

## Control of downy mildew with fungicides in *Lamium* “Purple Dragon”

### Author

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### Funding

Michigan Nursery and Landscape Association

### Introduction

Many Oomycetes impact the perennial plant industry. Their occurrence and the fact that they are difficult to control continue to be a problem. The Oomycetes include *Pythium*, *Phytophthora* and *Peronosporales* (downy mildew) species. Since the introduction of metalaxyl/mefenoxam-based fungicides in the early 1980s in Europe, many pathogen populations have been identified that are resistant to or have reduced sensitivity to these products. Resistance to metalaxyl/mefenoxam has not been widely reported or tested in the landscape industry, but it is a common phenomenon in agriculture in part due to the more intensive use of these products. Metalaxyl/mefenoxam resistance has been reported in potatoes (*P. infestans*; late blight, *Pythium ultimum*; *P. erythroseptica*; pink rot); cucurbits and peppers (*P. capsici*) and grapes (*Plasmopara viticola*). Failure of control of some oomycetes has been noted in Michigan e.g., downy mildew in *Rudbeckia* and *Lamium*. There has been much research on metalaxyl/mefenoxam resistance; however data are sparse on metalaxyl/mefenoxam

resistance management in perennials.

### Objectives

In the perennial plant industry in Michigan, oomycetes are controlled primarily by cultural practices such as water management or pathogen exclusion on seed stock, but fungicides are the primary protection measure. The objectives of this research are to develop methods to quickly determine the degree of insensitivity of these pathogens to metalaxyl/mefenoxam; especially for downy mildew, which cannot be cultured *in vitro*. In addition, alternative chemical interventions for control of oomycetes are evaluated, such as new fungicides e.g., Cabrio (pyraclostrobin). The methods developed will allow growers to choose an alternative product once the degree of sensitivity of the pathogen to metalaxyl/mefenoxam is determined, and avoid loss of plant material to

**Table 1.** Concentrations of Subdue MAXX and Cygnus 50WG fungicides that inhibited downy mildew in *Lamium maculatum* by 50% relative to the control.

Days after application of fungicide (DAFA)	EC50 value (ppm concentration)	
	Mefenoxam	Kresoxim-methyl
20	0.097	0.47
30	0.096	0.47
40	0.096	1.44
50	13.9	3.47

**Table 2.** Comparison of efficacy of different concentration rates of metalaxyl/mefenoxam (Subdue MAXX) and kresoxim-methyl (Cygnus 50WG) against downy mildew in *Lamium maculatum*.

Concentration of fungicide (ppm <sup>a</sup> )	Downy mildew severity index <sup>b</sup>							
	20 DAFA		30 DAFA		40 DAFA		50 DAFA	
1 Untreated	29.5	a <sup>d</sup>	47.0	a	66.0	a	85.0	a
2 Mefenoxam 0.01	19.0	b	20.5	c	27.0	c	42.0	b
3 Mefenoxam 0.1	0.0	c	0.0	d	0.0	e	18.5	bc
4 Mefenoxam 1.0	0.0	c	0.0	d	0.0	e	17.5	c
5 Mefenoxam 5.0	0.0	c	0.0	d	0.0	e	14.5	c
6 Mefenoxam 10.0	0.0	c	0.0	d	0.0	e	20.0	bc
7 Kresoxim-methyl 1.0	23.5	ab	37.5	b	53.5	b	71.5	a
8 Kresoxim-methyl 5.0	0.0	c	0.0	d	9.0	d	37.0	bc
9 Kresoxim-methyl 10.0	0.0	c	0.0	d	0.0	e	25.0	bc

<sup>a</sup> Fungicide doses of 0, 0.1, 1, 5, and 10 ppm mefenoxam (Subdue MAXX) mixed in water for a final application rate equivalent to 25 gal mixture/acre. Kresoxim-methyl (Cygnus 50WG) applied at 0, 0.1, 1.0 and 5.0 ppm

<sup>b</sup> Indices of 0 - 25 cover the range 0 - 2%; 26 - 50 cover the range 3 - 10%; 50 - 75 cover the range 11 - 50% foliar symptoms and > 75 cover the range 51 - 100% foliar symptoms and plant death.

<sup>c</sup> Days after application of fungicide.

<sup>d</sup> Means followed by the same letter are not significantly different at P = 0.05 (Tukey multiple comparison).

oomycetes. This report covers the efficacy of Subdue MAXX and Cygnus 50WG applied at increasing dose rates in controlled environments.

**Research methodology**

*Lamium maculatum* “Purple Dragon” plants with symptoms of downy mildew were obtained from a Michigan grower. These plants were overwintered outside at Michigan State University Plant Pathology Greenhouse complex (from October 30 to March 30). In early spring, the plants were potted into flats that contained 10 plants, replicated five times for a total of 50 plants per experimental unit. Fungicide doses of 0, 0.1, 1, 5, and 10 ppm mefenoxam (Subdue MAXX) were mixed in water for a final application rate of 25 gal mixture/acre and were applied with a precision fungicide applicator. Additional treatments of kresoxim-methyl (Cygnus 50WG) at rates of 0, 0.1, 1.0 and 5.0 ppm were applied as above. A single application of the fungicide treatments was made on April 4, 2003.

**Data analysis**

A downy mildew index was calculated by counting the number of plants (from each sample of 50 plants in the controlled environment), falling into class 0 = no visible symptoms; 1 = 1 to 2% of leaves with lesions; 2 = 3 to 10% of leaves with lesions; 3 = 11 to 50% of leaves and stems with lesions; 4 = 51% with lesions to 100% defoliation/plant death. The number of plants

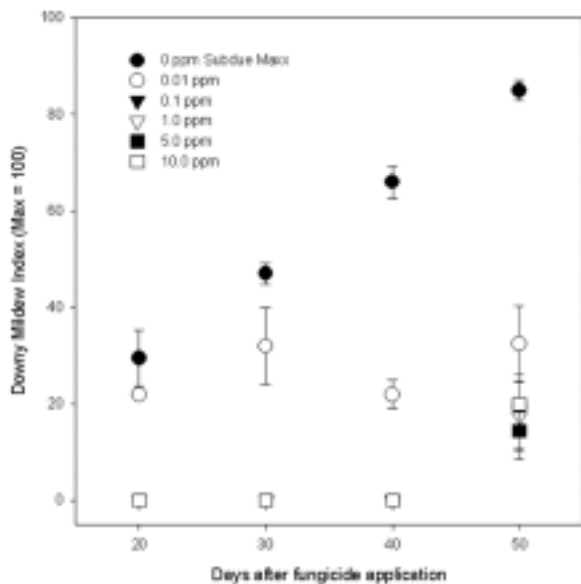
in each class was multiplied by the class number and summed. The sum was multiplied by a constant to express as a percentage. Indices of 0 to 25 cover the range 0 to 2% foliar symptoms; 26 to 50 cover the range 3 to 10% foliar symptoms; 50 to 75 cover the range 11 to 50% foliar symptoms and greater than 75 cover the range 51 to 100% foliar symptoms and plant death.

Downy mildew was evaluated 20, 30, 40 and 50 days after application of fungicides. The average index for each evaluation was expressed as a function of time after planting. In addition, at each evaluation date, the fungicide concentration that inhibited downy mildew by 50% relative to the control (EC50 value) was calculated to determine the duration of efficacy. The EC50 was calculated as percent inhibition of the downy mildew index of the treated plants relative to the untreated control. A regression analysis that expressed percent inhibition as a function of fungicide concentration was calculated and the concentration of the fungicide that inhibited downy mildew by 50% relative to the control was determined.

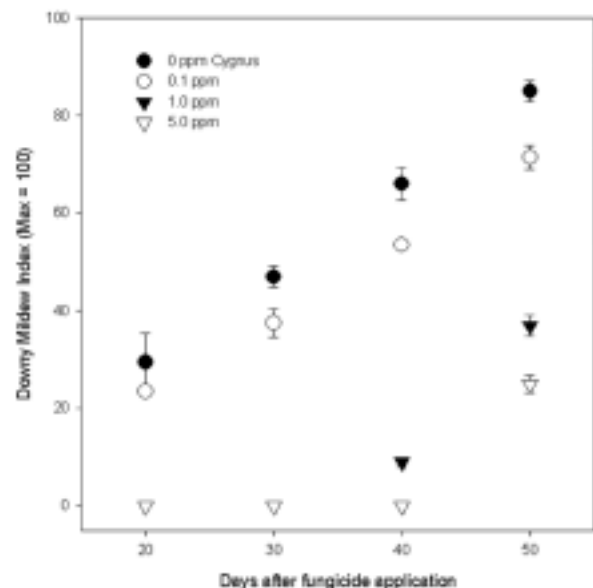
**Results and discussion**

Downy mildew slowly defoliated *Lamium* in the untreated control. By 50 days after application of the fungicides (DAFA) the downy mildew index was greater than 80, indicating that plants were exhibiting

**Figure 1.** Development of downy mildew on *Lamium maculatum*- Influence of time after application on efficacy of mefenoxam (Subdue MAXX) applied at increasing concentrations.



**Figure 2.** Development of downy mildew on *Lamium maculatum*- Influence of time after application on efficacy of kresoxim-methyl (Cygnus 50WG) applied at increasing concentrations.



between 51 and 100% defoliation (Figures 1 and 2). Treatments of 0.1 to 10 ppm mefenoxam (Subdue MAXX) gave effective control of downy mildew up to 40 days after treatment. The EC50 values indicated that both fungicides were effective against downy mildew, but that efficacy decreased with time (Table 1). By 50 days after application, the efficacy of Subdue MAXX at rates effective prior to the final assessment no longer effectively controlled downy mildew. Manufacturer recommended effective field rates (MRR) are between 8 - 16 ppm. The efficacy of Cygnus 50WG was reduced three-fold (MRR between 30 - 60 ppm) by 50 DAFA. Up to 40 days, Subdue MAXX applied at rates greater than 0.1 ppm was more effective than Cygnus 50WG at any rate. The efficacy of Subdue MAXX decreased 50 DAFA and application rates >0.1 ppm were not significantly different from Cygnus 50WG application rates of 5

and 10 ppm.

The occurrence of resistance to metalaxyl/mefenoxam was not noted in this experiment as the downy mildew affected plants responded to applications of Subdue MAXX. Although this was presumably a single isolate of a potentially more diverse population, it should be clear that if plants are observed up to about 40 days after application of Subdue MAXX, control of downy mildew will be obvious. However, as inoculum is rarely eliminated from the environment the re-appearance of downy mildew symptoms may be observed about 50 DAFA. Such a phenomenon should not be confused with resistance but is a normal occurrence as the efficacy of the fungicide is diluted through "weathering". Cygnus 50WG could be applied in rotation with Subdue MAXX to reduce the risk of development of resistance to Subdue MAXX. ☞

## ***Phytophthora* water molds in ponds and recirculated irrigation systems in Michigan nurseries**

### **Authors**

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### **Funding**

Center for Integrated Plant Systems (CIPS)

### **Background**

*Phytophthora* causes disease on a wide host range of plants. Historically, Michigan nurseries have been relatively free of these highly destructive plant pathogens, except when it arrived on rhododendrons or other stock from other states. In Michigan, introduced *Phytophthora* species often die during the severe winter and native soils seldom contain the pathogen. In contrast, nurseries in western and southeastern states have had continuous problems with infestations of this group of water molds.

Water management in nurseries has recently changed to include the capture of water run-off and the use of re-circulating water stored in ponds. Recently in Michigan, significant losses of lilac and rhododendron nursery stock occurred due to *Phytophthora* cankers and root rot diseases. (Photo, color insert, page 2A.) Preliminary evidence indicated that the source of the infections had been from the nursery's pond water. For example, two years ago a nursery lost over \$300,000 of lilacs from *Phytophthora* canker on the stems. Sprinkler systems had deposited pond water onto the stems during irrigation. The cankers and the pond water

contained the pathogenic water mold *Phytophthora cactorum*. Further sampling of seven ponds that summer yielded *Phytophthora* species.

### **Objectives**

In order to develop a management system to reduce and control *Phytophthora* diseases in the nursery, we needed to know what *Phytophthora* species could survive in the ponds over the winter. Managers would then know if they began each spring with clean ponds or infested ponds. It is remarkable that this type of information has not been determined in any state with a cold winter.

Research in vegetable crops has determined that species of *Phytophthora* forming a thick-walled spore, (Figure 1) can survive winter in soils in Michigan. Species of *Phytophthora* that cannot make these spores, and there are many such species, generally die during winter without the roots of the infected plant present.

Our research objective was to determine which *Phytophthora* species survived the winter in nursery ponds in Michigan; and whether the *Phytophthora* species that survived were species which formed the thick-walled spores or not.

### **Methods**

We sampled 11 ponds and also sampled nursery drains, run-off ditches and rivers used by nurseries for irrigation in eastern and western Michigan. Samples were taken in the fall and in the early spring.

The pathogenic water molds were captured and quantified by floating green pears in samples of the ponds' waters. Plant pathogenic water molds can infect green fruit whereas non-pathogenic species usually cannot. Swimming spores produced by fungal structures formed underwater, (Figure 2) are attracted to the fruit and penetrate the skin causing rot. (Photo, color insert, page 2A)

Pathogenic water molds known as *Pythium* also infect such fruit in high numbers. These pathogens are less important in causing damage of woody plants in the nursery, except during rooting of cuttings and seed germination. So, many *Pythium* species must first be identified and eliminated from the pond assays for accurate information on *Phytophthora* to be determined. We set about the task of separating *Pythium* species and identifying species of *Phytophthora* in the ponds. Identifying *Phytophthora* strains included using DNA sequence analyses to verify the microscope-based identifications. We sampled ponds late in the fall and early in the spring.

In order to verify that a *Phytophthora* strain in a pond in the fall is the same strain found in the early spring, we needed a means of identifying individual strains. We used a new DNA technology to "fingerprint" strains of pathogens, called AFLP analysis (amplified fragment length polymorphism), so that specific spring strains could be compared to specific fall strains. Dr. Kurt Lamour (of MSU plant pathologist Mary Hausbeck's lab) did the DNA fingerprinting AFLP analysis for this research. A picture from our studies of what a portion of an AFLP analysis looks like is shown below. Identical strains will have matching patterns (peaks) on the graph. The graph below (fig. 3) allows comparison of 7 strains of *Phytophthora gonapodyides*.

### Conclusions

Nursery ponds in the spring varied from nearly free of *Phytophthora* species to highly infested. The heavily infested ponds led our research effort to studying nearby rivers. A unique situation was found in Michigan that has never previously been reported in any state: *Phytophthora gonapodyides* was found to be occurring at very high levels in Michigan rivers and some ponds in the earliest spring samples.

There is little literature on this species of *Phytophthora* because it has only been recognized and studied in North America since 1989. Fortunately for the nursery industry, this species that is not pathogenic to plants, except for seedlings of some conifers. It is of no more concern than the many *Pythium* species discarded from the study, so we

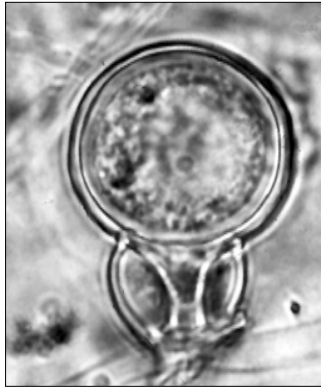
refer to it as "non-pathogenic" in this report. Surprisingly, this species does NOT form the thick-walled "winter" spore, yet it is abundant in rivers and ponds soon after the winter thaw. We discovered that in rare instances species that generally do not form the winter spore could infest our waterways. We speculate the fungus survives in plant debris and roots, or agricultural soils, and then sporulates profusely with early spring rains that drain into the rivers carrying the fungus. Alternatively, the fungus may actually survive in the ice-covered rivers, perhaps in plant debris, aquatic plants, or in roots of riverbank plants.

Unfortunately for our studies, the huge number of this "non-pathogenic" species overwhelms our trapping methods and our assays for the virulent species. The numbers also overwhelm our ability to track an individual strain of a pathogen in a pond in fall into the following spring. In microscopic identifications, *P. gonapodyides* appears identical to two important pathogenic species, *P. drechsleri* and *P. cryptogea*, which means that more expensive DNA methods must be used to separate the pathogenic from the non-pathogenic species. One outcome of our research, was the realization that useful studies of *Phytophthora* in recirculated water systems in Michigan will be much more difficult and costly than originally anticipated.

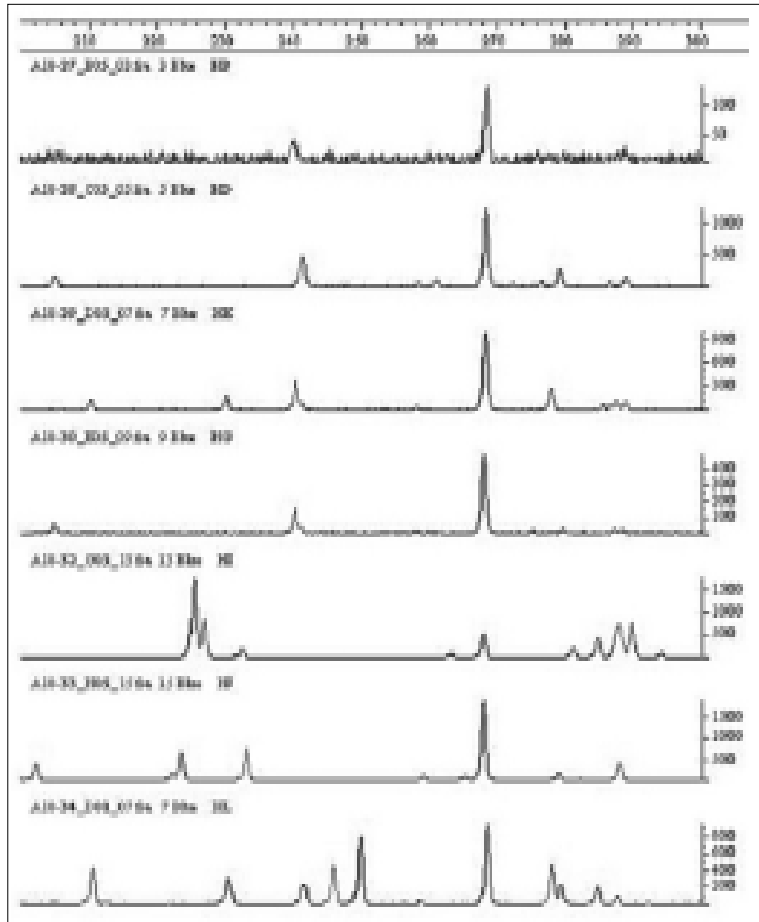
Pathogens found in ponds in early spring were species that formed the "winter" spore, including: *P. cactorum*, *P. citrophthora*, *P. nicotianae*, and a species tentatively identified as *P. humicola*. All of these species were rare and at very low numbers in the ponds in spring. Another pathogenic species, *P. drechsleri*, may have been present. Because we were unable to check these isolates with DNA tests, we remain uncertain. *P. drechsleri* generally would not form the winter spore. If it was present in spring, then it may have been the most abundant and widespread of the pathogenic species in the ponds. More research will be needed to verify this point.

At least one pond was found that had four species of *Phytophthora* present, however, most ponds had only the "non-pathogenic" *P. gonapodyides*. Drains and ditches in nurseries had very high numbers of *Phytophthora* present, but not any higher than rivers. When we compared water from immediately before and immediately after filtration through a diatomaceous earth-based system in a pump house, no discernable difference in numbers of *Phytophthora* were evident, even with moderately high numbers present. Commercial filtration units have been found to be ineffective in reducing *Phytophthora* concentrations in research in other states. ✍

**Figure 1.** Oospore, a thick-walled resting spore of *Phytophthora* sp.



**Figure 2.** Sporangiophores-structures that contain zoospores (swimming spores) of *Phytophthora* sp.



**Figure 3.** A picture from our studies of what a portion of an AFLP analysis looks like. Identical strains will have matching patterns (peaks) on the graph. The graph shows comparison of 7 strains of *Phytophthora gonapodyides*.

## Is a more virulent *Sphaeropsis* shoot blight of pine moving into Michigan forests and killing seedlings?

### Author

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Department of Plant Pathology

### Funding

Michigan Department of Natural Resources (DNR)

### Background

In response to urgent requests by some Michigan nurseries, the Michigan DNR funded a cooperative research project with our lab to investigate *Sphaeropsis* shoot blight. The shoot blight is the most damaging disease of pine in the Michigan landscape, especially on Austrian, Ponderosa, Mugo and Scots pines. Most nursery and landscape industry workers know this disease as “Diplodia tip blight.” The pathogenic fungus, *Sphaeropsis sapinea*, also causes “collar rot” on nursery seedlings. The symptom is a

canker at the soil line often followed by death of the seedling. The impetus for this investigation was concern that Michigan conifer seedlings were more frequently infected with this disease than nursery seedlings from neighboring states. There were other concerns in addition to potential loss of sales. For example, several hundreds of acres of forest stands of red and jack pine seedlings had recently died due to the pathogen in Michigan. Were the nursery seedlings the source?

The objectives of our study of nurseries and forests were to determine: 1) whether collar rot was a problem in the nurseries; 2) whether the pathogen was moving from the nursery to newly planted forest stands; and 3) whether a new and more virulent pathogen was moving into the forest stands.

The most important result: the Michigan nurseries examined in our studies had no detectable collar rot! A scientist from University of Wisconsin was brought

in to witness this. He left not only convinced that the examined nurseries were very nearly disease free but also, remarkably, that they were less diseased than those in two other nearby states. The Michigan DNR then took further steps to ensure the disease remains scarce by removing susceptible pines from nursery windbreaks. Enough said. Though, for those who enjoy the more complex stories that arise from research there is much more.

One major objective of the research was to determine whether a more virulent type of the fungus, (known as Type A) was moving into Michigan forests where a less virulent type (known as Type B) appeared to be native. Type A has been the cause of shoot blight in every diseased ornamental pine that I have examined over many years (1987-2002) in Michigan. A general consensus among foresters in the Lake States is that Type A was introduced to the region in the late 1960s and is now responsible for an increasing incidence and severity of the disease.

We examined seed lots, healthy seedlings grown in soil beds and in green houses, windbreak trees, and diseased and healthy forest stands, for presence of *S. sapinea* of Type A and Type B. A number of different methods were used to detect and identify *S. sapinea* in symptomless or diseased pines. The most successful and sensitive method was based on DNA. We were able to detect the DNA of the fungus in extracts of whole plant DNA. Amplification of fungal DNA present in the plants was accomplished using *S. sapinea*-specific primers. The identity of the fungus was verified by sequencing the DNA, and by RFLP (random fragment length polymorphism) analysis. Another method involved detection of fruiting bodies on diseased plant tissues and microscopic identification of the pathogen. Furthermore, *S. sapinea* was detected by isolation and cultivation on agar media and identification of Type A and Type B strains based on colony characteristics and growth rate.

Seeds taken directly from refrigerated nursery lots varied from clean to heavily infested with Type B, Type A and both. The infestation of the seed lots

probably occurred in the forest from which they were sampled because *S. sapinea* is a pathogen of green cones. Variation in climate from year to year can increase or decrease the amount of cone infection by *S. sapinea* in a stand. However, we cannot rule out the possibility of poor handling also increasing the amount of the pathogens. No obvious pattern was discernable in the data although it suggested that in some locations plantation-derived seeds are more infested than natural stand-derived seeds. Table 1 provides the first data on levels of *S. sapinea* in collected seed. It is unknown whether the fungus is on the surface, directly under the seed coat, or among the cells of the embryo. The results may vary from year to year. More precise information on the actual location where seeds were collected in a stand would be useful. These evaluations should be repeated in further studies before they are fully accepted.

Studies revealed that *S. sapinea* was present and latent in healthy green needles of bed-grown seedlings. The presence of the fungus in healthy seedlings confirmed that the fungus survives in pine tissues without causing disease until significant stress occurs to the plant, such as severe drought or hail. Other researchers in South Africa recently discovered the latent nature of the fungus. The fungus is now referred to as an endophyte. Seedlings from the same seed lot, but grown under greenhouse conditions with strict sanitation were found to be entirely free of the endophytic fungus. This discovery is very significant because it informs us that *S. sapinea*-free seedlings can be grown from moderately infested seed. Further research will help clarify the cultivation practices and/or fungicide application practices that encourage production of *S. sapinea*-free seedlings.

Studies of the extensive acreages of jack and red pine seedlings that died or were severely damaged by *S. sapinea* cankers produced unexpected results. The stands in both the lower and upper peninsulas of Michigan had approximately 85% infection (cankers) caused by Type B and 15% by Type A. Healthy

**Table 1.** Pine seed lots and *Sphaeropsis sapinea* infestation- dates in brackets are approximate.

Species	Lot number	Location	Forest	Year	Type A	Type B	Infestation
Red pine	MI0235	Toumee	Natural stand	1975	Yes	No	<u>Moderate</u>
Red pine	9703	Kalkaska Range	Natural stand	1996-7	Trace	No	<u>Clean</u>
Red pine	MI0232	Manistique	Plantation stand, 25 years old	(1997)	Yes	Yes	<u>Heavy</u>
Red pine	MI0233	Manistique	Natural stand	(2001)	No	No	<u>Clean</u>
Red pine	MI0234	Manistee -Osceola	Natural stand	(1999)	Trace	No	<u>Clean</u>
Jack pine	MI0206	Manistique	Natural stand	(2001)	No	Yes	<u>Moderate</u>
Jack pine	MI0208	SE of Gaylord near Mio	Plantation stand	(1996)	No	No	<u>Clean</u>
Jack pine	MI0211	Benzie- Manistee	Natural stand	(2001)	No	Yes	<u>Heavy</u>
Jack pine	MI0218	Brighton- Howell TIC	Plantation stand	1999	Yes	No	<u>Low</u>

foliage had primarily Type B endophytic infections. The general hypothesis was that cankers and death had not been seen when only Type B was present in the forests, therefore, the damage must be due to the introduced Type A. Further research will now be necessary to determine whether seedlings infected with Type A (as endophytes in healthy foliage) keep them after outplanting. Investigations will also be needed to determine whether *S. sapinea*-free seedlings remain so after outplanting. Additionally, seedlings with Type A endophytic infections should be compared with those having Type B endophytes for disease resistance under drought stress.

Significant new information on the *Sphaeropsis* disease in Michigan was rapidly discovered using

DNA detection technologies for fungi, enabling us to provide quick responses to industry concerning questions about collar rot. For example, this is the first report of detection and identification *S. sapinea* Type A and Type B in seed lots and, the first measurement of the level of infestation among lots. Additionally, the DNA-based methods made it possible to compare infestation of seed lots prior to sowing with presence of the fungus after sowing, in needles of healthy seedlings.

Dr. Mursel Catal from our research group joined the MSU Plant Diagnostic Clinic in 2003, and he has integrated this DNA capability into diagnoses of many plant diseases. Dr. Catal has been using the new methods for detection of fungal, bacterial, virus and nematode pathogens. ☞

## Protecting Michigan forests from the introduction of sudden oak death

### Authors

Gerard Adams and Mursel Catal, Department of Plant Pathology, MSU

### Funding

Michigan Department of Agriculture (MDA)

The new and devastating disease of trees and woody ornamentals known as sudden oak death (SOD) has been found in nurseries in California, Oregon, Germany and the Netherlands. The name of the disease comes from the rapid loss of tanoaks in the moist coastal forests of California. The disease is caused by a newly identified pathogen, *Phytophthora ramorum*, first reported in Germany on Rhododendron stock. The pathogen causes oozing cankers on the trunks of the trees and the cankers rapidly girdle the trees, killing them (see photos page 2A). Federal quarantines have been established to protect Michigan and other non-infested states from accidental introduction of this pathogen. However, prudence would warrant a careful watch over nursery stock imported from infested states because the methods for screening shipments of plants are error prone.

The potential impact of introducing *P. ramorum* into Michigan could be a massive devastation of our forestry industry and natural resources. Concern in Michigan is based on the known susceptibility of red oak, *Quercus rubra*. This tree is one of the most valued species in Michigan forestry. The impact could be compounded by loss of other woody plants in the forest ecosystem because the susceptibility of native Michigan plants remains unknown.

Early detection of the pathogen's introduction into Michigan is essential for any plans for eradication to succeed. The MDA began an initial survey of nurseries in 2001-02. They collected samples from

ericaceous plants, such as rhododendron, viburnum, camellia, and blueberry, as they arrived in Michigan from western nurseries. The samples collected included any leaves with necrotic areas and leaf spots. It has been discovered in California that the SOD pathogen causes small harmless infections on leaves of ericaceous plants. These infections cause lesions (see photo, page 2A) in which the pathogen sporulates. The spores are spread by rain. The pathogen is not known to sporulate on the oak trees.

The MDA brought approximately 100 samples to our laboratory to screen for the presence of *P. ramorum* in 2001-02. Screening techniques included incubating the leaves in moist chambers to induce sporulation, culturing of pathogens from the leaf lesions onto selective agar media, and molecular diagnostic tests. The molecular diagnostic tests were developed at the University of California and are based on highly sensitive detection of the pathogen's DNA in leaf tissue. We used molecular techniques and chemical extraction of the DNA of the pathogen sent to us from the California laboratory. The pathogen DNA served as a positive control for verifying that our screening tests were working.

Our screenings of the MDA samples were negative for the SOD pathogen. However, it will be important to continue screening nursery samples in future years and to keep a watchful eye on the health of oak trees near nurseries. Other species of *Phytophthora* were occasionally detected on leaves of the ericaceous plants. Careful analysis was needed to distinguish *P. ramorum* from these other *Phytophthora* species. Experience in California has shown that detection of *P. ramorum* by DNA technology is far more sensitive and precise than older methods based on culturing. ☞

## Phytophthora root rot of Fraser and other true firs in Michigan

### Authors

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### Industry partner

Michigan Christmas Tree Association

### Funding

Project GREEN

### Background

Fraser fir is viewed as a premium Christmas tree by the Michigan Christmas tree industry. Phytophthora root rot of Fraser fir, can be caused by several species of *Phytophthora* and is a limiting factor in production if trees are planted in low areas with poor soil drainage. Increased acreage of Fraser fir in Michigan has resulted in more trees being planted in unsuitable soils. In these situations, Phytophthora root rot can cause devastating losses in transplant beds as well as in plantations.

### Project objectives

The purpose of this study was to survey Michigan Christmas tree plantations and nurseries for root rot and identify the species of *Phytophthora* associated with diseased Fraser fir. This knowledge will allow us to institute management plans for Phytophthora root rot in Michigan Christmas tree plantations and to identify fir species resistant to Phytophthora root rot.

### Results

Similar to a results of a survey conducted in 1986, the most common species of *Phytophthora* found associated with Fraser fir plantings in Michigan were *P. citricola* and *P. cactorum*. Although *P. cinnamomi* is highly pathogenic to Fraser fir and is problematic in North Carolina, it was not recovered in Michigan in this or the 1986 survey. It has been suggested that *P. cinnamomi* is poorly adapted to the cooler temperatures of Michigan soils.

Of significance, however, was the finding of another species that has been difficult to characterize. Different aspects of this strain resemble both *P. megasperma* and *P. cryptogea*. Other *P. megasperma* isolates have not been able to cause disease on Fraser fir, but this novel strain is strongly pathogenic to Fraser fir and appears to be new to Michigan farms. Other species of *Phytophthora* including *P. medicaginis*, *P. europa*, *P. gonapodyides* and *P. nicotianae* were also found associated with the roots of dying Fraser fir, but their role in disease, if any, is not well understood.

Using the most pathogenic *Phytophthora* species from our recent survey, we inoculated various fir species in greenhouse tests. In these assays, it appears that Canaan and balsam fir were more tolerant of root rot than Fraser fir.

A unique opportunity to test the results of our greenhouse studies on root rot resistance in Canaan fir appeared during our survey. Both highly pathogenic and weakly pathogenic isolates of *Phytophthora* were present in the soil where a severe root rot epidemic was occurring. It was obvious at the outset that the root rot was associated with contaminated nursery stock from an out-of-state nursery since diseased trees abruptly stopped in the eighth row of a 14-row landing where nursery stock from one nursery was changed to that of another. The next six rows of Fraser fir were from an in-state nursery and no trees in these 6 rows showed symptoms of root rot. (Photo, color insert, page 2A.)

Furthermore, not only did the Fraser fir in this field originate from different nurseries, but the Fraser fir landings at one point were separated by a landing (16 rows) of Canaan fir. Similar to our greenhouse results, the Canaan fir did not develop root rot even though water drained from the diseased trees directly through the Canaan fir landing. Another landing of Fraser fir planted to the far side of the Canaan fir began to develop Phytophthora root rot later in the study.

This disease pattern strongly supports the probability that *Phytophthora* spores were carried by the drainage water through the Canaan fir planting to the Fraser fir landing on the far side. Yet, the passage of *Phytophthora* spores through the Canaan fir did not lead to any root rot of the Canaan fir suggesting some level of field tolerance in Canaan fir. These observations supported our greenhouse inoculation assays that suggested higher levels of resistance to Phytophthora root rot could be found with Canaan fir as with Fraser fir.

### Future directions for research

These results open two directions for future research. First, it may be possible to select balsam or Canaan fir with high levels of root rot resistance with better needle retention than currently available; or, second, it may be possible to graft Fraser fir to fir root stock with high levels of root rot resistance. This at first would appear to be prohibitively expensive. But, using these grafted trees to expand fir acreage into areas where root rot would normally be expected to infect trees could increase premium fir production acreage in Michigan. ☞

## Phytophthora root rot of white pine, a new disease for the state tree

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### Background

It is obvious to most travelers of Michigan roads that white pine is struggling along highways. Trees are turning chlorotic and/or red and are dying. It has been suggested that these trees have been stressed from drought, salt spray damage, and a harsh winter that included dehydration where roots could not take up enough water. During these times, it becomes imperative not to assume that all complaints of dying white pine are due to the same stress events throughout the state. One tree farmer in mid-Michigan began to notice dead white pines in his tree planting. These trees were 15 to 20 feet tall, were dying in groups and were not planted near a highway. Only the white pines were affected and dying and the interplanted spruce trees appeared healthy.

### Diagnosis

Several trips were made to the tree farm to determine the cause of the white pine deaths. It was obvious that the affected trees occurred in a pattern (focus) and that a large planting of healthy white pines was less than a hundred yards away. Based on the ground-level stumps, more than 20 dead white pines had been cut down in this particular location in the last two growing seasons. In early June, two more trees were dying and it appeared as if two more trees were candidates with their off-color gray appearing needles. Off-color needles (including gray-green, yellow and red), wilt, bark borers and bark fissures leaking sap were the only common symptoms on trees that had recently died. Roots were black and appeared to be more advanced in decay than the top of the trees. The root collar showed some necrosis and this appeared to be coming up from the roots

rather than down from the stem. Roots on trees beginning to show symptoms of off color needles did not appear to be severely affected.

Samples of the roots were taken back to the laboratory and checked for the presence of *Phytophthora* using a quick immunoassay (ELISA). Some roots appeared positive, however, nonpathogenic *Phytophthora* and other related water molds can confound these assays. Soil samples from around the roots were removed and using rhododendron leaves, *Phytophthora* isolates were baited out of the soil via the rhododendron leaves and cultured on a selective medium.

Using both traditional and new molecular methods, these isolates were characterized and identified as *Phytophthora cryptogea*. When placed in small wounds on the root collar of three-year-old, potted white pine in the greenhouse, death of the stems ensued and trees died within three weeks. This same *Phytophthora* species killed young Fraser fir when inoculated onto young fir in the same greenhouse in a parallel test.

### Significance

This is the first report of *Phytophthora* root rot of Eastern white pine. The trees were planted in a heavy clay soil that was wet in the spring and dry in the summer; the location was slightly lower than the surrounding area. The field had been in field crops prior to planting with conifers. Healthy trees in this disease focus had been dug and shipped, however, the grower indicated that no trees exhibiting symptoms were dug. *Phytophthora cryptogea* is not a new pathogen of conifers and it is possible that the presence of the pathogen, the stressed conditions of the white pine and the heavy, wet soils contributed to the expression of this new disease. In our tests, however, this species of *Phytophthora* appeared very aggressive on white pine and steps to monitor dead and dying white pines throughout the state, especially those trees recently transplanted, should be undertaken. ☞