early spring (and mild winter) moderating temperatures (70^0F!) experienced by much of this area. In Alabama, cold damage to landscape plantings (stem/trunk splitting) and the peach crop (estimates indicate over 50% of the peach crop was damaged by cold) did occur, but the snow that accompanied the cold temperatures helped

prevent more severe damage in the form of root freeze. Many nurseries in South Alabama that covered their plantings escaped much of the cold damage (J. Olive). We did have one report of greenhouse structural collapse from the weight of the snow accumulation. There was considerable damage to new spring growth (blossoms and foliage) on Japanese magnolias, boxwoods, some junipers, azaleas, etc.

Late spring reports indicated that southern blight (*S. rolfsii*) has been especially prevalent in many southeast areas.

Florida Report (G. Simone) - Incidence of CMV was again widespread in north and central Florida in the tobacco acreage. An unusual occurrence of black blotch or sooty blotch of clover caused by *Polythrincium trifolii* was noted this spring. Several unusual vegetable diseases were observed including *Cercospora nasturtii* leaf spot on water cress and Cercosporidium leaf spot of dill. An outbreak of Bean Golden Mosaic Virus of snap bean occurred in the Dade County bean production area. This disease was first diagnosed by the plant virus inclusion method and later verified by cDNA probes as a non-endemic strain of this geminivirus in Florida.

Increased reports of take-all patch of St. Augustinegrass caused by *Gaeumannomyces graminis* var. *graminis* were statewide in distribution - particularly from urban turf sites. This fungus has also been verified from centipedegrass in the panhandle region and zoysiagrass from central Florida. The teleopmorph has been detected from St. Augustinegrass crown tissue as well.

New diseases within the ornamental industry in Florida included tomato spotted wilt virus (L-strain) on Stokesia (Stoke's Astor) and Cucumber Mosaic Virus on Bougainvillea. New reports of a Cylindrocladium sp. root rot disease were documented on *Cycas revoluta* and *Jasmine* sp. Also a new spot disease on Desert Rose (*Adenium obesum*) was reported to be caused by *Corynespora cassiicola*. Oak scald has been defined by selective Azure A staining of mid rib cross sections from symptomatic nursery and landscape material in late May and early June. Both cases were verified by ELISA also.

Tennessee Report (B. Long) - In Tennessee, Beth Long reported greenhouse/nursery ornamentals with tomato spotted wilt virus, impatiens strain (impatiens necrotic leaf spot) on New Guinea and double impatiens; black root rot on pansy; gray mold (*Botrytis cinerea*) on a wide variety of bedding plants and potted plants. Beth commented that the extended periods of overcast cool weather had resulted in increased disease problems. Some other problems included Phytophthora root rot on holly, yew, juniper, and a downy mildew (*Peronospora sparsa*) epidemic in at least two nurseries. Several shipments of bare rooted plants (800+) from Texas and California were lost to this disease. With landscape ornamentals, Botryosphaeria, Nectria and Thyronectria cankers on woody ornamentals (probably a result of previous winter injury) were common. Also seen frequently were Phytophthora root rot on yew and juniper; anthracnose fungal leaf spot on ash, maple and oak; azalea leaf and flower gall on azalea and camellia; and Entomosporium leaf spot on photinia. With homeowner problems, bedding flower plants and vegetable transplants and seedlings were often seen with Pythium and Rhizoctonia damping off. On strawberry, common leaf spot, Phomopsis leaf spot and anthracnose were present. Also, Rhizoctonia brown patch on turfgrass was common. With field crops, Sclerotinia crown and stem rot was observed on a number of alfalfa and crimson clover samples. Wheat spindle streak and barley yellow dwarf virus were common finds on wheat this past spring. Most of the plants appeared to grow out of the spindle streak. Tobacco float bed's showed a variety of problems including Sclerotinia collar rot, angular leaf spot, bacterial soft rot, Pythium and Rhizoctonia stem rot, and blotch (also called scab) caused by *Microdochium tabacinum*, which is usually associated with cold injury on transplants.

North Carolina Report (T. Cresswell) - Tom reported that a major problem was identified as *Gymnosporangium clavipes* rust on Bradford pear in Charlotte, NC area landscapes. Some other significant problems included Dutch elm disease on American Elm, cultivar "Liberty"; Exobasidium diseases on azalea, camellia, rhododendron and horse-sugar (*Symplocos tinctoria*); Phytophthora crown rot (*Phytophthora cryptogea*) causing heavy losses of Gerbera daisy for a very large greenhouse (in spite of excellent sanitation practices). *Phytophthora parasitica* blight of Madagascar periwinkle (*Catharanthus roseus*) continued to be the limiting factor in use of this bedding plant. Incidence of chemical injury on roses and angular leaf spot (*Xanthomonas fragariae*) on strawberry was higher than the last few years. On the other hand, incidence of Impatiens Necrotic Spot Virus was lower than previous years but many growers were still experiencing losses. Late blight appeared frequently this spring on tomato and potato. A problem of leaf distortion and discoloration on bell pepper seemed to be of a genetic origin. It could not be linked to chemical injury, insect damage or any of the more common pepper viruses. Bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) was also a frequent problem on pepper as well as southern bacterial wilt on tomato.

Kentucky State Report (B. Eshenaur) - Rhizoctonia root and stem decay was prominent in some annual plantings, especially with impatiens and begonias. Brown patch was active in home lawn and golf course situations, and some new plantings were severely affected. Phytophthora root rot was observed on many ginseng plantings. Blue mold of tobacco showed up early this year on transplants, and has become currently prevalent throughout the state. Black shank of tobacco was also wide spread and causing stand losses in many fields. Powdery mildew was diagnosed on *Cornus Florida* (this is unusual for Kentucky). The affected mature leaves were showing red blotches while the small new leaves were slightly distorted and yellowed mottled in appearance.

Arkansas State Report (S. Vann) - Noteworthy diseases of this past spring included black rot, anthracnose, and dead arm on grapes; southern blight at an increased level on vegetables such as pepper, tomato, and beans; Entomosporium leaf spot at a high incidence (severely defoliated shrubs were commonly observed in homeowners landscape as well as commercial locations). With field crops, barley yellow dwarf virus was especially common in many varieties of wheat. Infection resulted in reductions of plant height, seed weight and numbers. Rust (leaf) was also quite common in many fields.

Mississippi State Report (M.V. Patel) - Due to weather conditions, several outbreaks of common diseases occurred in Mississippi. Powdery mildews on ornamentals, rust on zoysiagrass, oak leaf blister, blossom-end rot on tomato, southern stem blight on vegetables, ornamentals and soybeans, Entomosporium leaf spot on photinia, and rust on Mayhaw (Indian Hawthorn) were some of the commonly seen diseases. Also, Sclerotinia timber rot occurred in field grown tomatoes. A severe case of phoney peach was observed in one peach orchard. A manganese toxicity (low soil Ph) problem was observed on several fields of watermelon, cantaloupe and other garden vegetables.

Alabama State Report (J. Mullen) - Noteworthy early spring problems on landscape ornamentals included new growth cold damage to a variety of woody shrubs/trees. Later on this spring, Phytophthora aerial blight, aerial Rhizoctonia, and anthracnose have been problems on annual periwinkle. Also, Xanthomonas bacterial blight was identified on a landscape planting of geranium and we have seen one case of powdery mildew of dogwood. With greenhouse crops, disease problems have included Pythium root rot on bedding plants, Xanthomonas bacterial blight of geranium (only one incidence identified), late

blight on tomato, and aerial Rhizoctonia on begonia. As with Florida, take-all patch has become a frequently-seen problem on St. Augustine and also occasionally zoysia. Earlier this spring, we saw several incidences of cold damage to turf, especially in the Birmingham area. Peaches this year have suffered from cold damage, the usual brown rot, and the Phomopsis canker dieback which has been a recently-seen problem of the past 3 years. With field crops, some cold damage occurred early this spring on wheat and other small grains. Barley yellow dwarf virus was confirmed (P. Mask) by ELISA in several of the wheat areas of the state. Tomato spotted wilt virus has developed on peanuts and appears to be at a higher incidence than the low levels found in the two previous years. Diseases have been especially common with vegetables this spring. Earlier, bacterial leaf spot on strawberry was widespread in the state. Irish potato fields developed black leg, early blight, late blight, ozone injury, an especially severe level of soft rot in storage (possibly related to the wet conditions that occurred during harvest), and an especially severe incidence of soft rot in the field at harvest time with a question of possible herbicide effects. More recently, *Sclerotium rolfsii* has been seen state-wide causing problems with a variety of vegetables. As conditions have been dry, the usual white fungal mat typical of *S. rolfsii* has not been present. The most obvious symptom has been a dark, wet decay of the lower stem at the soil line. Also, TSWV, cucumber mosaic virus, potato virus Y, and tobacco etch virus have begun to show up on field-grown tomato crops. And, southern bacterial wilt (*P. solanacearum*) has been common on tomatoes as is usual at this time of year. Our most recent problems include greenhouse/nursery aerial Rhizoctonia on begonia, holly, azaleas, and pittosporum.

Central Region

Karen Rane

As is usually the case in the Midwest, the big story is the weather - this time the Great Flood of 1993. Thousands of acres of farmland are under water as the Mississippi, Missouri and other rivers in the region have overflowed their banks and broken levees. Even in states less affected by flooding, soils are saturated from unusually high rainfall. While wet weather usually means increased diseased plant samples in regional clinics, Paula Flynn of Iowa State University reports that the number of samples she has received has actually decreased as the rains have continued to fall - people are too busy keeping their homes safe and basements dry to spend much time on their landscape plants.

Agronomic Crops - Flooding damage resulted in premature kill of wheat in Kansas. In addition, leaf rust, tan spot, various viruses, scab, and Septoria diseases were all present in significant amounts in Kansas wheat fields (decreased yield is predicted for the state). In Nebraska, an estimated $2 million in wheat was lost to wheat streak mosaic virus in one county alone. Seedling blights in corn and soybeans were found throughout the region, due to excessively wet soils. Other corn problems include rust (Nebraska, Indiana, Kansas, Iowa) anthracnose (Indiana, Iowa), crazy top (Kansas) and potassium deficiency (Illinois). Hail injury and subsequent smut infections were reported from Kansas and Indiana. As expected under wet conditions, Phytophthora rot of soybeans has been reported from Indiana and Missouri. In Illinois, Pythium was found more often than Phytophthora attacking soybeans in saturated fields.

Ornamentals - Heavy rains contributed to an increased incidence of all types of foliar diseases in trees and shrubs throughout the Midwest. Taphrina disease samples (oak leaf blister, plum pockets,

peach leaf curl) increased in Indiana, Iowa, Kansas and Missouri. Oak and ash anthracnose were severe in Minnesota, and in Missouri, these diseases were active later than normal in the growing season. Dogwood anthracnose, caused by *Discula destructiva*, was confirmed for the first time in Indiana. Within one month, infected landscape dogwood samples were received from nine counties extending from the southern part of the state to the Michigan border. In Nebraska, an increased incidence of ash rust was reported. Apple scab is causing severe defoliation of susceptible crabapples for the third consecutive season in Minnesota, and is severe in many other parts of the Midwest region. Root stress problems attributed to saturated soil conditions have been found in both woody and herbaceous ornamentals throughout the region. Other ornamental problems of note include brown rot of chokecherry and serviceberry (Kansas), Thielaviopsis root rot on fuschia (Indiana), growth-regulator herbicide injury (attributed to windy spring conditions in various parts of the region) and an increase in Dutch elm disease samples (Iowa, Illinois). Ash yellows has been confirmed on green ash, white ash, black ash and common lilac in Minnesota. An unusual fungal canker disease of green ash, nicknamed "coin disease", has resulted in the destruction of hundreds of trees in Minnesota. The cankers are reddish in color, and range from the size of a pencil eraser to a quarter in diameter. If you have any information on this disease, please contact Cindy Ash at the University of Minnesota (phone #:612-625-7022).

Vegetables - Bacterial spot, caused by *Xanthomonas campestris pv. vesicatoria*, has become a serious problem this year in some processing tomato fields in Indiana. Other vegetable problems in the region include bacterial spot on pepper (Illinois, Indiana), and Phytophthora rot of cabbage (Illinois), peppers (Kansas, Indiana, Missouri) and tomato (Indiana). In Kansas, zucchini yellow mosaic virus has been detected in pumpkin earlier in the growing season than in years past, and yield losses are expected.

Other crops - An increased incidence of foliar diseases on fruit crops has been observed throughout the region, again due to the extremely wet weather. Bacterial leaf spot was reported on peach and apricot in Missouri, while increases in scab and cedar apple rust were reported on apples across the Midwest. Turf problems include Pythium blight (Illinois), and Ascochyta leaf blight (Iowa).

Southwest Region

Steven Koike

In Nevada, the incidence of fire blight increased this year due to a cool, wet spring. Turf diseases were also abundant and widespread during the spring, including *Ascochyta* species, leaf blight, anthracnose, *Typhula* snow mold, and fairy rings. Tomato spotted wilt (lettuce strain) was confirmed on Pittosporum in southern Nevada. The diamond scale pathogen, *Sphaerodothis neowashingtoniae*, was identified for the first time in this state on *Washingtonia filifera*.

At present, this is the warm, dry season in most of the agricultural areas in Arizona. Environmental conditions are generally favorable for powdery mildew on several different crops. Powdery mildew (*Erysiphe cichoracearum*) was present on some late lettuce plantings, and cantaloupe fields were infected with *Sphaerotheca fuliginea*. *Pythium* and *Rhizoctonia* were found on stunted cotton seedlings. Bermudagrass, grown as a seed crop in Arizona, was infected with rust (*Puccinia cynodotis*). Finally, several trees in a commercial peach orchard in Yuma showed symptoms of severe decline and eventual death of the trees. Removal of soil around the trunk revealed the absence of bark. Cause of the problem was gophers, a large four-footed "pathogen".

In California, the biotic disease situation is fairly routine. However, the period of unusually hot weather in June and July resulted in the development of some abiotic disorders associated with the elevated temperatures. Tip burn was prevalent on lettuce, cabbage, Chinese cabbage, spinach, and other vegetable crops. Extremely hot temperatures in vegetable transplant greenhouses apparently caused unusual deformities and death of plant growing points in cauliflower and celery.

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FEATURES

LIGHT MICROSCOPY FOR PLANT VIRUS DETECTION
Part 1. Materials, Methods, and Utility

G.W. Simone, R.G. Christie, J.R. Edwardson

The sample box arrives with the normal mail delivery to the diagnostic lab. The package is opened to reveal one or two vine tips of a cucumber. The diagnostician looks in vain for some symptom of a bacterial or fungal pathogen that will allow the initiation of a standard processing protocol for pathogen isolation and identification. No such luck! This sample has a chlorotic mottle with some growth distortion, as well as areas of a strong mosaic pattern. Is this plant affected by a viral pathogen - if so, which one?

No other diagnostic challenge is so beset with frustration as the pursuit of viral etiology in the diagnostic facility. Apart from normal frustrations associated with inadequate sample quality/quantity and incomplete sample information, there is still a more deep-seated frustration in handling viral disease suspects. If you step back from the sample processing bench for a moment, perhaps you will perceive the major difference in pursuing viral disease diagnoses from other pathogen groups. Let's examine the second box in this morning's mail. A client has submitted several tomato suckers with dark, irregularly shaped lesions and evident watersoaking around the lesions. Being familiar with your geographical area, you formulate the following null hypothesis:

H_O: This tomato is affected with a foliar bacterial disease.
H_A: This tomato is not affected by a foliar bacterial disease.

With the null hypothesis set up, you proceed with a processing protocol to test this hypothesis. These tests proceed from the general to the more specific; bacterial streaming followed by isolation onto bacterial growth media and perhaps bacterial characterization tests will amply test your null hypothesis. If the light microscopy examinations for a bacterial stream were negative and cultural efforts failed to recover a population of pathogenic bacteria, you have disproven the H_O and <u>proven</u> the H_A. This tomato is not affected by a foliar bacterial disease.

Most diagnosticians would have proceeded with the establishment of a second testing model when the bacterial flow test failed. The text of the new model:

H_O: This tomato is affected by a foliar fungal pathogen.
H_A: This tomato is not affected by a foliar fungal pathogen.

This test would be proceeding simultaneously with the null hypothesis dealing with bacterial pathogens. The point of this example is that the negative processing results (ie. flow test and culturing) have disproven the H_O and proven the H_A with a high confidence level.

Returning to the first sample of the morning, the cucumber with viral disease symptoms. Knowing the crop and your endemic diseases of cucumbers, you realize there are seven known possible viruses in five virus groups that may be involved. For your area, these viruses are cucumber mosaic virus (CMV - - cucumovirus), tomato spotted wilt virus (TSWV – tospovirus), squash mosaic virus (SqMV – comovirus), cucumber green mottle virus (CGMV – tobamovirus), and the three potyviruses (Papaya ringspot virus - PRSV, watermelon mosaic virus II - WMVII, zucchini yellow mosaic virus - ZYMV). Recognizing suspect

viral symptoms, you formulate the following null hypothesis:

H_O: This cucumber is affected with a viral disease.
H_A: This cucumber is not affected with a viral disease.

This processing is ready to begin to test this hypothesis but with what processing protocol? What virological method can compare in a genuine sense to the bacterial flow test or direct microscopic examination of tissue for fungi? Review the methods available to a diagnostic laboratory that must consider timeliness, accuracy, and cost of a diagnosis:

1) Symptomatology – Can define etiology but is not viable for specific virus identification – especially with a mixed infection.

2) Sap transmission – Too time and space consumptive for a diagnostic lab.
Defining a new virus would be a nightmare.
Dealing with a mixed infection would be frustrating.
A negative answer for the H_O does not prove the H_A.

3) Electron microscopy – If accessible, would define a potyvirus or a tobamovirus with some ease.
Would not separate among the three potyviruses.
May not define a mixed infection with an non-rod shaped virus easily.
May miss a 'new' virus if not rod-shaped.
A negative answer for the H_O does not prove the H_A.

4) Serology – If all seven antisera area available, this method will prove the H_O, expose mixed infections, but may miss a 'new' virus.
If all antisera are not available, positive results will not pick up a 'new' virus or all components of a mixed infection.
Negative results for the H_O will not prove the H_A.

5) ds-RNA analysis - Neither widely available nor affordable.
Positive results will prove the H_O but without specific identification to virus.
Positive results may miss a mixed infection.
Negative results do not disprove the H_O nor prove the H_A.

The true source of frustration associated with defining plant viral infections is our inability to apply test procedures to the null hypothesis that will result in as high a confidence level (regardless of test results) as we experience with bacterial and fungal diagnostic methods. This avenue is not as dismal as it first appears! Efforts over the last 30 years have provided a strong database for the incidence of virus-induced inclusions in plant cells and the methodology to detect them using the light microscope. Light microscopy of virus-induced inclusions is the needed 'general' protocol to test the null hypothesis, H_O: Is there a virus in this plant sample? The use of this method for null hypothesis testing will allow high confidence whether results are negative or positive. It will define 'new' viruses, pick up mixed infections, and provide sufficient confidence to prove the H_A: There is no plant virus in this sample.

One of the surprising aspects of the plant virus inclusion technology is its minimal demand on facilities and equipment. Every diagnostic lab is already set up for light microscopy, hence the compound and stereo microscopes already exist. The only other expense of consequence is the optional use of a microwave for staining. This tool is fast appearing in most facilities as well. The likely expense associated with this technology revolves around the three stains, two solvents, two mounting media, and the fine-tipped forceps. A complete list of support equipment is presented in Table 1. Sources of the specialized materials (stains, etc.) are listed in Appendix 1 with vendor addresses and phone numbers presented in Appendix 2.

Table 1. Basic materials needed to implement light microscopy for plant virus inclusions.

Equipment

1. Compound light microscope with an oil immersion objective and at least 10X ocular lenses (15-16X oculars preferred) to achieve a minimum of 1000X magnification.
2. Stereo microscope with ample working space or an illuminated table magnifier (1.5-4X).
3. Microwave -- non-food use unit (optional).
4. glass microscope slides
5. fine tipped forceps
6. razor blades
7. disposable pipettes & suction bulbs
8. staining receptacles (watch glasses, depression slides, cavity (spot) plates, etc.)

Reagents

1. Calcomine Orange 2RS
2. Luxol Brilliant Green BL
3. Azure A
4. 2-methoxyethanol = Ethylene Glycol Monomethyl Ether = Methyl Cellusolve
5. dibasic sodium phosphate (Na_2 HPO_4-$7H_2O$)
6. Triton X-100
7. 2-methoxyethyl acetate
8. ethanol (95%)
9. Euparol mounting medium
10. Euparol Vert. mounting medium

Plant virus inclusions are direct intracellular evidence of virus infection. They may consist of aggregated virus particles, aggregated coat protein, virus directed non-structural proteins and, in some cases, mixtures of these. They may also be made up of altered host constituents. The appearance of these inclusions differs from surrounding cytoplasm and organelles in structure and staining reactions. Virus inclusions have been induced by all plant viruses studied cytologically to date. Inclusions induced by a specific virus maintain a characteristic appearance over a host range. When properly stained, most inclusions can be readily detected with a light microscope. Light microscopic recognition of inclusion types offers a reliable, practical, and economical method for identifying virus diseases at the group level and can often lead to a specific diagnosis when the virus host range is considered.

Research with the electron microscope has resolved the distinctive structure and composition of many inclusions. Once these inclusion features were described at the ultrastructural level, stains were designed which were capable of detecting and differentiating many of the same features in the light microscope. The ability to identify a particular inclusion type with both the light and electron microscope has enabled inclusions to be described in terms common to both levels of microscopy. For instance, an inclusion shown to consist of virus particles with electron microscopy can be similarly identified in the light microscope as a virus aggregate, even though individual particles cannot be resolved by light microscopy. Although in this two-part article, we deal exclusively with how inclusions appear in the light microscope, the descriptions have their basis in electron microscopy as well. Simple, rapid light microscopic techniques designed to select and differentiate inclusions induced by a wide range of viruses infecting many host species have been described in detail previously.[5]

Tissue Sampling Strategy

The quality of the plant sample will often determine the choice of diagnostic method, the amount of replication, and the probability of success in any diagnostic cycle. Sample quality and quantity are often determined by a second party - not by the individual responsible for plant disease determination. The

concerns of sample quality and quantity are especially important if maximum utility is to be derived from the plant virus inclusion method.

The viral groups of greatest importance primarily represent those possessing inclusions generally distributed throughout plant tissues. A quality plant sample, therefore, must consist of sufficient unwilted symptomatic plant canopy to allow processing by epidermal strips or sections through leaf mesophyll or vascular areas. Although most diagnostic cycles result in the definition of "known" viruses in documented hosts, unknown viruses or undocumented virus/host combinations do exist in the field. For this reason, complete symptomatic plants or subsampled plants (i.e., new growth, old growth, root system) should be submitted for processing. Additionally, the submission of "healthy" control plant material is very useful for individuals just adopting viral inclusion methods. The efficiency of this method is highly correlated with knowledge of plant anatomy, cytology, and light microscopy. Processing healthy plant material will familiarize the investigator with normal plant cytology, such as the presence of microbodies, plant crystals, plastid morphology, chromatin distribution in the nuclei, nucleolus number, etc. Familiarity with the normal cell constituents and their staining reactions will allow faster detection of the viral induced inclusion(s) amid the diversity present in the plant cell.

Plant virus inclusions are dynamic - not static. They develop and degrade overtime. In the initial stages they may be small and difficult to detect. However, over time they increase in size and, in some cases, complexity. When conditions are favorable, they reach a "mature" state where they display their most characteristic appearance. It is usually at this stage that they are most valuable for diagnosis. For this reason it is of utmost importance that different stages of infected tissue be examined to assure that such stages are included in the sample.

Certain environmental conditions, such as temperature and light, can affect the rate at which the inclusions develop. The relative tolerance or resistance of the host genome is also important. In tolerant hosts, inclusions may mature and reach their characteristic forms even though symptoms are not evident. On the other hand, resistant hosts can delay or even arrest inclusion development. In such cases there are also few or no symptoms evident, and the inclusions are harder to detect. However, proper sampling will still reveal the characteristic inclusions, although they are reduced in number and limited in distribution. Such information can be very valuable to programs breeding for resistance to viral diseases.

The choice of infected plant material can be critical to detection of inclusions, since symptom expression may not always be correlated with presence of inclusions. Inclusions may be difficult to detect when chlorosis or necrosis is severe because they may not have reached the size of stage or development necessary for recognition or they may have begun to disintegrate in dying cells. Often, inclusions are prominent just before symptom expression or in tissues with mild or undetectable symptoms. Therefore, samples should be taken not only from areas with symptoms but also from tissues of varying ages, regardless of symptom expression.

The epidermal cells of leaves, flowers, and fruit are often easy to prepare for observation and can be rich sources of inclusions induced by viruses of many groups. These tissues should be the starting point on the search for inclusions. If inclusions are not found in the epidermis, then the vascular tissues of the veins, stems and roots should be examined. In some cases, it is necessary to examine such tissues as apical meristems and lateral buds. Specific directions for preparing all these tissues have been presented in a previous study.[5]

Virus Inclusions Characteristic for Group

No viruses are known that do not induce inclusions. Inclusions are now considered as a main characteristic of most currently recognized plant virus groups.[13,14,20] Virus groups are defined as a collection of viruses and virus strains, each of which shares with the type member all, or nearly all, the main characteristics of the group.[15] An inclusion is characteristic for a group when it is induced by most group members, and is similar to those induced by other members in structure, composition, intracellular location, tissue location and staining reactions. The inclusions induced by some viruses are so unique that they are diagnostic for a particular virus.

Members of the potyvirus group (the largest of the plant virus groups) are among the most commonly encountered viruses in crops and weeds. Therefore, it is very important to become familiar with the different types of inclusions induced by viruses of this group. The most distinctive inclusion types induced by the potyviruses are the cytoplasmic cylindrical inclusions.[11] These inclusions are coded for by the viral genome[9] and are considered as diagnostic at the group level.[10] At early stages of infection they appear first at the cell periphery where they increase in number and in size.[4] As infection progresses, they begin to accumulate in groups in the central portions of the cell. Eventually, they are found only in large masses. In some cases, this process is arrested and the inclusions never reach the massing stage, but instead remain at the cell periphery. The recognition of the inclusions at the peripheral stage can be important in the diagnosis of potyvirus infections.

The potyviruses have been subdivided based on differences in cylindrical inclusion structures as seen in thin section.[11,12] Two different structural components of potyvirus cylindrical inclusions can be recognized in the light microscope. The first of these appears tubular in shape. In the electron microscope these inclusions are described as scrolls in cross section. The tubular structures can be likened to a group of needles laid side by side. If the needles are oriented so that the tips point toward the viewer, then they would appear as a group of dots. When the focus of the microscope is changed, the dots remain visible as dots, while the needles oriented on their sides leave the field of view. The second type recognizable in the light microscope consists of plate-like structures. These structures are described as laminated aggregates in terms of the electron microscope. When a group of plates belonging to an individual cylindrical inclusion are viewed from the side, they appear as a group of parallel lines. When such an inclusion is seen from the end, it would look like an asterisk. As the focus of the microscope is changed the plates shift position, but they still appear as lines. Therefore, simply by changing the focus of the microscope, it is possible to distinguish between the tubular and plate-containing cylindrical inclusions. Certain potyviruses induce only the tubular components, while others induce only the plate-like structures. In addition, there are those that induce both types, as in the case of viruses such as turnip mosaic, a member of Subdivision III.[12] Many potyviruses can be distinguished in the light microscope based solely on the differences in cylindrical inclusion structure.

The tubular types of cylindrical inclusions can in some respects resemble groups of virus paracrystals. Paracrystalline virus aggregates are induced by a number of potyviruses, as well as by viruses of other groups. The cylindrical inclusions can be distinguished from paracrystals based on differences in their staining reactions in Azure A. The virus paracrystals, which contain RNA, will stain red with Azure A, while the proteinaceous cylindrical inclusions, which lack RNA, will not stain.

Viral Aggregates

Many virus aggregates can be characteristic for a group, although not necessarily diagnostic. Aggregation of virus particles is apparently a common phenomenon among plant viruses. Aggregates can occur in the cytoplasm, vacuoles, and nuclei. They may vary considerably in size, but are usually sufficiently large to be detected in the light microscope. Virus aggregates, like all virus inclusions, go through developmental stages. During early stages of infection, they may be small and ill defined, while at later stages they may disintegrate or be present only in isolated cells or tissues. Therefore, it is important that the aggregates be detected at a stage of infection where they are plentiful, and where they display their most characteristic appearance. The Azure A technique is well suited for this purpose, because it stains viral aggregates vividly and allows extensive areas of epidermis, mesophyll and vascular tissue to be searched for their presence.

Two virus groups, the tobamoviruses and the potexviruses, both characterized by elongate virus particles, induce virus aggregates that are characteristic for their respective groups. The aggregates induced by certain viruses within each of these groups differ sufficiently in structure that they can be used to distinguish the virus inducing them from other group members. Polyhedral viruses also induce virus aggregates that can be useful for diagnosis, especially when their structure and location are considered along with the presence of additional inclusion types.

The aggregates of polyhedral virus particles are not as easy to detect as those induced by the elongate viruses. This is because they are not as uniformly distributed in the tissues and do not persist as long. This is especially true of virus aggregates located in the cell vacuoles, as in the case of cucumber mosaic virus (CMV). During early stages of infection, CMV crystals are abundant and easy to detect. In older tissues with long standing infections the inclusions are fewer and widely dispersed, although in such cases, abundant inclusions can still be found in very young leaves of the same plant. A distinctive feature of CMV inclusions is that they often appear as hollow shells.[4] This feature has proved to be of diagnostic value.

Additional Inclusions to the Type Inclusion of the Virus Group

Besides the inclusions that are characteristic for the group, i.e., those in common with other group members, many viruses induce additional inclusions that can be useful in separating groups and in some instances viruses within groups. Additional inclusions may be unique and diagnostic themselves, as in the case of the nuclear inclusions induced by tobacco etch virus isolates[3] and the cytoplasmic laminate inclusion components (LIC) induced by potato virus X.[21] Additional inclusions may also differ in some respect from inclusions induced by other groups, and may be used to differentiate viruses whose characteristic inclusions may be similar to those in other groups. For instance, both the carlaviruses and the nepoviruses induce vacuolate, irregular inclusions that are characteristic for their groups. However, the carlaviruses induce additional paracrystalline inclusions, while the nepoviruses induce additional crystalloid virus aggregates.[4] Differences between the two additional inclusion types are readily apparent and allow separation of members of these two groups.

Certain potyviruses induce irregular, cytoplasmic inclusions that are proteinaceous but also have an RNA associated with them. These inclusions are also products of the viral genome. Their constituent protein has been demonstrated to be involved with aphid transmission of the virus.[7] This protein does not aggregate into inclusions in all potyvirus infections. The presence of such inclusions in addition to the characteristic cylindrical inclusions can be used to separate many potyviruses.

A number of plant viruses also induce nuclear inclusions. Such inclusions may be virus aggregates, virus directed non-structural proteins, coat protein shells, or membranous structures. Certain potyviruses, such as tobacco etch virus, induce nuclear inclusions so distinctive that their presence can even be used to distinguish among closely related strains.[3] These inclusions are products of the viral genome and represent aggregations of two proteins that have putatively been designated as a viral polymerase[8] and a protease.[2] They are usually distinctive and well defined in shape. A number of viruses closely related to bean yellow mosaic virus also induce distinctive nuclear inclusions that differ in structure sufficiently to be useful for diagnosis.

Viruses in several groups induce distinctive nuclear inclusions that consist of virus aggregates. Nuclei containing these inclusions are often swollen and distorted. The inclusions themselves often have no distinctive shape, but can be distinguished based on their staining reactions. In the light microscope, nuclear aggregates containing RNA will stain red to violet in Azure A. Nuclear aggregates induced by the geminiviruses will stain blue in Azure A since they contain DNA. The distinct color of these nuclear inclusions coupled with the fact that they are associated with vascular tissues and not generally distributed in other plant tissues make them diagnostic for the geminivirus group.

Inclusions With Altered Host Constituents

A number of plant viruses induce inclusions that contain altered organelles and other cytoplasmic constituents. Such inclusions can be very useful for diagnosis both by themselves and when considered along with other characteristic inclusions. The distinctive clumping of plastids induced by the tymoviruses is an example of altered plant organelles themselves being diagnostic for infections at the group level.[16] Multivesicular bodies, derived from pre-existing cellular components, such as peroxisomes,[19,17] mitochondria,[18] and plastids,[1] have been suggested to be of diagnostic value for the tombusvirus infections.[18] In healthy tissues, microbodies (peroxisomes) contain a protein that stains green with the

O-G combination but remains unstained in Azure A indicating the presence of protein and the absence of RNA. In tomato bushy stunt-infected tissue the microbodies increase considerably in volume, often reaching the size of small plastids. In addition to the protein present, these inclusions are also found to stain a light red with Azure A, indicating the presence of RNA. Such inclusions appear to be unique to the tombusvirus group. The tombusviruses also induce both cytoplasmic and nuclear virus aggregates both of which stain red with Azure A. The presence of these latter two inclusion types taken together with the multivesicular bodies, whatever their particular origin, appears to be a diagnostic feature of the tombusviruses.

Inclusions In Vascular Tissues

Four virus groups; the closteroviruses, geminiviruses, luteoviruses, and the plant reoviruses induce inclusions associated principally with the living cells of the vascular system. Therefore, techniques designed for exposing these tissues, such as sectioning or abrading are necessary.[5] Inclusions induced by viruses in all of the above mentioned groups are best detected using the Azure A staining procedure.

The closteroviruses can be distinguished from the other vascular inhabiting viruses based on the presence of characteristic paracrystalline aggregates located in the cell cytoplasm. In addition many large, vacuolate, intensely red-violet staining cells are also present. The geminiviruses are characterized by their distinctive, blue staining nuclear inclusions, which are aggregates of DNA containing virus particles. These inclusions are diagnostic for infections by viruses in the geminivirus group.[6] Both the luteoviruses and the reoviruses induce virus aggregates in the cytoplasm. In addition to the virus aggregates reoviruses also induce dense, spherical viroplasms in the leafhopper-born subgroup (Fijiviruses), and an amorphous, elongate viroplasms in the planthopper-transmitted Phytoreovirus subgroup.[22] It can be readily seen that by using the five aspects for describing inclusions we can distinguish the vascular inhabiting virus groups from each other as well as from those virus groups whose inclusions are generally distributed.

Two virus groups, the comoviruses and sobemoviruses, form large blockages of xylem elements. These inclusions have been demonstrated through electron microscopy to consist of masses of virus particles. They stain red-violet in Azure A and can be seen at relatively low magnifications in the light microscope. These characteristic xylem-located inclusions, when considered with the other inclusions, make it possible to separate the comoviruses and sobemoviruses from viruses in all other groups.

It should be mentioned that certain other pathogens of the vascular system also stain with Azure A. Mycoplasma appear granular in the light microscope when viewed in longitudinal sections and are located in the sieve elements where masses of them often block the sieve plates. These organisms stain red-violet in Azure A. Fastidious plant bacteria, such as *Xylella fastidiosa*, which also stain red-violet, are limited to the xylem elements and are easily discerned by their bacilliform shape in transverse sections. Both the mycoplasma and the fastidious bacteria stain green with the O-G method.

Stain Preparations

Differential stains have been developed to provide rapid penetration and uniform distribution, even into relatively thick tissue pieces. One stain is a combination of two dyes, one orange (Calcomine Orange 2RS) and one green (Luxol Brilliant Green BL), that will here be subsequently referred to as the O-G combination. This combination differentially stains plant organelles and inclusions containing protein. Another staining solution, Azure A, is designed to detect inclusions containing nucleic acids. When used under the conditions described,[1] Azure A is metachromatic, rendering virus inclusions containing ribonucleic acid (RNA) red-violet and deoxyribonucleic acid (DNA) blue.

The powdered formulations of all three stains are prepared in 2-methoxyethanol (ethylene glycol monomethyl ether). The preparations of the O-G stain proceed as follows:

a) Dissolve 1 gm of Calcomine Orange 2RS in 100 ml of 2-methoxyethanol. Stir thoroughly and filter.
b) Dissolve 1 gm of Luxol Brilliant Green BL in 100 ml of 2-methoxyethanol. Stir thoroughly and filter.
c) Both stains are stable when stored in tightly capped, brown bottles.

The O-G combination can be pe-mixed in one bottle in the following ratio: one part distilled water; one part Calcomine Orange, and eight parts Brilliant Green. This O-G combination stain solution is stable if kept under the same conditions.

The preparation of the Azure A stain for nucleic acids and nucleoproteins is similar to that of O-G. The preparation is as follows:

a) Dissolve Azure A powder into 100 ml of 2-methoxyethanol to constitute a 0.1% dye (gm/100ml). Azure A powders vary in dye content so check the label.
b) This stain is stable if kept tightly sealed in a brown bottle. Prepare a 0.2M solution of dibasic sodium phosphate ($Na_2 HPO_4$-$7H_2O$). Remember to use the hydrated (not anhydrous) form. The final staining solution should be made fresh each time and not reused. Mix one part of phosphate solution to nine parts of Azure A for effective staining.

Remember to use polyvinyl, single-use gloves and adequate ventilation when preparing or using the stains or solvents in this technique. Consult the manufacturer's material safety data sheets for further information.

The methods for removing epidermal peels and/or sectioning mesophyll or vascular tissues has been amply covered in the past[5]. These methods have not varied. The staining procedures have changed markedly in recent years due to the introduction of the microwave to reduce slide preparation time. A review of the staining protocols follows. Remember when employing this technique, its reliability (as with any other technique) is dependent upon a replication factor. The diagnostician must replicate epidermal strips and sections from symptomatic tissue across different tissue ages. This effort placed into slide preparation maximizes the efficiency of that time spent in microscopic examination for plant viral inclusions.

View each sample as a total unknown. Do not insert a bias in pre-guessing a particular virus. Sample tissues broadly because there are many unknowns in the environment; either new viruses, old viruses removed from their geographical zone, or old viruses in new hosts. Do not bias the method by incomplete processing.

A standardized slide preparation has evolved over the years that offers the greatest efficiency for this technique. A plant virus inclusion preparation is actually three preparations. Take a spot plate or three depression slides and prepare your staining solutions as follows:

a) Place O-G stain into well or slide "A".
b) Place 2% Triton X-100 into well or slide "B".
c) Place nine parts of Azure A with one part of 0.2M dibasic sodium phosphate into well or slide "C".
d) Disperse strips and sections into preparations "A", "B" and "C".
e) Microwave 10-15 seconds at full power. Use a microwave not destined for food preparation. Enclose a beaker of water during use. If a microwave is not available, allow solutions to stain for 10-15 minutes at room temperature.
f) Remove O-G stain from preparation "A" and Azure A from preparation "C" with disposable pipettes and replace with a 70:30 mixture of 2-methoxyethyl acetate (MeA): ethanol (ETOH).
g) Rinse once or twice with the 70:30 mixture until excess stain is removed.
h) Replace 70:30 MeA:ETOH with pure MeA as these stains are insoluble in this solvent. This step is useful when a break is needed in the schedule or when more permanent preservation of color is desired in these slides.
i) Mount preparation A into Euparal Vert (green color) and preparation C into Euparal (straw colored).
k) Remove the Triton X-100 from preparation B with a disposable pipette. This 2% solution is a plastid solubilizing agent that will dissolve plastids from cells while improving visibility of obscure inclusions.
l) Follow Triton X-100 in preparation B with O-G stain and microwave for 10-15 sec. or stain at room temperature for 10-15 minutes.
m) Remove O-G from preparation B with a disposable pipette and replace with 70:30 MeA:ETOH.

n) Rinse once or twice with 70:30 MeA:ETOH until excess stain is removed.
o) Same as in h) above.
p) Mount tissues from preparation B in Euparal Vert (green color) in the middle position on the microscope slide.
q) Examine for inclusions.

A procedural flow chart is presented in Appendix 3.

After examination of these slides, identified preparations can be retained as permanent slides if stored in a cool, dark slide storage area. Even when stains fade, these mounts can be redissolved in ethanol and the tissues reprocessed through the staining sequence and remounted.

It is difficult to find a technique that does not have exceptions. The use of Azure A to stain nucleic acids and nucleoproteins does not stain the crystalline, paracrystalline, or angled-layer aggregate inclusions induced by such tobamoviruses as TMV unless heat is applied during staining. In samples where a tobamovirus fall within the realm of possible etiology, the staining step with Azure A is recommended to be done with heat (ca 60^0C) for several minutes. A modified flow chart is present in Appendix 4.

Diagnosis with Virus Inclusions

Diagnosis of plant viral diseases does not differ from that conducted with any other pathogen group. This diagnostic process is a deductive one that logically proceeds in the following manner:

a) identification of the host species
b) perception of plant symptoms that imply viral etiology
c) access to a relevant plant disease index to focus the direction of investigation
d) choice of investigatory techniques to define pathogen etiology
e) literature confirmation for a "known" viral pathogen
f) Application of Koch's postulates for investigation of an unreported virus or virus/host combination.

Selection of plant inclusion methodology offers a strength above all other viral diagnostic technologies. This method is the only unbiased one available to answer the fundamental diagnostic hypothesis: "Is there a virus present in this sample?" Plant viral inclusions define viral etiology regardless of viral particle morphology, nucleic acid composition, or transmissibility requirements.

The presence of a particular viral induced inclusion can establish that a virus is present in a particular sample and thus eliminate from consideration other conditions that may mimic viral symptoms, e.g., pesticide damage. The next step is to compare the types of inclusions present with those characteristic of different virus groups. If an unknown virus is found to induce inclusion types with similar characteristics to those of a particular group, it can be assumed that the virus belongs to that group. Placing a virus within a group eliminates from consideration all viruses outside the group and at the same time allows inferences to be made about properties that the virus may have in common with group members. This is especially important in cases where the virus in question is undescribed and information on its properties is lacking.

When using inclusions for diagnosis, five distinctive inclusion features need to be considered in describing them. These are: 1) their structure; 2) their composition, e.g., protein or nucleoprotein; 3) their intracellular location; 4) their tissue location; and 5) their reaction to differential stains. Inclusions can be distinguished from one another based on differences in one or more of these criteria.

Practice in the examination of plant tissues will build expertise with this technique. The staining reactions of both O-G and Azure A stains must become familiar to the diagnosticians in both healthy and diseased tissue. Reactions of these stains in healthy tissues is presented in Table 2. Recognizing the natural cell constituents by their staining reaction will aid in focusing more rapidly on suspect viral inclusions. Characteristic inclusion types by virus group, tissue location, and staining reactions will be presented in Part II of this feature with September issue of PDQ.

Table 2. The Staining Reactions of Host Cell Constituents Present in Both Healthy and Virus Diseased Tissues.

Host Cell Constituents	Staining Reactions	
	Azure A Stain	O-G Stain
Cell wall	Colorless	Yellow-green
Chromatin	Blue	Green
Cytoplasm	Colorless	Yellow-green
Inorganic crystals (druses, raphides, etc.)	Colorless	Colorless
Microbodies & Microcrystals	Colorless	Green
Nucleolus	Red-violet	Green
Nucleoplasm	Clear	Orange
Plastids	Colorless[a]	Yellow-green
P-protein (phloem)	Colorless	Green
Starch Granules	Colorless	Colorless

[a] The cytoplasm and plastids may stain reddish in color in diseased cells.

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APPENDIX 1 SOURCES OF MATERIALS FOR PLANT VIRUS INCLUSION STAINING

Azure A –	Aldrich Chemical Co. cat. #86,104-9 ICN cat. # 150 416 Rt Kodak Lab Chemicals cat.# 120-6168 Sigma Chemical Co. cat. # A2918
Calcomine Orange 2RS –	Pylam Products Co., Inc. cat. #102
Euparal –	Carolina Biological Supply cat. # 86-1890
Euparal Vert.	Carolina Biological Supply cat. # 86-1910
Luxol Brilliant Green BL -	Aldrich Chemical Co. cat. #27,726-6
2-methoxyethanol –	Fisher Scientific cat. # E182 Aldrich Chemical Co. cat #27,048-2 Kodak Lab. Chemicals cat. # 136 4520
2-methoxyethylacetate –	Aldrich Chemical Co. cat. # 30,826-9 Kodak Lab. Chemicals cat. # 118 8267
Dibasic Sodium phosphate – ($Na_2 HPO_4 7H_2O$)	Aldrich Chemical Co. cat #. 22,199-6 Fisher Scientific cat. #S 373-500 ICN cat. #191441 RT Kodak Lab. Chemicals cat. #110 3639 Sigma Chemical Co. cat. # S 9390
Triton X-100 –	Aldrich Chemical Co. cat. # 23,472-9 Fisher Scientific cat. # 1C 26280-1 ICN cat. # E807423 Kodak Lab. Chemicals cat. #136 4637 Sigma Chemicals Co. cat. #X-100
Cavity (spot) plates –	Fisher Scientific cat. #13-748B, 13-745-5 Thomas Scientific cat. #7812-C22, 7812-G17 VWR Scientific cat. #53636-105, 53632-002
Fine tipped tweezers -	Ernest F. Fullam, Dumont #3 sharpened stainless steel tweezers cat. #13020

APPENDIX 2. LIGHT MICROSCOPY OF PLANT VIRUS INCLUSIONS: VENDOR LIST

Aldrich Chemical Co., Inc.
1001 West Saint Paul Avenue
Milwaukee WI 53233
1 (800) 558-9160 (U.S. & Canada)
1 (800) 962-9591 FAX U.S. & Canada

Carolina Biological Supply Co.
2700 York Road
Burlington NC 27215
1 (800) 334-5551
(919) 584-3399 FAX
or
c/o Powell Laboratories Division
19355 McLoughlin Blvd.
Gladstone OR 97027
1 (800) 547-1753
(503) 656-4208

Ernest F. Fullum, Inc.
P.O. Box 444
Schenectady NY 12301
(518) 785-5533

Fisher Scientific (regional offices)
711 Forbes Avenue
Pittsburgh PA 15219-4785
1 (800) 766-7000
1 (800) 926-1166 FAX

ICN Biomedicals, Inc.
3300 Hyland Avenue
Costa Mesa CA 92626
1 (800) 854-0530
1 (800) 334-6999 FAX

Kodak Laboratory Chemicals
Bldg. 70
Eastman Kodak Co.
343 State Street
Rochester NY 14650
1 (800) 225-5352

Pylam Products Co., Inc.
1001 Stewart Avenue
Garden City NY 11530
1 (800) 645-6096

Sigma Chemical Co.
3050 Spruce Street
St. Louis MO 63103
1 (800) 325-3010
1 (900) 325-5052 FAX

VWR Scientific (regional offices)
P.O. Box 13645
Philadelphia PA 19101-9711

Sigma Chemical Co.
3050 Spruce Street
St. Louis MO 63103
1 (800) 325-3010
1 (900) 325-5052 FAX

APPENDIX 3. GENERAL PROCEDURE FLOW CHART

1) Sample A
↓
Orange-Green stain
↓
Microwave 10-15 seconds or stain 10 min at room temperature
↓
Rinse 2 or 3 times until clear with 70:30; 2MeA:EtOH
↓
Place in 100% 2MeA (optional) for 1-2 min at room temperature.
↓
Mount in Euparal Vert (green color)
↓
Examine slide for inclusions

2) Sample B
↓
2% Triton X-100
↓
Microwave 10-15 seconds or stain 10 min at room temperature
↓
Remove Triton X-100 Replace with the Orange-Green stain
↓
Microwave 10-15 sec. or stain 10 min. at room temperature
↓
Rinse 2 or 3 times until clear with 70:30; 2MeA:EtOH
↓
Place in 100% 2MeA (optional) for 1-2 min at room temperature.
↓
Mount in Euparal Vert (green color)
↓
Examine slide for inclusions

3) Sample C
↓
Azure A stain combined with dibasic sodium phosphate
↓
Microwave 10-15 seconds or stain 10 min at room temperature
↓
Rinse 2 or 3 times until clear with 70:30; 2MeA:EtOH
↓
Place in 100% 2MeA (optional) for 1-2 min at room temperature.
↓
Mount in Euparal (straw color)
↓
Examine slide for inclusions

APPENDIX 4. PROCEDURAL FLOW CHART FOR TOBAMOVIRUSES.

Sample 1

↓

Azure A combined with dibasic sodium phosphate (room temperature)

↓

Rinse 2 or 3 times until clear with 70:30; 2MeA:EtOH

↓

Place in 100% 2MeA (optional) for 1-2 min at room temperature.

↓

Mount in Euparal (straw color)

↓

Examine for clear crystalline inclusions

Sample 2

↓

Azure A combined with dibasic sodium phosphate (heat ca 60°C in the stain for 1-2 minutes)

↓

Rinse 2 or 3 times until clear with 70:30; 2MeA:EtOH

↓

Place in 100% 2MeA (optional) for 1-2 min at room temperature.

↓

Mount in Euparal (straw color)

↓

Examine for stained (red/violet) crystalline, paracrystalline, angled-layer aggregate inclusions

COMMITTEE MEETING

November 1993

I have outlined below a tentative agenda for the November APS Diagnostics Committee Meeting in Nashville. Please take a look and contact me if you have suggestions/additions. Also, please start thinking about nominations for the new Vice-Chair (Vice-Chair 1994; Chair-1995) for the committee. As I understand from Carol Windels, APS Senior Councilor at Large, the Vice-Chair should be nominated from the existing 'pool' of committee members, if possible. See listing at the end of the agenda. (She did say, though, that exceptions could be made to this rule). Nominations and elections will be held at the committee meeting in November. If you plan to nominate someone, please contact the individual ahead of time to be sure he or she will be willing and able to serve if elected.

1. Introductions

2. Committee Membership/Meeting Attendance

   a. Roll call of Committee - J. Mullen

   b. Designation of new incoming (93-94) committee members.

   c. Designation of outgoing (92-93) committee members.

   d. Distribution of Diagnostics Committee List of 1992, 1993, 1994.

   e. Circulation of attendance sheet for committee records.

   f. Introductions of committee members and visitors.

3. Review of minutes of Portland Meeting - J. Mullen.

4. Old Business

   a. Registry of Plant Pathologists

   b. Diagnostic Booth and Diagnostic Posters/Discussion Session at the International Congress of Plant Pathology at Montreal, July 28 - Aug. 6 - C. Semer.

   c. Diagnostic Lab Roster - C. Sutula.

   d. Diagnostic Committee sponsored events at Portland.

      1. Rapid Diagnostic Assays for Plant Pathogens Workshop at Portland (S. Miller).

      2. Diagnostic Committee Poster at Portland - J. Mullen

      3. Plant Disease Diagnostic Contest - Portland - B. Eshenaur

   e. Other Old Business

5. New Business

   a. Diagnostics Committee Account with APS - J.Mullen.

   b. Diagnostic Manual Subcommittee - C. Semer.

c. PDQ Report - G. Simone, G. Ruhl.

d. Diagnostic Committee sponsored events at Nashville.

1. Rapid Diagnostic Assays for Plant Pathogens Workshop - S. Nameth, S. Miller.
2. Diagnostics Committee Poster - P. Bachi.
3. Pythium Species Identification Workshop - J. Mullen, E. Long, P. Bachi.
4. Plant Disease Diagnostic Contest - B. Eshenaur.
5. Diagnostics Social.

e. Possible Future Events for Committee Sponsorship.

1. Turfgrass Disease Workshop.
2. Phytophthora sp. Workshop III.
3. Pythium sp. Workshop II.
4. Xylem-limited Bacteria Discussion.
5. Fusarium sp. Workshop.
6. Molecular Technique Workshop or Discussion.
7. Rapid Diagnostic Assays for Plant Pathogens Workshop.
8. Diagnostics Committee Poster.
9. Plant Disease Diagnostic Contest.
10. Diagnostics Social.

f. Nominations & Elections of New Vice Chair 1994 (Chair 1995) - J. Mullen.

g. Other New Business.

h. Adjournment

J. Mullen

Diagnostic Committee Members - 1992-1993

Colette Beaupre	91-93	Sharon Douglas	92-94
Jan Hall	91-93	James Blake	93-95
Karen Rane	91-93	Mike Likens	93-95
Jim Sherald	91-93	Bob McGovern	93-95
Brian Eshenaur	92-94	Jackie Mullen, CH	93-95
Paula Flynn	92-94	Paul Bachi, V-CH	94-96
Barbara Corwin	92-94	Chuck Semer, IPC	91-93

Diagnostic Committee Members - 1993-1994

James Blake	93-95	Elizabeth (Beth) Long	94-96
Sharon Douglas	92-94	Karen Flynnt	94-96
Brian Eshenaur	92-94	David Roberts	94-96
Paula Flynn	92-94	Lauri Kenyon	94-96
Mike Likens	93-95	Paul Bachi, CH	94-96
Barbara Corwin	92-94	J. Mullen, IPC	93-95
R.J. McGovern	93-95	, V-CH	93-94

---

*92-94, etc. indicates years, by committee meeting, that members serve. (Actually, term for 92 meeting begins just after 91 meeting).

PROGRESS WITH THE PYTHIUM SPECIES IDENTIFICATION WORKSHOP

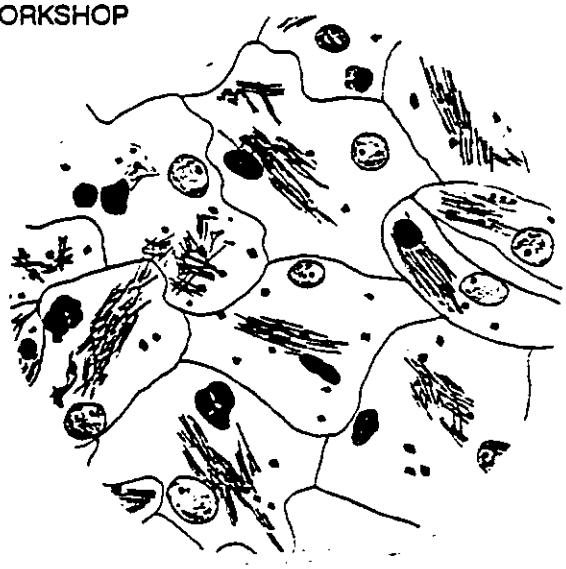
As of this writing (July 16), the Pythium workshop is filled. The 30 slots went rapidly! I am keeping a list of the 'too late applicants'. We may want to consider a 'repeat performance' if the interest level is there.

In early September, I will send out information regarding workshop local arrangements which are being handled mostly by Beth Long & Paul Bachi.

- We will include information on a few hotels/motels (hopefully - economical) in the area of the agriculture center, a travel/lodging questionnaire to help us arrange for transportation needs, an area map, and a list of workshop registrants.

- A schedule for the workshop activities will be mailed in September or early in October.

VIRUS INCLUSION WORKSHOP



Dates January 24-26, 1994

Place University of Florida
Florida Extension Plant Disease Clinic

Registration Fee $300.00 - Limit 9

Hosts Gary W. Simone, Ph.D., Associate Professor, Richard E. Cullen, Senior Biologist, Plant Pathology Department, Richard G. Christie, Senior Biologist, Department of Agronomy, Mark D. Gooch and Valerie Jones, Biologists, Plant Pathology Department, University of Florida, I.F.A.S.

Plant virus inclusions are valuable for diagnosing viruses at the group level, and in some instances can be used to identify a specific virus. They can be detected with a light microscope when properly stained. Inclusions induced by a specific virus have the same characteristic appearance across a host range. The procedures are simple, rapid and inexpensive and can save valuable antisera as well as direct in the selection of proper techniques for identifying plant virus diseases.

Course Description:
A 3 day introductory course for scientists, diagnosticians, and/or technicians who have no previous experience or limited experience with virus inclusion identification. "Hands-on" labwork will include virus inclusion identification of potyviruses, tobamoviruses, potexviruses, cucumoviruses, comoviruses, tomato spotted wilt virus, and geminiviruses. Other groups will be demonstrated through the use of prepared slides and kodachrome slide presentations. Staining techniques, tissue selection, and tissue preparation will be covered. All materials will be provided including use of a compound microscope for each participant. A start-up kit including stains and a monograph of virus inclusions will be supplied to participants.

Due to limited space and facilities, interested individuals must pre-register for this limited enrollment workshop.

For additional details, course agenda, or registration, please contact:

Dr. Gary W. Simone
Florida Extension Plant Disease Clinic
University of Florida
P.O. Box 110830
Gainesville, FL 32611-0830

Phone 904-392-1994
FAX 904-392-3438

Registration

The registration fee for this 3 day workshop is $300.00 per person. Registration includes the costs of preparation of infected plant material for at least 12 viruses representing eight major virus families. In addition, each participant will receive a revised Plant Virus Inclusion Monograph, starter kit of plant virus inclusion stains and reagents, and a pair of watchmaker's fine forceps for tissue stripping. Shuttle service from airport and hotel to the workshop each day and lunch trips is provided. Refreshments during the day are complimentary. Registration does not include meals, lodging, or travel-related costs.

Attendance is limited to the first 9 individuals that confirm interest to FEPDC staff by phone or FAX. Registration form and fee must be received no later than January 7, 1994 to confirm a place in this workshop. Please complete the lower portion of this registration form and return this with remittance to:

Florida Extension Plant Disease Clinic
University of Florida
Bldg. 78 Mowry Rd.
Gainesville, FL 32611

Make registration check payable to: University of Florida – SHARE - Foundation

A registration receipt can be procured at the start of the workshop.

Registration fees will not be deposited until the day of the workshop.

PLANT VIRUS INCLUSION WORKSHOP REGISTRATION
(Detach, complete, and return)

Name: _____

Date: Jan. 24 - 26, 1994

Institution: _____

Address: _____

_____

Phone: _____ FAX: _____

Arrival by: Air____ Car____
If air, Airlines_____ Flight#_____
Date/Time of Arrival_____
Will you need shuttle from airport? (circle) Yes No
Lodging Selection (circle)

Budget Inn Cabot Lodge Knights Inn Super 8 Motel

Will you need daily shuttle service? (circle) Yes No

The Plant Disease Clinic: Service Delivery, Version 2000.1

The APS meeting in Nashville on November 7-11 will include a colloquium by the Extension Committee entitled "Extension Plant Pathology: Meeting the Challenges Beyond 2000". Part of this colloquium is entitled "The Plant Disease Clinic: Service Delivery, Version 2000.1". When I was invited to present this topic, I had very defined views for extension clinic services in the future based upon trends here in Florida and elsewhere. What I have perceived may differ sharply from the reality across the United States so I'm asking for some assistance through the following survey before I compose my presentation.

The attached survey is designed to be handled on a per laboratory basis and is not limited to State Extension facilities. I realize that my requests for data from 10 years ago may tax some memories but I will appreciate your efforts in this regard. These data will allow me to plot some trends among diagnostic laboratories especially as regards funding, staffing, sample demand, etc. Please take the time to photo duplicate these pages and fill them out so that my effort in November can be as accurate as possible! Thanks for the help.

G.W. Simone

Survey of Plant Diagnostic Laboratories for Projection of Future Service Levels

Please return a copy per laboratory of this survey to:

Dr. Gary W. Simone
Plant Disease Clinic
Bldg. 78, Mowry Road
University of Florida
Gainesville, FL 32611

1. Survey completed by:

2. Name of diagnostic laboratory with address:

3. Classification of laboratory (check one):

   Extension ☐ State Regulatory ☐ Private/Commercial ☐

4. What numbers of samples have been processed in this facility in the following years:

   1980 _____ 1985 _____ 1990 _____ 1992 _____

5. What percentage of the yearly sample total is submitted by the following clientele groups?

   1980: Commercial Ag. & Hort. _____ Homeowner: _____ Research: _____

   1990: Commercial Ag. & Hort. _____ Homeowner: _____ Research: _____

6. If you do not have percentages of the year's samples, can you identify a trend in clientele between 1980 and now? (Check the appropriate)

Commercial Ag. & Hort.	☐ up	☐ level	☐ down
Homeowner	☐ up	☐ level	☐ down
Research	☐ up	☐ level	☐ down

7. How many FTE positions were involved with the lab in:

1980 _____

1990 _____

8. What is the highest educational level attained by each FTE or fraction of an FTE presently employed in the facility in 1993?

High School _____ M.S. _____

B.A./B.S. _____ Ph.D. _____

9. Do you utilize any of the following non-paid staff support in the facility?

Master Gardener	☐ yes	☐ no	# hour/wk _____
Graduate student	☐ yes	☐no	# hour/wk _____
Other volunteers	☐ yes	☐ no	#hour/wk _____

10. Do you have a specific Clinic-directed budget? (check one)

☐ yes ☐ no

11. Ignoring inflation, how has budgetary support for the facility changed?

Since 1980: ☐ increased ☐ level ☐ decreased

12. How do you view your budgetary support in the following areas?

Large equipment (>$500.00 ea)	☐ high	☐adequate	☐ low
Expendable materials (<$500.00 ea)	☐ high	☐adequate	☐ low
Hourly labor	☐ high	☐ adequate	☐ low

13. Do you have a charge policy per sample in place today?

☐ no ☐ yes

If no, do you plan one soon?
☐ yes ☐ no

If yes, when did it start (year)? _____

Is the fee a flat or graduated charge? _____

If flat fee, how much per sample? $_____
If graduated, what is the range? $_____

Do you guarantee a turn around time for charge samples? ☐yes ☐no

14. What percentage of your yearly budget is generated by sample charges?
_____%

15. Are all sample diagnoses routinely accompanied by a "control" answer?

☐ yes ☐ no

16. What answer delivery systems are used within your facility and what is the percentage of total samples for which each is used?

☐ Hard copy mail ☐ Computer mail ☐ FAX ☐ Phone ☐ Verbal (In-person)

_____% _____% _____% _____% _____%

17. What pathogen groups accounted for what percentage of the year's samples in 1980 and 1992?

	Bacteria	Fungi	Viruses	Nematodes	Abiotics
1980	____%	____%	____%	____%	____%
1992	____%	____%	____%	____%	____%

18. In 1992, what was your relative usage of the following techniques for bacterial pathogens?

Symptoms	_____%
Microscopic flow test	_____%
Culture	_____%
Physiological characterization	_____%
Hypersensitivity	_____%
Biolog	_____%
Serology	_____%
Fatty acid analysis	_____%

19. In 1992, what was your relative usage of the following techniques for fungal pathogens?

Symptoms	_____%
Signs	_____%
Direct Microscopic Exam	_____%
Culture	_____%
Serology	_____%
Speciation	_____%

20. In 1992, what was your relative usage of the following techniques for viral pathogens?

Symptoms	_____%
Electron Microscopy	_____%
Plant Virus Inclusion	_____%
Sap Transmission	_____%
Serology (Ouchterlony, ELISA, etc.)	_____%
ds-RNA analysis	_____%
c-DNA probes	_____%

21. In 1992, what was your relative usage of the following techniques for fastidious bacteria?

Symptoms	_____%
Light Microscopy	_____%
Serology	_____%
Culture	_____%

22. In 1992, what was your relative usage of the following techniques for nematode pathogens?

Direct Tissue Extractions	_____ %
Baermann Funnel	_____ %
Soil Sieving	_____ %
Speciation	_____ %
Race Determination	_____ %

23. Does your facility forward samples to other labs for processing?
(private or state - in-state or out-of-state)

☐ yes ☐ no

24. What percentage of total yearly samples were "farmed out" of the facility for completion in:

1980: _____ %
1993: _____ %

25. **COMPLETE THIS SECTION IF YOU ARE AN EXTENSION-FUNDED DIAGNOSTIC FACILITY ONLY.**

26. Is your facility and/or equipment shared with a research or teaching function?

(Check one) ☐ yes ☐ no

27. Is there a State Regulatory facility in your state? ☐ yes ☐ no

If yes, how many samples/yr. are processed? _____

28. In 1980, were there plant disease diagnostic facilities in your state **in the private sector**?

☐ yes ☐ no
If yes, do some exist in 1993? ☐ yes ☐ no

If yes, have the number of these facilities changed in 1993?
☐increased ☐level ☐decreased

29. Do diagnostic staff FTEs share split responsibilities with teaching or research?

Teaching ☐ yes ☐ no
Research ☐ yes ☐ no

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Guidelines to Contributors

Submission Format

Articles are preferred submitted on diskette (5.25 or 3.5) – especially the longer Feature Articles. Electronic submission will allow greater consistency among type fonts and sizes and improve the appearance of the publication. We use Word Perfect 5.1 on IBM hardware, but have the capability of converting most word processing software. Please send a copy of the article on the software you use (be sure to identify the software); please also send an ASCII file to use in case we have problems with the conversion. Label disks with your name and address and job file name. All disks will be returned. Please include a hardcopy printout as well.

Articles will also be accepted in a hardcopy format by surface mail or FAX. Where secretarial time allows, shorter articles will be retyped. Longer articles, however, may be used camera-ready. Please follow the Manuscript Format instructions that follow.

Manuscript Format

The title of the article is printed in bold letters (mixed case), is placed 1 1/2 inches from the top of the page, and is centered. Skip one line then center your name, then center the institution of your affiliation on the following line. Your name and affiliation should be printed in mixed case.

The top margin will be 1 1/2 inches on the first page and 1 inch for each page thereafter. One inch margins should be used on the remaining sides. Page numbers should be lightly pencilled in at the bottom of each page.

Paragraph or section headings should be in bold print or underlined. Skip the next line and then begin the paragraph; paragraphs are separated by blank lines.

Lines are single-spaced. The article should be printed on a letter quality printer or typewriter; dot printing will not reproduce well and should be avoided.

Latin binomials should be italicized rather than underscored if possible.

Length

Feature articles should be a minimum of 5 pages. Aside from this limitation, articles may be of any length as long as they remain focused on the topic selected.

Illustrations

Our ability to reproduce illustrations is limited; line drawings reproduce most faithfully. Original black and white photographs (prints only) may be used if they are of high quality. Illustrations should be mounted on a separate page, with their captions mounted below.

Fact Sheets

Contributed Fact Sheets from states extension/research units or other agencies for inclusion with PDQ are gratefully accepted. Send two (2) originals to Gary W. Simone (Editor) for appropriate listing in the next issue. If sufficient copies of the publication are available, send 225 copies to Gail Ruhl – Managing Editor so that they can be compiled with the issue.

References

Use at your discretion. If articles are referred to in the text, please cite them at the end of your article using a standard format such as that used in Plant Disease. If references are not cited, related articles may be listed under the heading "Bibliography".

Green, S.K.
Guidelines for Diagnostic Work in Plant Virology
1991. Technical Bulletin 15. 2nd ed. Asian Vegetable Research and Development Center. 63 p. $10.00. ISBN:92-9058-0003-10.

This soft bound bulletin is worth some commentary. Few of us have the time or funds to seek out the international publications available from such vendors as Agri-Bookstore. This title was worth the time. In its second edition (I missed the first one), this publication reads like a well-honed study guide to the diagnostic methodology used in virology. The text is well spaced, almost in outline form and brings to my mind what I wish I had in studying for my oral exam years ago! If copyright laws weren't so sensitive these days, I would be inclined to turn this publication into a series of overlays for a teaching machine to cover such topics as viral symptomatology, types of virus transmission (in vivo and in vitro), viral diagnostic methods, virus storage, and virus disease management options. Granted the diagnostic methods do not include c-DNA probe use or ds-RNA analysis but the rest of the topics are quite current. The sections are broken by short lists of examples for types of transmission or particular vector species. There are a few, excellent line drawings and a series of appendices representing viruses for major crop species with select biological and physical characteristics and group designations. Although its a small text, its large on utility.

Wang, C.J.K. and R.A. Zabel.
Identification Manual for Fungi From Utility Poles in the Eastern United States.
1990. American Type Culture Collection. 356p. $30.00. ISBN 0-93-0009-31-2.

So....how many utility pole samples does your Clinic receive yearly? All kidding aside, this title did not peak my interest on the first pass. When I had a chance to see it, my initial impression changed. Although this text will never get overly worn in pursuit of plant disease diagnoses, it does represent a compact treatment of wood decay fungi affecting primarily Douglas-fir and southern yellow pine. The fungi are grouped by taxonomic subdivision. The Basidiomycetes are arranged in a synoptic key treating such features as colony color on agar, growth rate, oxidase test results, types of generative hyphae, and special propagative structures. The photographs of mycelial colonies are accompanied by good line drawings of vegetative and propagative structures. Descriptions of each fungus are fully accompanied by the authority and citations.

The other fungal subdivisions are similarly treated but are more extensively illustrated with black and white composite photomicrographs of excellent quality. Although only a few of these fungi are plant pathogens, many species discussed are commonly encountered in isolation efforts from plant tissues. The photomicrographs justify the book price. Additionally, when that next fungal mat comes in from a landscape mulch layer or the bark component of a containerized ornamental, you might just have a chance of defining the saprophyte!

Matthews, R.E.F.
Fundamentals of Plant Virology
1992. Academic Press. 403 p. $59.95 ISBN 0-12-480558-2

This publication is a much abbreviated overview of R.E.F. Matthews Plant Virology (1991). If you own the third edition of Plant Virology, save some of your book budget and pass by this title. This treatment focuses on concepts of virology as might be needed for an introductory plant pathology or virology course. The type style is larger than in Plant Virology with the page format being more spacious. The concepts are simply and concisely presented with good supporting figures and tables. There are only eight pages of literature citations compared with the 118 pages in the 3rd edition of Plant Virology.

Most Clinics should own the more encyclopedic treatise represented by the 3rd edition of Plant Virology. If your Clinic library supports a teaching curriculum, consider adding this text to help with lecture preparation or as a recommended text for the introductory course.

Johnson, John M. and George W. Ware
Pesticide Litigation Manual
1992 edition. Clark Boardman Callaghan Environmental Law Series. $85.00. ISBN: 0-87632-798-6.

Florida is still faced with a long road ahead beset with Grower vs. E.I. DuPont litigation. When this title was advertised, I bought it immediately in an effort to better equip myself for the legal arenas ahead. This book contains an excellent overview of pesticide regulation and a excellent insight into what pesticide law really says, what is required, and what is or is not complied with by the pesticide industry. Liability is another well discussed topic with actual case histories for illustrations. Midway through this text I discovered more about the legal arena that I ever wanted to know and am firmly convinced this is a profession out of control and beyond the comprehensions of a logical, rational mind!

The greatest surprise in this publication was that more than half of the 300+ pages are devoted to pesticide technology. Topics like pesticide vocabulary, formulation types, modes of action for the different types of pesticides by group (e.g. insecticides, etc.) and by function (e.g. invertebrate control, algicides, etc.). Additionally, there are good overviews on pesticide resistance, toxicity, non-target species toxicity, safe handling & storage, and drift problems. Completing this book are a series of extensive appendices dealing with pesticide trade name, common chemical name, use patterns, and toxicology as well as a strong glossary.

The soft bound format of this book and the absence of conventional pagination are disappointing features in view of the price. Considering the paucity of literature on pesticide litigation, I encourage its purchase.