

# **Plant Pathology and Nematology**

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**Fungi Associated with Naturally Occurring Leaf Spots and Leaf Blights in  
*Hydrangea macrophylla***

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**Index Words:** Ornamentals, diseases, *Myrothecium roridum*, *Phoma exigua*, *Botrytis elliptica*, *Corynespora cassicola*.

**Significance to Industry:** Bigleaf hydrangea or garden hydrangea (*Hydrangea macrophylla*) cultivars are popular flowering landscape shrubs used in full sun and shade gardens. Foliage diseases that infect hydrangea can impact the appearance, health and market value of these plants. Ninety bigleaf hydrangea cultivars of grown in full sun and in 60% shade were evaluated at the Otis Floyd Research Center in McMinnville TN. Plants grown in the shade were severely infected with the powdery mildew disease while those in the full-sun were primarily infected with leaf spots and leaf blights caused by fungi. This paper reports on the foliage diseases that occurred naturally on bigleaf hydrangea in McMinnville, TN.

**Nature of Work:** While hydrangeas are known to need only moisture and shade to thrive, most cultivars benefit when they receive dappled direct sunlight, or are exposed to morning sun (7). Two sets of ninety commercial cultivars of *H. macrophylla* were grown in 26.5 L (7 gal.) plastic containers. Out of the 90 cultivars, 69 were *H. macrophylla* subsp. *macrophylla*, 18 were *H. macrophylla* subsp. *serrata* and 3 were hybrids between the two subspecies. One set was maintained under 60% shade cloth, micro-irrigated using spray stakes; the second set was maintained in full sun, and watered using overhead sprinkler system. All plants were fertilized with a 19-5-9 Osmocote Pro fertilizer (Scott's-Sierra Horticultural Products Co., Maryville, OH), at a rate of 143 g per pot. Plants were arranged in a completely randomized block design with a replication of three individual plants per cultivar in the shadehouse and four plants for each cultivar in full-sun. Plants were monitored for disease symptoms that developed from natural airborne inocula.

Foliar diseases were diagnosed using disease symptoms and signs of the pathogens. The causal agents were isolated on Potato Dextrose agar (PDA) and clean cultured. Isolates from different disease symptoms were evaluated for pathogenicity by inoculating healthy leaves of three susceptible cultivars 'Nikko Blue', 'All Summer Beauty', and 'Blue Bird'. The detached leaves in-vitro assay technique was used (4). Disease-free detached leaves were placed in clear plastic containers lined with double layer tissue papers that were kept dripping wet to create a moist chamber. Leaves were inoculated with different isolates using agar plugs (2 cm. diam.) containing young mycelia cut from 10-14 day-old cultures. Each half leaf was inoculated with one isolate with a replication of four leaves. Sterile PDA agar plugs with no mycelia were used as

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controls. Following inoculation, the moist chamber were placed in clear plastic bags to maintain 90-100% moisture content, incubated at 20°C, 23°C, and 26°C, and monitored for disease development over 14-21 days. A factorial experimental design with isolates completely randomized at each temperature was used. Pathogenic isolates were re-isolated from the lesions that developed on the detached leaves to complete Koch's Postulate. These isolates were evaluated for virulence on different cultivars using the in vitro technique described above.

Pathogenic fungi were characterized using DNA sequence analysis and morphological features observed under a compound microscope. Genomic DNA was extracted from conidia and mycelium using DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA). DNA amplification was performed using Polymerase Chain Reaction (PCR) and universal primer pairs, ITS1/ITS4 following standard PCR procedures with minor modifications. Sequence analysis was done by the Davis Sequencing Inc. at Davis, CA (<http://www.davissequencing.com>). The sequences obtained were compared with all sequences of ITS region in the GenBank closest sequence similarity by using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>).

**Results and Discussion:** Powdery mildew was the most common disease on plants grown in the shade starting in late July. Powdery mildew was not observed in full sun, but significant amounts of leaf spots and leaf blights developed in some cultivars grown in the shade. Leaf spots and leaf blights were common on plants in the full-sun beginning in early June. Early defoliation was associated with leaf spots/ leaf blights in the full-sun and in the shade. Although many fungi were isolated on affected leaves, seven fungi were found to be pathogenic to hydrangea.

Four fungi were isolated from necrotic lesions that were characterized by small circular leaf spots with ash colored centers and prominent purplish to dark-brown margins (Fig 1a, b). This disease was more common on plants grown in full-sun than in the shade. Only a few cultivars in the shade developed a significant amount of this disease. Lesions began mostly as purplish discolorations that later became necrotic. All four fungi that were isolated from these necrotic lesions were pathogenic on hydrangea. Lesions that developed on detached leaves did not develop ash colored centers or purplish margins. Based on DNA sequence analysis of the ITS region and morphological features, these fungi were identified to be *Cercospora* spp., *Phoma exigua* (GenBank Accession No. EU343124), *Myrothecium roridum* (GenBank Accession No. AJ301994 and AJ608978) and *Glomerella acutata* (Anamorph: *Colletotrichum acutata*). While *Cercospora* spp. and *G. acutata* produced very small circular leaf spots, pin-head size in diameter on 'All Summer Beauty' and 'Nikko Blue', *Phoma exigua* and *M. roridum* produced larger lesions (Fig 1 c). DNA sequence for *Cercospora* spp. matched that of *C. fukushiana* (GenBank Accession No. EF600954) and *C. penzigii* (GenBank Accession No. DQ835072) by 100 %. Symptoms presented in Fig. 1 (a-b) resembled that described for *Cercospora* leaf spot, also known as frog-eye leaf spot caused by *C. hydrangeae* (1). *Myrothecium roridum* is an important pathogen that causes leaf spots and leaf blights on more than 150 different hosts, but this is the first report that this fungus causes foliar diseases on hydrangea. This pathogen caused

damage to the foliage and reduced the aesthetic value of infected plants (5). Symptom produced by *M. roridum* on hydrangea leaves is similar to those reported on other crops (5). Further studies on the economic value of this pathogen in hydrangea are needed.

Three fungi were isolated from larger circular to irregular shaped necrotic lesions with concentric rings of alternating brown and slightly lighter brown rings with an ash-grey center (Fig. 2a). These lesions were observed on many plants in full-sun from early June through September. These lesions were also observed on flowers (Fig. 2b). On detached leaves, these fungi produced dark brown necrotic lesions characterized by concentric rings of dark and slightly lighter color without the ash colored center (Fig 2 c). Based on spore morphology and ITS region DNA sequence analysis, these fungi were identified as *Corynespora cassiicola* (GenBank Accession No. FJ184988), *P. exigua*, and *M. roridum*. *Corynespora cassiicola* was the most aggressive of the three; it produced lesions on detached leaves in 3-4 days after inoculation while it took 4-5 days for *M. roridum* and *P. exigua* to produced similar symptoms in (Fig 2c). *Corynespora cassiicola* was pathogenic on 26 cultivars it was evaluated on, including 'Blue Billow', 'Nikko Blue', Gen. Vicom.Vibraye', 'Holstein', 'Lemon Wave' and 'Todi' (Fig 2c). *Myrothecium roridum* and *P. exigua* have not been evaluated on all 26 cultivars, but *M. roridum* was pathogenic on 12 cultivars. These symptoms resembled that described for anthracnose also known as target leaf spot caused by *Colletotrichum gloerisporoides* L (1).

Two additional fungi were isolated from lesions that had symptoms that consisted of light-brown to grey-brown necrotic lesions with or without dark-brown margins surrounded by chlorotic halo (Fig 3a-b). These symptoms were observed on plants in the full-sun and on a few cultivars in the shade. Pathogenicity for these fungi was confirmed on 'Nikko blue', and 'All Summer Beauty' at 23 and 26°C (Fig 3c-d). One of the fungi was identified as *Phoma exigua*, and the other as *Botrytis elliptica* (Gene Bank Accession No. EU519207) and *Sclerotinia sclerotiorum* (Gene Bank Accession No. EU530000). *Botrytis elliptica* is also an important pathogen in *Lilium* spp (6). . This fungus was isolated from plants grown in full-sun and in shade, during the later part of the summer (from August to October) when the climate was hot and humid. The fungus was also isolated in the shade where it caused severe dieback and killed some plants. *Botrytis cinerea* was not observed in this study and it becomes a problem on hydrangea during cool and wet weather (1). *Phoma exigua* and *B. elliptica* have not been reported on hydrangea in the United State.

From dark-brown circular to irregular necrotic lesions on the foliage of plants grown in full-sun and on their lower leaves of shaded plants, two other fungi were isolated (Fig 4a-b). They are *P. exigua* and *Alternaria* sp. Pathogenicity of the these fungi was confirmed on 'Nikko blue', 'All Summer Beauty' and 'Blue Bird' (Fig 4c-d) and identified based on morphological features and DNA ITS region sequence analysis.

All observed leaf spots/leaf blights impacted the appearance of infected plants. Leaves inoculated with clean agar plugs did not develop lesions, thus validating the

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pathogenicity tests on detached leaves. Results of this study showed that hydrangea leaf spot and leaf blight occurred as a disease complex (Figs 1a, 2a, 3b, 4b). This observation suggests that careful evaluation of all components of the disease complex need to be considered in any evaluation for disease resistance in natural environment and not simply use a reported disease symptoms. All characteristics of observed symptoms were not reproduced on detached leaves, and there were minor variations in different cultivars. This may be attributed to time needed for development of all symptoms characteristics, environmental factors and/or host/parasite interactions in the disease complex. Anthracnose (*C. gloerisporoides*) and cercospora leaf spots (*C. hydrangeae*) are common in the Southeast United States (1), but these pathogens were not isolated. Out of the fungi that occurred naturally in the McMinnville area, *C. cassicola*, *P. exigua*, and *M. roridium*, reproduced symptoms similar to that described for *C. gloerisporoide* (Fig 2a). *P. exigua* has been reported as a pathogen of hydrangea in Italy where it caused severe defoliations and impacted the aesthetic value of the infected plants (2). To the best of our knowledge, *P. exigua* has not been reported in hydrangea in the United States. Although *Botrytis cinerea* has been reported to cause gray mold on hydrangea pathogen (1), it was not isolated in our study. The *B. elliptica* isolated in this study was highly aggressive on some cultivars in the open-sun and in the shade and some cultivars were resistant in the pathogenicity tests. *Alternaria* is a common secondary pathogen and was pathogenic on some cultivars and its symptoms resembled that from *P. exigua*. The advantage of using detached leaf assay is the ability to screen large populations of genotypes against individual and multiple pathogens using smaller amount of inoculum, and less space to maintain test plants (4). Reports that the incidence and severity of cercospora leaf spots is reduced when plants are grown under the shade is supported by this study, since plants in full-sun had more leaf blight and leaf spot than plants in the shade (3).

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Figs 1- 4 a-b: Disease symptoms on *Hydrangea macrophylla* observed on plants growing in open sun (1- 4a-b) and reproduced on detached leaves by *Phoma exigua* (Fig 1c) and *Myrothecium roridum* (Fig 2c-d); reproduced by *Corynespora cassicola*, *P. exigua*, and *M. roridum* (Fig 3d); reproduced by *P. exigua* and *Botrytis elliptica* (Fig 4c); and reproduced by *P. exigua* and *Alternaria* sp (Fig 4 c- d).

## Comparison of Resistant and Susceptible Flowering Dogwoods in Pathogenesis of *Discula destructiva*

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**Key Words:** Dogwood anthracnose, flowering dogwood, 'Cloud 9', 'Appalachian Spring'

**Significance to Industry:** 'Appalachian Spring', a resistant cultivar to dogwood anthracnose, was released nearly ten years ago. However, the resistant mechanisms are unknown. This report demonstrates a cuticle resistance which significantly inhibits conidial germination and germ tube growth.

**Nature of Work:** Flowering dogwood (*Cornus florida* L.) is one of the most popular ornamental trees in eastern United States. Flowering dogwood has been severely impacted by dogwood anthracnose since the disease first appeared in 1976 (2) and significant mortality has occurred in the southeastern United States (3, 4, 5). Differences in levels of disease resistance were observed among flowering dogwood lines (1, 8, 9). Understanding the mechanism of the resistance in flowering dogwood is essential in order to determine breeding strategies and manage the disease. Thus, it is very essential to understand early stages of the pathogenesis. Cultures of *Discula destructiva* were isolated from infected dogwood twigs and leaves. Terminal leaves were wounded and inoculated with a conidial suspension. Each inoculated leaf was enclosed in Petri dishes with moistened filter paper, which were placed at 20° with a 12/12h light/dark cycle. One-centimeter leaf segments were harvested 2, 3, 4 days after inoculation (dai). Leaf segments were then placed into a clearance solution (0.15% trichloroacetic acid [wt/vol] in ethyl alcohol/chloroform, 4:1 [vol/vol]) to stop the growth of the fungi and to remove all chlorophyll. The solution was exchanged once during the next 48 h. To stain fungal structures for light microscopy, leaf segments were stained in a freshly prepared Coomassie blue solution (0.6% Coomassie brilliant blue R 250 [wt/vol] in methanol/15% trichloroacetic acid [wt/vol] in H<sub>2</sub>O, 1:1 [vol/vol]) for 15 s, washed in water and mounted in 50% glycerol [vol/vol], examined under a light microscope. At the same time, the percentages of germinated conidia were calculated in 1 and 2 DAI under the light microscope.

**Results and Discussion:** At 24 h after inoculation, the percentage of germinated conidia was not significantly different on leaves of susceptible and resistant cultivars (Table 1). At 48 h after inoculation, the percentage of germinated conidia on susceptible and resistant leaves was significantly different, which implies that the cuticle is less compatible for conidia germination on 'Appalachian Spring'. Furthermore, the growth of germ tube was also significantly suppressed on 'Appalachian Spring' (Table 2). This type of inhibition of hyphal growth on resistant cultivars has been reported for powdery

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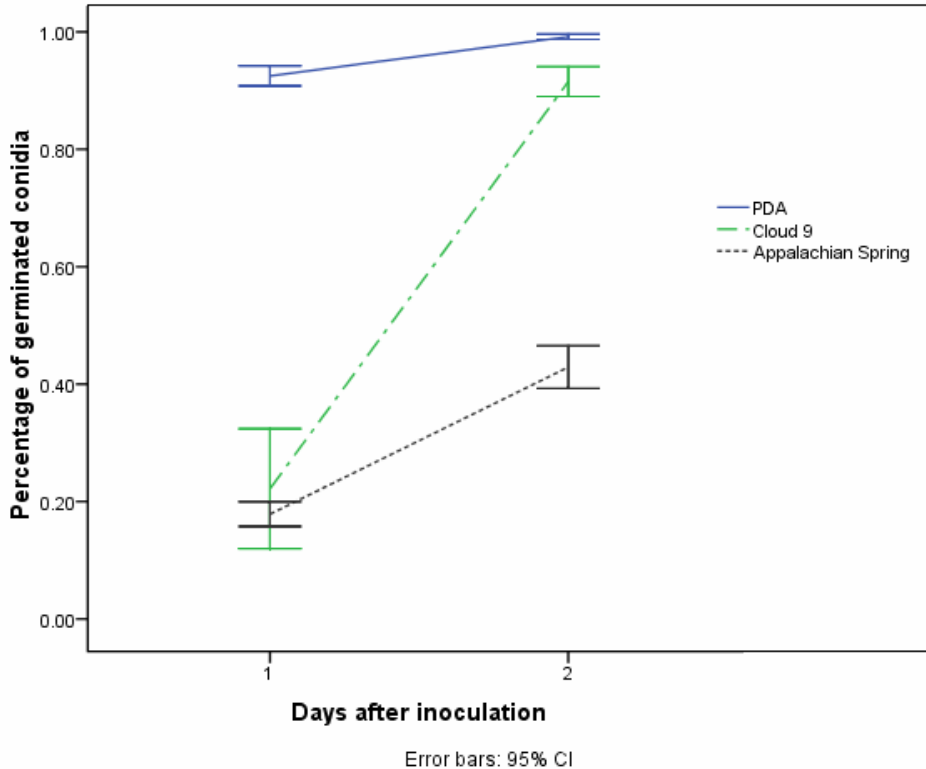
mildew of dogwood and sweet peas (6, 7). This strategy decreases inoculum potential of dogwood anthracnose. Further research will focus on how the host resistance histologically affects the infection events of *D. destructiva* on flowering dogwood.

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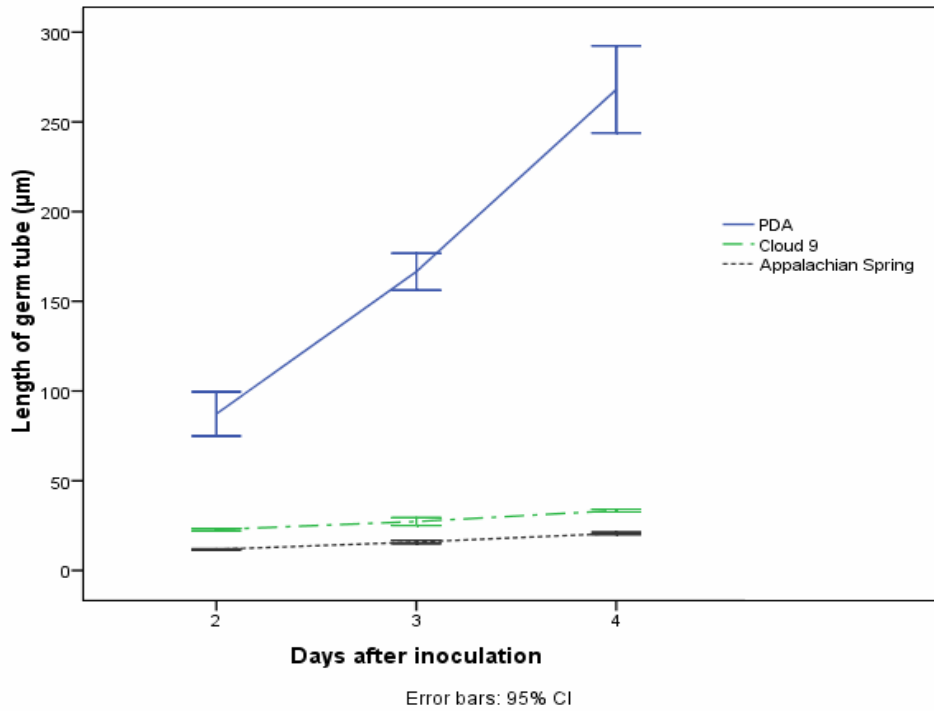
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**Table 1. Comparison of percentage of germinated conidia of dogwood anthracnose on PDA, susceptible and resistant cultivars.**



**Table 2. Comparison of germ tube length of dogwood anthracnose on PDA, susceptible and resistant cultivars.**



## Fungicidal Resistance of *Corynespora cassiicola* from African violet

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**Significance to Industry:** Fungicide applications are relied upon exclusively for control of *Corynespora cassiicola* on African violets in greenhouse production. Reduced efficacy of fungicides labeled for use on African violets has the potential to lead to increased grower costs. Identifying fungicidal resistance of *C. cassiicola* should help to determine the best selection of fungicides for disease control.

**Nature of Work:** African violets (*Saintpaulia ionantha*) are among the most popular indoor houseplants around the world. *Corynespora* leaf spot, caused by *C. cassiicola*, is a relatively new pathogen on African violets that is identified by rapidly expanding lesions on the leaf surface and petioles (5). The disease occurs in all stages of production and can result in thousands of plants being discarded daily (5). Because cultural management strategies are unknown, fungicide applications are used for control. Preliminary experiments found that Cleary 3336 (thiophanate-methyl) and Daconil (chlorothalonil) were successful in controlling *C. cassiicola* on African violet but chlorothalonil is not used due to phytotoxicity issues (7). Since Cleary's 3336 was recommended for control, fungicide application rates have had to be increased for continued suppression of the pathogen populations. Continuous use of benzimidazole fungicides may lead to a reduction in pathogen tolerance and compromise efficacy and lead to management difficulties and/or increased economic losses (8). The objective of this experiment is to quantitatively determine the in-vitro resistance of greenhouse collected *C. cassiicola* isolates to four different classes of fungicides.

**Materials and Methods:** Leaves of African violets symptomatic for *Corynespora* leaf spot were collected during a disease outbreak in September 2007 at a large African violet production facility. Symptomatic leaves were individually placed into sterile plastic bags. Excised lesions were placed in an aqueous solution of 10% sodium hypochlorite and 5% ethanol for 30 seconds and then rinsed in sterile water for 10 seconds (2). Leaf samples were then placed in Petri dishes containing 25 ml of potato dextrose agar (PDA) amended with chlortetracycline hydrochloride and streptomycin sulfate (2). The plates were placed in an incubator at 28 ±1°C for 48 hours that has alternating 12 hour light and dark cycles as described by Silva et al. (6). After incubating for 48 hours, 5-mm-diameter plugs of mycelium growing from the leaf tissues were obtained using a sterile cork borer and transferred to Petri dishes containing PDA. Cultures were incubated as previously described until cultures had grown sufficiently to be identified using colony and conidial morphology (6).

PDA (potato dextrose agar) was amended with technical grade thiophanate-methyl, iprodione, azoxystrobin or fludioxonil so that the a.i. of each fungicide was 0, 0.01, 0.1, 1, 10, or 100 µg a.i. / ml. For the strobilurin treatments, salicylhydroxamic acid (SHAM)

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was diluted in methanol and acetone (1/1 [vol/vol]) and added at a concentration of 100µg/ml to each fungicide concentration to inhibit alternative respiration as described by Avila-Adame et al. (1). Strobilurin treatments were screened in the presence and absence of SHAM. Mycelial plugs of each isolate were obtained using a 5-mm-diameter cork borer from the margins of actively growing, five-day-old cultures and placed upside down on the agar surface of media amended with a fungicide treatment or agar without fungicide (control) (4,6). Each fungicide concentration/ isolate combination was replicated three times. Cultures were incubated for 11 days at 28 ±1°C in complete darkness (6). Mean colony diameter of each of the 51 isolates was determined by taking two measurements at right angles with the original mycelial plug subtracted from each. The experiment was repeated once.

**Results and Discussion:** Most isolates of *C. cassiicola* isolates were sensitive at rates greater than 0.01µg/ml of thiophanate-methyl (Fig.1). Isolate designated Or.4-2 displayed the least sensitivity to the fungicide at 0.01µg/ml. All isolates showed reduced growth to iprodione (Fig.2) as concentration increased but all isolates in both trials still grew at a mean of 12% at the 100µg/ml concentration. Isolate Black 3-1 possessed the greatest tolerance to iprodione by growing at 140% of the control at 0.1µg/ml. All isolates had a reduced sensitivity to all concentrations of fludioxonil (Fig.3) and were able to maintain means of 16% and 9% of control at 100µg/ml for trials one and two respectively. Isolate Or. 18-1 had the least amount of sensitivity to fludioxonil and was able to maintain growth of 63% of the control at 100µg/ml. All isolates of *C. cassiicola* were tolerant to increased concentrations of azoxystrobin (Fig.4) and showed 40% and 37% mean growth for trials one and two respectively at 100µg/ml without the addition of salicylhydroxamic acid. Isolate 17B-3 possessed the greatest tolerance of any other isolate to azoxystrobin by growing at 69% of the un-amended control at the 100µg/ml concentration. All isolates had a reduced tolerance to the azoxystrobin plus salicylhydroxamic acid (Fig.5) treatments. The mean growth at 100µg/ml for trials one and two were 40% and 38%, respectively. Isolate Or. 4-3 grew with the least sensitivity to azoxystrobin (+SHAM) by growing at 117% of the un-amended control at 10µg/ml.

The overall amount of growth resulting at the 100µg/ml concentrations within the iprodione, fludioxonil, and azoxystrobin treatments demonstrates that these isolates of *C. cassiicola* are less sensitive to those fungicides than they are to thiophanate-methyl. Although these isolates have a high sensitivity to thiophanate-methyl, it's important to monitor shifts in sensitivity. Changes in sensitivity could occur due to an increased level of selection pressure on pathogen populations exposed to thiophanate-methyl applications. Reduced efficacy to azoxystrobin and thiophanate-methyl is very important to commercial greenhouse and nursery producers because their site-specific activity can be quickly overcome by small genetic mutations within the pathogen and result in fungicide resistant pathogen populations. Due to the insufficient efficacy of azoxystrobin to these fungal isolates, it's not recommended for control of *C. cassiicola*.

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Figure 1. Average relative growth (expressed as % growth of control plates) of all isolates and largest isolate of *C. cassiicola* on agar amended with thiophanate-methyl.

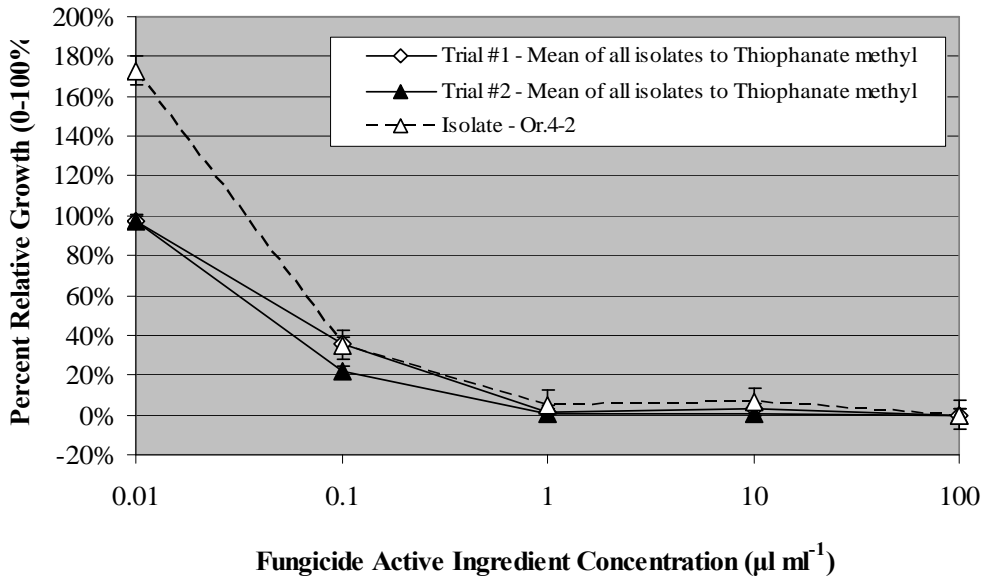


Figure 2. Average relative growth (expressed as % growth of control plates) of all isolates and largest isolate of *C. cassiicola* on agar amended with iprodione.

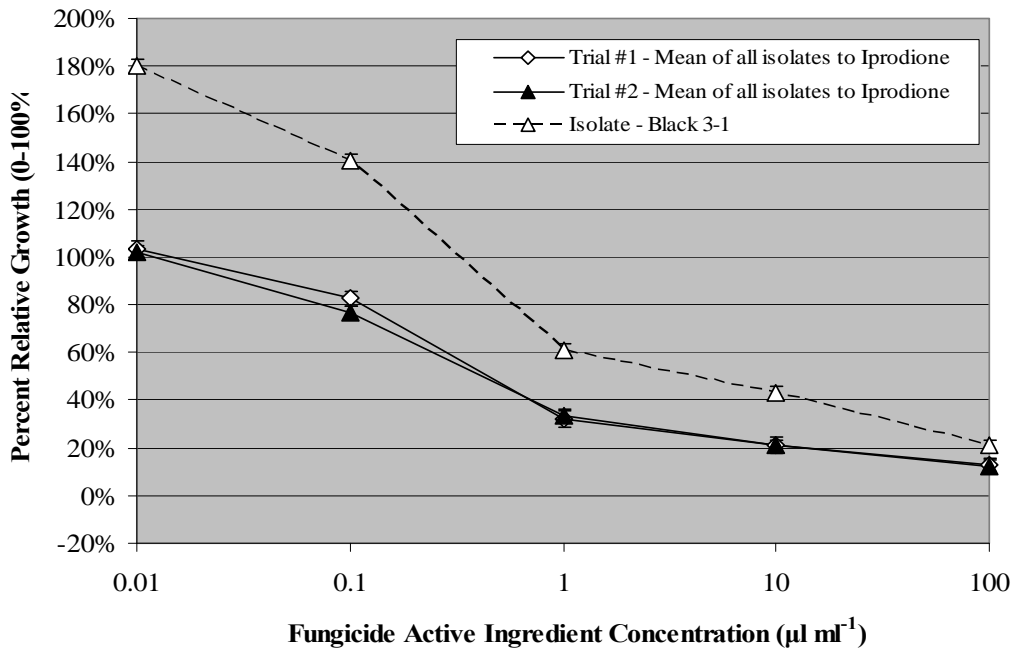


Figure 3. Average relative growth (expressed as % growth of control plates) of all isolates and largest isolate of *C. cassiicola* on agar amended with fludioxonil.

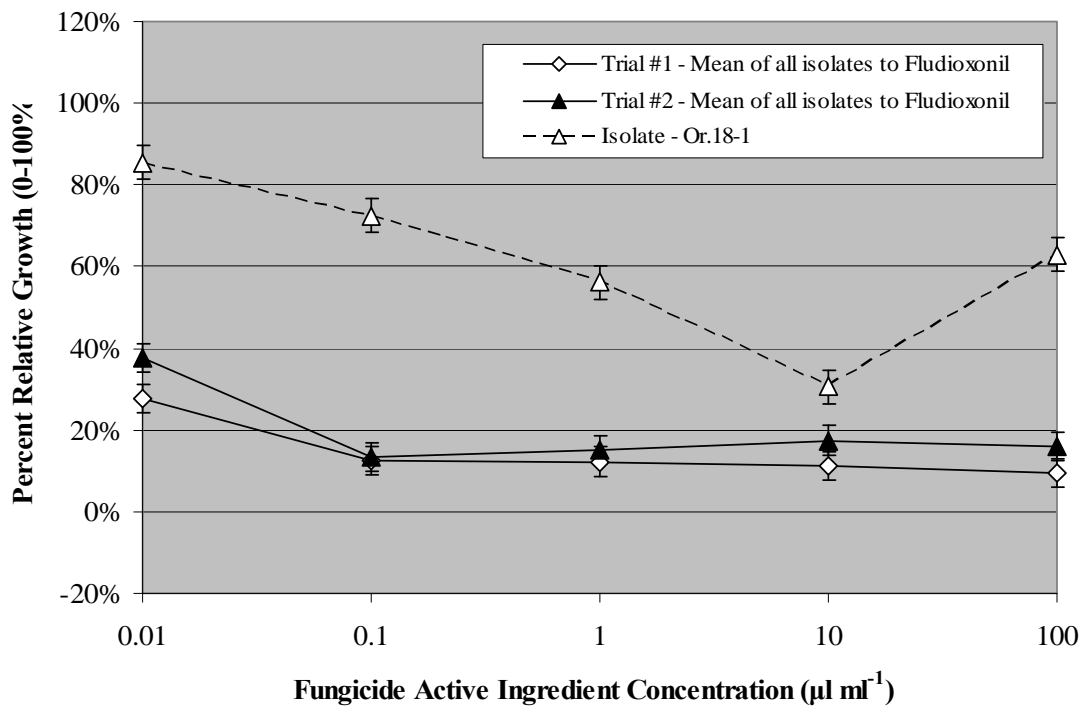


Figure 4. Average relative growth (expressed as % growth of control plates) of all isolates and largest isolate of *C. cassiicola* on agar amended with azoxystrobin.

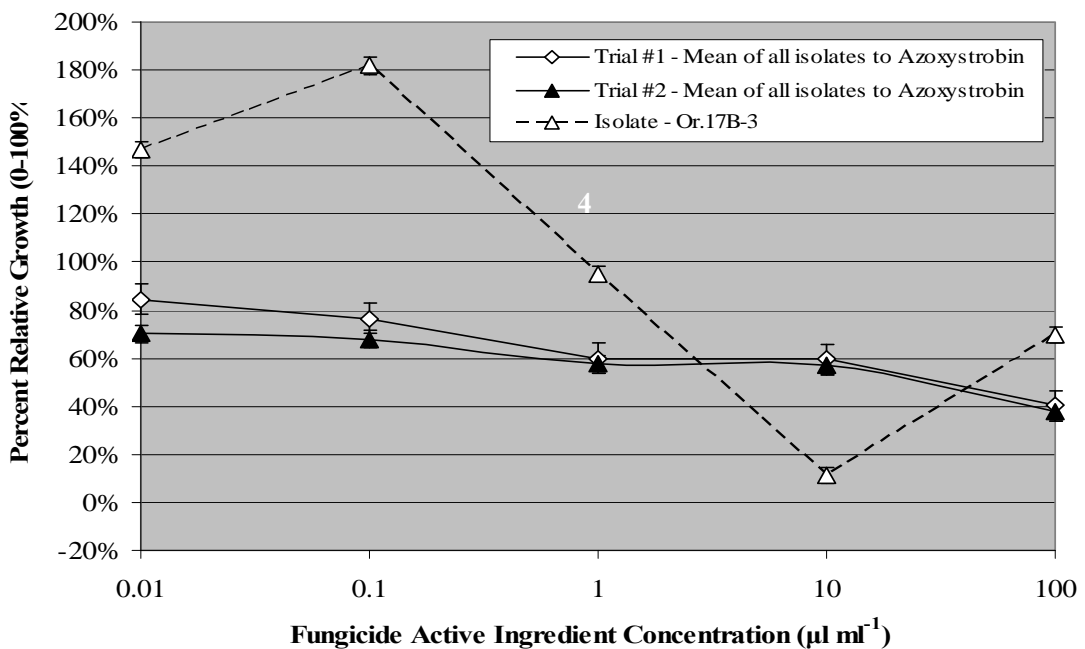
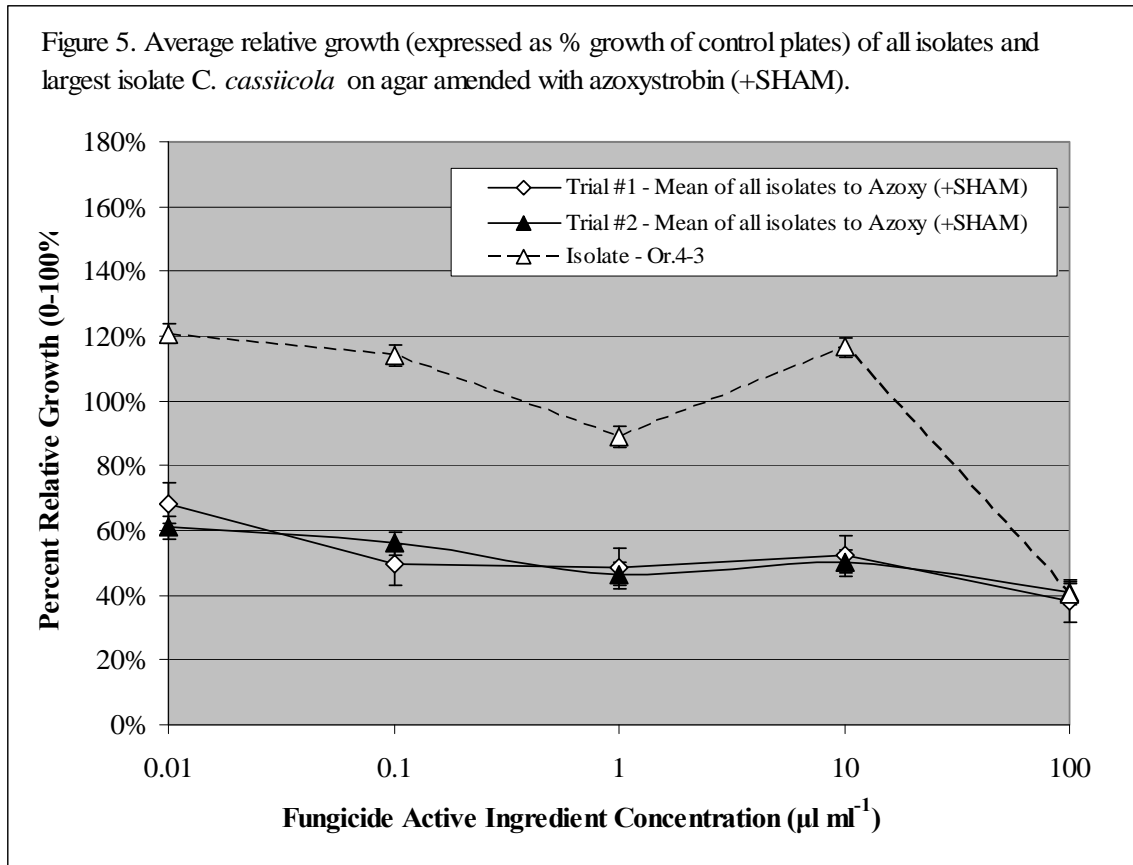


Figure 5. Average relative growth (expressed as % growth of control plates) of all isolates and largest isolate *C. cassiicola* on agar amended with azoxystrobin (+SHAM).



## Chemical and Hot Water Treatments to Control *Rhizoctonia* on Infested Azalea Stem Cuttings

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**Index Words:** Disinfectant, disinfestant, fungicide, propagation, sanitation, thermotherapy

**Significance to Industry:** Azalea web blight occurs annually on some azalea cultivars. We have discovered that spring shoot growth used for propagation of stem cuttings can harbor the pathogen, thus the pathogen is unsuspectingly propagated with the plant. The goal of this study was to evaluate and select disease control methods that eliminate the pathogen from cuttings without damaging plant tissue.

**Nature of Work:** Azalea web blight is caused by binucleate *Rhizoctonia* species, primarily by anastomosis group (=AG) P (previously AG U) (5). In the process of collecting samples from the field (5), it was noticed that azalea plants with web blight symptoms were randomly distributed within each block of a cultivar, and regularly distributed across many cultivars within a nursery, even less susceptible cultivars. Similar distribution patterns were reported by Frisina and Benson (3). The mechanisms by which *Rhizoctonia* spp. are spread in agricultural field and row crops is generally known; however, that knowledge does not fully explain the wide dispersion of *Rhizoctonia* among azalea cultivars. We discovered that 5 to 20% of new shoot growth in the upper canopy of the plant can be colonized by *Rhizoctonia* during the spring when new growth is harvested for propagation (Copes, *personal communication*). This information helps explain the distribution pattern of *Rhizoctonia* in azaleas grown in ornamental plant nurseries.

Best management practices for propagation of pathogen-free plants includes the use of clean rooting media, collecting cuttings from disease-free plants, and use of sanitized facilities. If pathogen-free stock plants cannot be guaranteed, the generally accepted practice is to treat stock plants with fungicides and/or dip cuttings in a disinfestant or fungicide solution (2,6). Generally, fungicides have been more effective than disinfestants for treating ornamental plant propagation material (1,4). However, a number of treatment methods, including use of disinfestants, fungicides, heat and UV radiation, have been employed for disinfesting various plant materials, such as fruits, ornamental plants (bulbs, corms, and stem cuttings), seeds, and vegetables.



The objective of this work was to evaluate efficacy of disinfesting methods (commercially available disinfestants, fungicides, and hot water) for eliminating *Rhizoctonia* from azalea stems. The first step was to find which method(s) caused significant reduction in the recovery of *Rhizoctonia* AG P from azalea stems. The second step was to evaluate a broader range of factor levels to see if efficacy could be improved. To achieve these objectives, a series of small experiments were performed and each experiment was repeated. Data analysis included categorical, probit, and regression methods as appropriate.

Pathogen control was assessed with stems that had been inoculated with an isolate of binucleate *Rhizoctonia* AG P. Each control method had a little different application criteria. For disinfestants, inoculated stems were fully submersed in the solution for 10 minutes. For fungicides, stems were submersed in the solution for several seconds, but allowed to air dry for 2 hours. For hot water, stems were submersed for the specified time period (30 seconds to 45 minutes). Treated stems were placed on water agar to determine a percentage of recovery or absence of the pathogen.

Potential plant damage from hot water was assessed with stems that had green leaves. After submersion in hot water for the specified time period, stems were placed in a humid chamber for 24 hours to allow visible expression of leaf tissue damage. Overall damage was calculated from the number of leaves expressing no, moderate, and severe leaf damage.

**Results and Discussion:** Several rates of disinfestants (sodium hypochlorite, hydrogen dioxide, quaternary ammonium chloride) and fungicides (chlorothalonil plus thiophanate methyl, and flutolanil) did not eliminate *Rhizoctonia* from stem cuttings. These results were disappointing, but demonstrate the importance of experimental evaluations. Recovery of *Rhizoctonia* was eliminated by submersing stems in 122°F (50°C) water for 21 minutes and in 131°F (55°C) water for 5 minutes, but was not reduced by submersing stems in 113°F (45°C) water for up to 45 minutes. Minor leaf damage resulted from submersion of stems in 131°C water for 5 minutes and in 122°F water for up to 40 minutes. The level of plant damage was judged to be low enough that rooting would not be negatively affected; however, this assumption will be critically tested during spring 2009, at the time when most nurseries would perform this task. The margin of error in time between killing the pathogen and severely damaging plant tissue is narrower at 131°C than at 122°F (Figure 1). Severe leaf damage occurred when stems were submerged in 131°C water for greater than 7 minutes.

Although hot water submersion is the only treatment to date that has effectively eliminated *Rhizoctonia* from azalea stems, further studies with fungicides are planned. Based on results from bench top studies, the application of fungicides to plants prior to collecting stem cuttings showed some potential for eliminating *Rhizoctonia* from azalea stems. Several fungicide timing patterns will be evaluated in field trials for the purpose of preventing growth of the fungus up onto the current year's shoot growth. Additional laboratory studies are planned to determine if surfactants and/or application methods can improve chemical efficacy.

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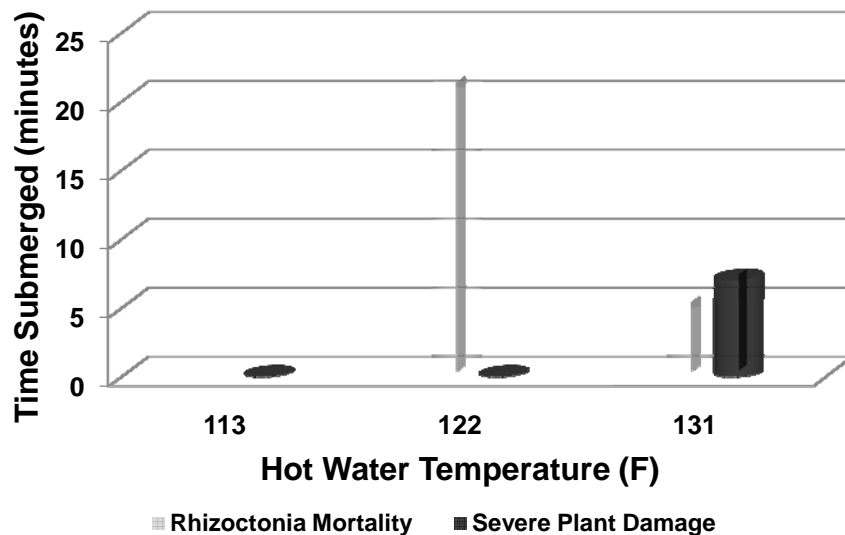


Figure 1. Minutes required to eliminate *Rhizoctonia* from azalea stems and to cause severe leaf damage. A zero value means the pathogen was not eliminated or the leaves were not damaged. Thus at 122°F, severe leaf damage never occurred within the maximum time tested (40 minutes).

## Seasonal Presence Of *Rhizoctonia* Species In Container-Grown Azalea

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**Index Words:** Survival, persistence

**Significance to Industry:** To develop new control strategies against azalea web blight, it is important to understand seasonal changes in the population of *Rhizoctonia* fungi, including at times of the year when disease symptoms are not present. The goal of monitoring pathogen populations in symptomless plants is to identify times for implementing management strategies.

**Nature of Work:** *Rhizoctonia* web blight is caused by binucleate *Rhizoctonia* that belong to several anastomosis groups (=AG), with the primary pathogen belonging to AG P (previously classified as AG U) (3). Web blight symptoms develop annually from early-July to early-September on container-grown azaleas (*Rhododendron* spp.) in the southern and eastern United States (1,2).

In nurseries, we have observed that *Rhizoctonia* web blight is randomly distributed in blocks of azalea cultivars of varying susceptibility yet regularly distributed across a nursery. It is not known how the pathogen becomes so widely dispersed and whether the pathogen persists year-to-year on individual plants in containers. The main objective of this project was to determine seasonal recovery levels of binucleate *Rhizoctonia* species in individual container-grown azaleas. A second objective was to evaluate the potential for new control strategies based on changes in population levels of the fungus.

In 2005 and 2006, 'Gumpo' azalea plants with a incidence of 35 to 60% diseased leaves per plant were collected from nurseries and maintained under overhead irrigation. Completely blighted leaves were collected during late summer and 30 leaves enclosed in a nylon mesh bag and placed on the bark medium surface in each pot. Disease symptoms disappeared during the fall with the growth of new leaves and no new development of symptoms, thus plants appeared healthy from October to July. Live stems, dead leaves, and pine bark substrate with roots were sampled in December, February, and May. At each sample period, 10 plants were destructively divided into eight horizontal zones [3 zones of branches (0 to ¾ inch, 1 ½ to 2 ¼ inch, and 3 ½ to 6 inch from the pine bark), 1 zone of detritus (dead leaves), 4 zones of pine bark (top 1 ¼ inch layer, middle 2 ¾ inch layer, 1 ¼ inch diameter central core from the middle layer, and 1 ¼ inch bottom layer)]. From a single plant, 15, 30, and 84 samples of asymptomatic live plant stems, blighted leaves, and bark media nuggets, respectively, were plated on an agar medium to assess the frequency of seasonal recovery of *Rhizoctonia* species. Four additional sample periods were done in June and July, at

which time only plant stems were sampled following the procedures previously described. A total of 3600 isolates were recovered over 2 years.

**Results and Discussion:** Recovery frequency was high from the blighted leaves (66 to 98%) at all sample dates. *Rhizoctonia* species were recovered more frequently from the top 1 ¼ inch of bark media (17 to 55%) and the lowest ¾ inch of plant trunk (68 to 100%) than from bark media and stem zones at a greater distance from the dead leaf layer. Recovery levels were lowest in May from the middle and lower zones of bark media and in June from stems in the upper-most canopy. Despite the population decline during early summer, the fungus was recovered from 5 to 20% of the current year's stems in early June, the time when stems are commonly collected for propagation at many commercial nurseries. Thus, the pathogen can be unsuspectingly carried into the propagation house on cuttings.

Identification of the anastomosis group of *Rhizoctonia* was conducted for 171 isolates by sequencing the internal transcribed spacer region (ITS) of the nuclear encoded ribosomal DNA and by pairing isolates with tester isolates to determine anastomosis reactions. Most isolates (95%) were binucleate species and represented six anastomosis groups (A, G, K, P, R, S). The predominate *Rhizoctonia* group recovered was AG P, which is the primary group associated with web blight (3).

Our research suggests that once individual container-grown azalea plants become colonized by binucleate *Rhizoctonia* AG P that the fungus persists on bark substrate, on plant surfaces, and in live tissue from year-to-year in the container. Once plants become infested, fungicides are the most practical control approach. An alternative strategy would be to investigate the potential of producing cuttings not colonized with the fungus and maintaining disease-free plants throughout the production process. This type of approach would depend on the use of effective sanitation and handling methods throughout the entire production cycle. An important step in achieving this strategy would be developing methods to eliminate *Rhizoctonia* from the cuttings (see article by Copes and Blythe in this proceedings issue). Additional research is planned to evaluate control during propagation and the level of benefit associated with sanitation methods at different plant handling activities.

The type of exploratory research being presented here is of practical significance, because it provides important information that will lead to the development of economical and effective disease control technologies. Development of a comprehensive sanitation program potentially could benefit control of a number of unrelated diseases. Of all the agricultural plant production systems, ornamental plant production is the most complex. Ornamental plant systems are unique with frequent irrigation, radiant absorptive black surfaces, organic media, and high human activity. In-depth, long-term research is needed to properly develop cost-effective cultural, sanitation, and chemical controls tailored for the complexity of ornamental plant production systems.

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## Unknown Leaf Spotting and Defoliation of Knock Out Roses

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**Index Words:** Shrub rose, Pink Knock Out, Blushing Knock Out, Rainbow Knock Out, Double Knock Out, Knock Out, Bayer Disease Control, Daconil Ultrex, Immunox, Compass, tebuconazole, chlorothalonil, myclobutanil, trifloxystrobin.

**Significance to Industry:** Abundant brightly colored blooms along with a high level of disease resistance have made Knock Out brand roses the most widely planted roses in residential, leisure, and commercial landscapes across the South. In 2008, a distinctive yellowing or chlorosis of the leaves in the inner canopy of several Knock Out cultivars in a simulated landscape planting in Brewton, AL. Numerous small circular to irregular green spots with an uneven or feathery edge appeared on the yellowed leaves, which eventually were shed. Leaf yellowing and premature defoliation appeared to progress from lower stems near the base of the plant upward through the plant canopy towards the shoot tips. This disorder intensified from the July through September rating dates. While Knock Out and Blushing Knock Out roses suffered the heaviest premature defoliation, some canopy thinning was also seen on Pink Knock Out and Double Knock Out. Little if any leaf yellowing and no premature defoliation was found on Rainbow Knock Out. Symptoms identical to those described above have also been noted in landscape plantings of the Knock Out rose in Auburn, AL. Occurrence of the above disorder is not influenced by fungicide treatment. Of the five cultivars, Rainbow Knock Out proved to be most susceptible to *Cercospora* leaf spot. In contrast, little if any leaf spotting attributed to this disease was noted on the other four cultivars.

**Nature of Work:** The original Knock Out rose is a landscape phenomenon across the South. In addition to a cascade of bright red blooms, this cultivar has proven highly resistant to black spot and *Cercospora* leaf spot in Alabama (1). The Knock Out rose is one of the few no-(fungicide) spray rose for southern landscapes. Pink Knock Out, Blushing Knock Out, Rainbow Knock Out, Double Knock Out and the recently released Yellow Knock Out rose reportedly share the desirable horticulture and disease resistance characteristics with the original Knock Out rose. In Tennessee (2), Pink Knock Out showed a high level of resistance to both black spot and *Cercospora* leaf spot. In a previous study, Hagan *et al.* (1) noted that leaf canopy on Knock Out was not as dense as other disease resistant shrub roses. Since neither black spot nor *Cercospora* leaf spot was observed, the premature leaf shed and sparse canopy was attributed to chlorothalonil phytotoxicity or heat stress (1). We initiated this study to further study the disease resistance of the Knock Out series roses as well as assess their sensitivity to retail fungicides such as Immunox and Bayer Disease Control along with the commercial fungicides Daconil Ultrex and Compass. All of the above fungicides have activity against black spot, *Cercospora*-incited leaf spot diseases or both.

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On 14 Feb 2008, bare-root roses were transplanted into raised beds in a Benndale (A) sandy loam soil on 8 ft centers with 13 ft between rows. Prior to planting 600 lb/A of 5-10-15 analysis fertilizer plus 40 lb/A of minor elements were applied to the beds and incorporated. After a drip irrigation system was installed on 19 Feb, the beds were mulched on 1 Apr with aged pine bark. An application of 0.4 oz/plant of ammonium nitrate on 7 Apr was followed by a 16 July applications of 1.1 oz/plant of 16-4-8 analysis fertilizer. Pre-emergent weed control was obtained with 28 Feb and 9 Sep applications of 2 qt/A of Surflan + 1.0 lb/A of Gallery. Sedgehammer at 1.3 oz/A was applied to the beds on 17 Jun to control yellow nutsedge. Escape weeds were pulled by hand. Fusilade at 2 qt/100 gal of spray volume was applied for grass control on 4 Jun. The study consisted of a split plot with four replications with rose selections as the main plot and the four fungicide treatments and non-treated control as the split-plot treatments. Fungicide treatments were applied at 4-wk intervals starting on 18 Apr until 1 Oct to drip with a CO<sub>2</sub>-pressurized sprayer. Cercospora leaf spot (CLS) incidence was visually rated using a modified 1 to 10 Florida peanut leaf spot rating scale where 1 = no disease, 2 = very few lesions in canopy, 3 = few lesions noticed in lower and upper canopy, 4 = some leaf spotting and  $\leq 10\%$  defoliation, 5 = lesions noticeable and  $\leq 25\%$  defoliation, 6 = lesions numerous and  $\leq 50\%$  defoliation, 7 = lesions very numerous and  $\leq 75\%$  defoliation, 8 = numerous lesions on few remaining leaves and  $\leq 90\%$  defoliation, 9 = very few remaining leaves covered with lesions and  $\leq 95\%$  defoliation, and 10 = plants defoliated on 19 Jun, 22 Jul, 21 Aug, 24 Sep, and 11 Nov. Significance of treatment effects was tested by analysis of variance and means were separated using Fisher's least significant difference (LSD) test (P=0.05).

**Results and Discussion:** Leaf yellowing and premature loss of those yellowed leaves was first noticed primarily on the Knock Out and Blushing Knock Out roses on July 7. Upon closer examination of the symptomatic plants, numerous small circular to irregular green spots or 'islands' with an uneven or feathery edge appeared on the yellowed leaves, which eventually were shed. While the symptoms superficially resembled those of black spot, no fungal mycelia or fruiting bodies were associated with the green 'islands'. Leaf yellowing and premature defoliation appeared to progress from lower stems near the base of the plant upward through the plant canopy towards the shoot tips. This disorder intensified between the first observations of the damage on July 7 through the September 24 rating date.

At all three rating dates, the highest level of premature defoliation was noted on Knock Out and Blushing Knock Out roses (Table 1). As indicated by ratings 4.0, Knock Out and Blushing Knock Out suffered nearly 30% premature defoliation by the September 24 rating date. Defoliation ratings for both cultivars were lower at the earlier than later rating dates. While less sensitive compared with the above roses, Pink Knock Out also had relatively high defoliation ratings on September 24. Defoliation levels on Double Knock Out were similar to Pink Knock Out at two of three rating dates. Defoliation attributed to this unknown disorder was not observed on Rainbow Knock Out.

At the August and November rating dates, defoliation ratings for the unknown disorder were similar for all fungicide treatments and the non fungicide-treated control (Table 2). On 24 September, the level of premature defoliation was higher for the non-treated control than the Bayer Disease Control- and Immunox-treated roses but similar to those for Daconil Ultrex and Compass 50W. On cultivars that suffered noticeable disorder-related damage, defoliation ratings were similar for all fungicide treatments and non-treated control on Pink Knock Out and Double Knock Out but not for Blushing Knock Out and Knock Out (data not shown). While the defoliation ratings for the Immunox-treated Pink Knock Out roses were lower compared with the non-treated controls and Compass 50W-treated roses, the Bayer Disease Control-treated plants had lower ratings than the Daconil Ultrex, Immunox, and non-treated controls.

The dominate disease observed, particularly at the latter two rating dates, was *Cercospora* leaf spot. A zonate leaf spot caused by a *Phomopsis* sp. was also noted. Black spot was not observed. When averaged across fungicide treatments, Rainbow Knock Out had the highest disease rating at two of the three rating dates (Table 3). At the September 24 and November 5 rating dates, disease ratings for Double Knock Out were significantly higher compared with Blushing Knock Out, Knock Out, and Pink Knock Out, which remained nearly disease-free. All fungicides treatments were equally effective at all rating dates in preventing the development of *Cercospora* leaf spot on Rainbow Knock Out (data not shown). When compared with the non-treated control, significant reductions in leaf spotting were also obtained on November 5 on Double Knock Out with Daconil Ultrex and Compass 50W.

With the notable exception of Rainbow Knock Out, Knock Out series roses have a high level of resistance if not immunity to *Cercospora* leaf spot. Black spot was not diagnosed on any Knock Out series rose. Results of this study confirm recent observations in Tennessee (2) that Pink Knock Out is highly resistant to both of the above diseases. Although Rainbow Knock Out did suffer from *Cercospora* leaf spot-related leaf spotting and a low level of premature defoliation, noticeable symptoms of this disease were not evident until mid-fall. For the remainder of the year, disease symptoms were unobtrusive. In contrast, the leaf yellowing and premature defoliation attributed to the unknown disorder, which appeared in early to mid-summer, was observed into early November. While Pink Knock Out and Knock Out were most sensitive to this disorder, some leaf yellow and defoliation was also seen on Blushing Knock Out and Double Knock Out. The Rainbow Knock Out rose was not affected by this disorder. In an adjacent shrub roses planting at the same location, symptoms similar to those described here were found on the several Knock Out series rose cultivars but not any of the other rose selections (data not shown) as well as in a landscape planting of non fungicide-treated Knock Out roses on the campus of Auburn University. In Tennessee, Mynes et al. (2) may have noted but not recognized this disorder in a simulated landscape planting of Pink Knock Out rose. While they reported little if any black spot or *Cercospora* leaf spot development on this cultivar, a low level of premature and possibly not disease-related defoliation was reported.

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In contrast to Hagan *et al.* (1), damaged attributed to this unknown disorder was not linked with phytotoxicity to the fungicide chlorothalonil. The characteristic leaf bronzing and marginal leaf burn associated with chlorothalonil burn was not observed any of the Knock Out series roses. With two exceptions, defoliation ratings for all fungicide treatments and non-treated control were similar on all five Knock Out series roses. An unknown leaf spot disease or a hypersensitive response to a known disease also may account for the premature leaf yellowing and defoliation. At one rating date, some reduction in defoliation on Blushing Knock Out and Knock Out roses was obtained with either Immunox or Bayer Disease Control, respectively. However, all of the fungicides screened included a broad range of activity against potential plant pathogenic fungi, particularly the causal fungi of black spot and *Cercospora* leaf spot. In addition, no sign of any plant pathogenic fungi or potential arthropod pest was associated with the yellowed, senescing leaves. In an adjacent planting of shrub roses at the same study location, symptoms similar to those described here were found on the Knock Out series roses but not any of the other shrub rose selections (data not shown) as well as in a landscape planting of non fungicide-treated Knock Out roses on the campus of Auburn University.

A lack of adaptability to the hot, humid summer weather patterns or micronutrient deficiency are other possible explanations for the leaf yellowing and premature defoliation noted on Knock Out series roses in this and previous years at this study location and other sites in Alabama. Additional observations as well as leaf mineral assays will be conducted in 2009 in an attempt of identify the cause of this unknown disorder in Knock Out series roses.

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Table 1. Defoliation ratings for unknown disorder on each Knock Out series rose cultivar at three rating dates in 2008.

Knock Out Rose	Rating Date		
	August 21	September 24	November 5
Pink Knock Out	1.9 b	3.3 b	1.9 b
Blushing Knock Out	3.1 a	4.0 a	2.8 a
Rainbow Knock Out	1.0 c	1.0 d	1.0 c
Double Knock Out	1.9 b	1.9 c	2.0 b
Knock Out	2.6 ab	4.0 a	2.6 a

Defoliation due to unknown disorder was visually estimated using a 1 to 11 scale where 1 = no defoliation, 2 = 1 to 10%, 3 = 11 to 20%, 4 = 21 to 30%, 5 = 31 to 40%, 6 = 41 to 50%, 7 = 51 to 60%, 8 = 61 to 70%, 9 = 71 to 80%, 10 = 81 to 90%, and 11= 91 to 100% premature defoliation.

Means in each column that are followed by the same letter are not significantly different according to Fisher's least significant difference (LSD) test (P=0.05).

Table 2. Defoliation ratings for unknown disorder when averaged across Knock Out cultivars and segregated by fungicide treatments at three rating dates.

Fungicide Treatments	Rate per 100 gal	Rating Date		
		August 21	September 24	November 5
Bravo Ultrex 1.4 lb	1.4 lb	2.2	3.0 ab	2.1
Bayer Disease Control	0.6 gal	1.9	2.4 b	2.0
Immunox	0.8 gal	2.2	2.5 b	2.0
Compass 50W	2 oz	2.1	2.9 ab	2.1
Non-treated Control	---	2.3	3.3 a	2.2

Defoliation due to unknown disorder was visually estimated using a 1 to 11 scale where 1 = no defoliation, 2 = 1 to 10%, 3 = 11 to 20%, 4 = 21 to 30%, 5 = 31 to 40%, 6 = 41 to 50%, 7 = 51 to 60%, 8 = 61 to 70%, 9 = 71 to 80%, 10 = 81 to 90%, and 11= 91 to 100% premature defoliation.

Means in each column that are followed by the same letter are not significantly different according to Fisher's least significant difference (LSD) test (P=0.05).

Table 3. Cercospora leaf spot ratings for the Knock Out series roses at three rating dates in 2008.

Variety	CLS Disease Rating		
	21 Aug	24 Sep	5 Nov
Pink Knock Out	1.1 b	1.1 b	1.1 c
Blushing Knock Out	1.1 b	1.0 b	1.1 c
Rainbow Knock Out	1.4 a	1.5 a	2.2 a
Double Knock Out	1.1 b	1.4 a	1.4 b
Knock Out	1.1 b	1.0 b	1.0 c

Cercospora leaf spot (CLS) was assessed using the 1 to 10 Florida peanut leaf spot rating scale.

Means in each column that are followed by the same letter are not significantly different according to Fisher's least significant difference (LSD) test (P=0.05).

## Reaction of Summer and Perennial Flowers to the Southern Root Knot Nematode *Meloidogyne incognita* race 3

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**Index Words:** root knot nematode resistance, bedding plants, amaranthus, begonia, celosia, coleus, cosmos, globe amaranth, gourd, lantana, marigold, melampodium, petunia, portulaca, salvia, scabosia, sunflower, tickweed, verbena, vinca, zinnia.

**Significance to Industry:** Damaging populations of the southern root knot nematode are a relatively common pest of summer annual and perennial flowers across the South. Since chemical treatments are no longer a control option, establishing annuals and perennials that are resistant to this nematode offers the most effective and least costly method of control. In this study, selections of amaranthus (*A. caudatus* cv. Candelabra), cosmos, globe amaranth (*G. globosa* cv. All Around Purple), lantana, marigold, melampodium, salvia (cv. Victoria), scabosia, sunflower (cv. Italian White and Angel's Halo), tickweed, vinca, and zinnia were shown to have a high level of resistance and in some cases immunity to the southern root knot nematode (*Meloidogyne incognita* race 3). Amaranthus (*A. hypochondriacus* cv. Pygmy Torch), begonia, celosia, coleus, globe amaranth (*G. haageana* cv. Strawberry Fields), gourd, petunia, portulaca, salvia (cv. Vista White) and sunflower (cv. Claret, Giganteus, and Chocolate Cherry) proved to be moderately to highly susceptible to attack by this nematode.

**Nature of Work:** Across the South, the southern root knot (*M. incognita*) and to a lesser extent the peanut root knot (*M. arenaria*) and Javanese root knot (*M. javanica*) nematodes are the most widespread and damaging plant parasitic nematode pests of annual and perennial flowers. Damaging populations of the above nematodes are found primarily on sites with well drained soils where root knot susceptible flowers or vegetables have been grown for an extended time period. Due to the once widespread production of cotton, race 3 of the southern root knot nematode may be among the most common of the four races of this nematode.

Nematode control options for residential, leisure, and commercial landscape plantings are limited. Registrations of effective pre-plant nematicide treatments such as methyl bromide and metam sodium (Vapam) have been cancelled. Techniques such as soil solarization are of limited effectiveness and are difficult to implement, particularly for beds planted year-round to annuals or perennial flowers. For landscape plantings, establishing root knot resistant or immune annuals and perennial flowers is the most effective strategy for avoiding damage on sites with established root knot nematode populations (1). However, limited information concerning the host status of many popular annual and perennial flowering to specific races of the southern root knot nematode, particularly race 3, is available. Previously, Walker *et al.* (4) identified

selections of ageratum, marigold (*T. erecta* and *T. patula*), and salvia (*S. splendens*) resistant to the above nematode. In contrast, the roots of most begonia, all celosia (*C. plumosa*) and pansy, along with a dianthus (*D. chinensis*), verbena (hybrid), and vinca selection were heavily galled by race 3 of the southern root knot nematode (4). Resistance to race 3 of the southern root knot nematode has also been demonstrated in several lantana selections (5). The objective of this study was to assess the susceptibility of summer annuals and perennial flowers to race 3 of the southern root knot nematode in a simulated landscape planting.

The site selected for this study had previously been cropped to summer vegetables and has a resident root-knot nematode population. A greenhouse host range study established that the southern (cotton) root knot nematode (*M. incognita* race 3) was present. In late April 2006 and 2007, the Benndale sandy loam soil (<1% organic matter) at the Brewton Agricultural Research Unit was worked to a raised bed. While the beds were mulched with aged pine bark in 2006, black plastic mulch was installed over the beds in 2007. Except when plant numbers were limited, four of each of test flowers or vegetable standards was planted in a 1-ft square on 9 May 2006 and 22 May 2007. Drip irrigation tape was installed immediately and the plants were watered as needed. Calcium nitrate or potassium nitrate was injected into the irrigation stream at approximately 2-week intervals from 22 May to 27 July 2006 and 29 May to 9 July 2007. Weeds were controlled with a hoe or were hand pulled. The experimental design was a randomized complete block with four replications. Soil samples for a nematode assay were collected from each plot on 28 July 2006 and 15 August 2007, and then processed using the sugar-water flotation method. On 7 August 2006 and 15 August 2007, one plant from each replicate was collected and the level of nematode damage was assessed using a 1 to 6 root knot gall rating scale. Due to exposure of soil samples during storage to high ambient air temperatures, juvenile counts for the 2007 study are not reported. Significance of treatment effects was tested by analysis of variance and Fisher's protected least significant difference (LSD) test ( $P=0.05$ ).

**Results and Discussion:** High summer temperatures in 2006, which contributed to the low vigor and poor survival of begonia, coleus, and annual salvia, may be responsible for the discrepancy between low root knot juvenile nematode counts for the above summer annuals and their high root gall ratings. Highest root-knot juvenile counts were recorded for portulaca, celosia, and pepper cv. Sweet Pepper, which was included as the susceptible standard. Juvenile counts for the remaining annuals and perennials did not significantly differ. Rather than the heavy galling seen on most susceptible host plants, the root system of the celosia cv. Fresh Look Mix disintegrated. Highest root gall ratings were recorded for the coleus, petunia, and verbena selections. Given their high juvenile counts, gall ratings for portulaca and pepper were lower than anticipated. No root galling was noted on globe amaranth, lantana, tickweed (*C. tinctoria*), vinca, zinnia, or either marigold selection.

In 2007, equally high root gall ratings were recorded for coleus, three celosia selections, cucumber, globe amaranth cv. Strawberry Fields, and both gourd selections. Amaranth

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cv. Pigmy Torch, begonia, bell pepper, petunia cv. Pink Wave, portulaca, purpletop verbena, and sunflower cv. Claret, Giganteus, and Chocolate Cherry also suffered considerable root galling. The second amaranth selection, cv. Clandelabra (*A. caudatus*) had a significantly lower gall rating compared with cv. Pigmy Torch (*A. hypochondriacus*). While the globe amaranth cv. Strawberry Fields (*G. haageana*) suffered moderate to heavy root galling, the root system of the other globe amaranth All Around Purple (*G. globosa*) were gall-free. The level of root galling on both of the petunia cultivars as well as purpletop verbena and verbena cv. Quartz Scarlet was similar. As noted above, moderate to high gall ratings were recorded several sunflower cultivars. In contrast, no root galling was noted on the sunflower cv. Italian White and Angel's Halo. Other largely gall-free summer annuals included the selections of globe amaranth, American and French Dwarf marigold, melampodium, salvia, tickweed, vinca, and zinnia. The gall rating for cosmos and scabosia did not significantly from those of the above gall-free summer annuals.

As expected, the host status of summer annual and perennial flowers to the southern root knot nematode (*M. incognita* race 3) significantly differed. Study results confirm observations by Walker et al. (4) that the American and French Dwarf marigold selections have a high level of resistance if not outright immunity to this race of the southern root knot nematode. Previously, no galling or reproduction of the southern root knot (*M. incognita* race 1), peanut root knot (*M. arenaria* race 1) or Javanese root knot (*M. javanica*) nematodes was noted on American (*T. erecta*) and French Dwarf marigolds (*T. patula*) (2,3). As reported by Goff (2), cosmos showed a high level of root knot resistance. In contrast to observations by Walker et al. (4), the absence of root galling in both years shows that annual vinca (*Cathranthus roseus*) probably is a very poor host for race 3 of the southern root knot nematode (*M. incognita* race 3). While Goff (2) observed that vinca (*C. roseus*) was highly resistant to an unnamed root-knot species, light to moderate galling attributed to the peanut root knot nematode was noted on the roots of vinca cv. Bright Eyes by McSorley and Frederick (3). In 2006, no root galling along with low juvenile counts confirms the report by Williams-Woodard and Davis (5) of the poor root knot host status of lantana. Over the study period, tickweed and the hybrid Profusion series zinnia selections did not suffer any southern root knot-related root galling. In addition, neither of the *Z. augustifolia* selections were damaged by the southern root knot nematode (*M. incognita* race 3) in 2007. McSorley and Frederick (3) also showed that zinnia (*Z. elegans*) was a poor host for the southern root knot nematode (*M. incognita* race 1). Goff (2) lists zinnia (*Z. elegans*) and *Coreopsis* sp. as having a high level of root knot resistance. The high level of root knot resistance that was noted for melampodium cv. Melanie in 2007 had not been previously reported.

While Goff (2) reported that salvia [sage] (*Salvia splendens*) has a high level of root knot resistance, differences in the host status as indicated by the level of root galling of salvia selections were noted in this and a previous study (3). Here, salvia cv. Vista White suffered moderate root galling in 2006, while cv. Victoria in 2007 remained gall-free. In contrast to a previous report (2), significant differences in the reaction of globe amaranth, amaranthus, and sunflower selections to the southern root knot nematode

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(*M. incognita* race 3) were seen. As previously reported (2) the globe amaranth selections (*G. globosa*) suffered no root galling in either year in contrast to Strawberry Fields (*G. haageana*), which was heavily galled in 2007. While amaranthus as reflected in the high gall rating for Pygmy Torch (*A. hypochondriacus*) was listed by Goff (2) as being susceptible to root knot, Candelabra (*A. caudatus*) proved to be moderately resistant to the southern root knot nematode (*M. incognita* race 3). While three sunflower selections as expected (2) suffered moderate to heavy root galling, sunflower cv. Italian White and Angel's Halo displayed a high level of root knot resistance.

Based on the moderate level of root galling seen in both years, portulaca may be more susceptible to root knot than indicated by Goff (2). Study results agree with Walker et al. (4) that begonia, verbena (hybrid) and celosia selections are susceptible to the southern root knot nematode (*M. incognita* race 3). In addition to the southern root knot nematode (*M. incognita* race 3), celosia is an excellent host for southern root knot nematode (*M. incognita* race 1), as well as the peanut root knot (*M. arenaria* race 1) and Javanese root knot nematodes, while coleus and verbena (hybrid) were moderately to heavily galled by the two latter root knot species (3). Coleus (2,3) and gourd (2) have previously been listed at highly susceptible to several root knot nematode species.

Overall, the American and French Dwarf marigold selections along with annual vinca, lantana, tickweed, melampodium, and several zinnia selections proved to be highly resistant if not immune to attack by the southern root knot nematode (*M. incognita* race 3). Other annuals and perennials that showed little or no root galling included cosmos, salvia cv. Victoria, globe amaranth All Around Purple (*Gomphrena globosa*) and sunflower cv. Italian White and Angel's Halo. In contrast, begonia, celosia, coleus, gourd, petunia, portulaca, verbena, as well as several selections of salvia and sunflower cultivars proved as susceptible to southern root knot nematode (*M. incognita* race 3) as the cucumber and pepper standards.

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Table 1. Reaction of selected summer annual and perennials to race 3 of the southern root knot nematode (*M. incognita*) in 2006.

Common Name	Cultivar	Root knot	
		No. juveniles <sup>z</sup>	Root rating <sup>y</sup>
Begonia ( <i>Begonia x semperflorins cultorum</i> )	Gin	35 <sup>x</sup>	5.5
Celosia ( <i>Celosia plumosa</i> )	Fresh Look Mix	950	---
Coleus ( <i>Solenostemon scutellarioides</i> )	Wizard Rose	6	6.0
Globe amaranth ( <i>Gomphrena globosa</i> )	Buddy Purple	115	1.0
Lantana ( <i>Lantana camara</i> )	Spreading Sunset	23	1.0
Marigold [American] ( <i>Tagetes erecta</i> )	Discovery Yellow	1	1.0
Marigold [French] ( <i>Tagetes patula</i> )	Safari Orange	5	1.0
Pepper ( <i>Capsicum annum</i> )	Sweet Banana	798	4.0
Petunia ( <i>Petunia x hybrida</i> )	Salmon Dreams	447	5.8
Portulaca ( <i>Portulaca grandiflora</i> )	Sundial	1157	3.8
Salvia ( <i>Salvia splendens</i> )	Vista White	237	3.5
Tickweed ( <i>Coreopsis tinctoria</i> )	--	8	1.1
Verbena ( <i>Verbena x hybrida</i> )	Quartz Scarlet	276	5.8
Vinca ( <i>Catharanthus roseus</i> )	Raspberry Cooler	14	1.0
Zinnia ( <i>Zinnia augustifolia x elegans</i> )	Profusion Cherry	7	1.0
LSD (P=0.05)		477	0.8

<sup>z</sup>Numbers of root-knot nematode juveniles (J2) per 100 cc soil.

<sup>y</sup>The level of root-knot galling on the roots was rated on a 1 to 6 galling index where 1 = no galling to 6 = severe root galling.

<sup>x</sup>Means separation was according to Fisher's protected least significant difference (LSD) test ( $P \leq 0.05$ ).



Table 2. Reaction of selected annual and perennial flowers to race 3 of the southern root knot nematode (*M. incognita*) in 2007.

Common Name	Cultivar	Root rating*
Amaranth ( <i>Amaranthus caudatus</i> )	Candelabra	1.7**
Amaranth ( <i>Amaranthus hypochondriacus</i> )	Pygmy Torch	4.3
Begonia ( <i>Begonia x semperflorins cultorum</i> )	Prelude Scarlet	4.3
Bell Pepper ( <i>Capsicum annum</i> )	Crimson Gold	4.3
Celosia ( <i>Celosia plumose</i> )	Fresh Look Gold	5.3
Celosia ( <i>Celosia plumose</i> )	Fresh Look Yellow	5.1
Celosia ( <i>Celosia plumose</i> )	Bombay Orange	5.0
Coleus ( <i>Solenostemon scutellarioides</i> )	Wizard Pastel	5.3
Cosmos ( <i>Cosmos sulfurous</i> )	Orange	1.3
Cucumber ( <i>Cucurbita pepo</i> )	Bush Slice	5.5
Globe Amaranth ( <i>G. globosa</i> )	All Around Purple	1.0
Globe Amaranth ( <i>G. haageana</i> )	Strawberry Fields	4.5
Gourd ( <i>Cucurbita pepo</i> )	Wings and Warts	5.8
Gourd ( <i>Cucurbita pepo</i> )	Small Fruited Mix	5.3
Marigold [American] ( <i>Tagetes erecta</i> )	Park's Whopper Yellow	1.0
Marigold [French] ( <i>Tagetes patula</i> )	Janie Flame	1.0
Melampodium ( <i>Melampodium paludosm</i> )	Melanie	1.0
Petunia ( <i>Petunia x hybrida</i> )	Pink Wave	4.0
Petunia ( <i>Petunia x hybrida</i> )	Pink Dreams	2.9
Portulaca ( <i>Portulaca grandiflora</i> )	Sundial Yellow	3.4
Purpletop Verbena ( <i>V. bonariensis</i> )	--	4.1
Salvia ( <i>Salvia splendens</i> )	Victoria	1.0
Scabosia ( <i>Scabosia atropurpurea</i> )	Grandmother Pincushion	1.7
Sunflower ( <i>Helianthus annus</i> )	Claret	4.0
Sunflower ( <i>Helianthus annus</i> )	Giganteus	3.8
Sunflower ( <i>Helianthus annus</i> )	Italian White	1.0
Sunflower ( <i>Helianthus annus</i> )	Angel's Halo	1.0
Sunflower ( <i>Helianthus annus</i> )	Chocolate Cherry	4.3
Tickweed ( <i>Coreopsis tinctoria</i> )	--	1.0
Verbena ( <i>Verbena x hybrida</i> )	Quartz Scarlet	2.8
Vinca ( <i>Catharanthus roseus</i> )	Jaio Dark Red	1.0
Vinca ( <i>Catharanthus roseus</i> )	Coconut Cooler	1.0
Zinnia ( <i>Zinnia augustifolia</i> )	Star Orange	1.0
Zinnia ( <i>Zinnia augustifolia</i> )	Crystal Orange	1.0
Zinnia ( <i>Zinnia augustifolia x elegans</i> )	Profusion Orange	1.0
LSD (P<0.05)		1.3

\*The level of root-knot galling on the roots was rated on a 1 to 6 galling index where 1 = no galling to 6 = severe root galling.

\*\*Means separation was according to Fisher's protected least significant difference (LSD) test ( $P \leq 0.05$ ).

## Rust Diseases in Ornamental Grasses

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**Index Words:** *Puccinia emaculata*, *Panicum virgatum*, *P. amarum*, resistance, symptom, pathogen

**Significance to Industry:** Native, perennial grasses are very popular ornamental plants in landscapes because they are normally resistant to pests and drought tolerant. In this paper, we reported the outbreaks of rust on ornamental grasses in landscapes and nurseries in Tennessee and North Carolina, and described disease symptoms, pathogen characteristics, host-pathogen interactions. These results provide us basic knowledge of the potential threat of rust disease in ornamental grasses and the possible impacts of potted grasses movement on the dynamics of the pathogen population structure and changes of host susceptibility.

**Nature of Work:** Switchgrasses (*Panicum virgatum* L.) and bitter grasses (*P. amarum* Elliot) are increasingly popular landscapes because they are drought tolerant, minimum fertilizer application, colorful forage and inflorescences, and resistant to pests. Occurrence of rust disease caused by *Puccinia emaculata* Schwein on forage switchgrasses has been reported in Iowa and Tennessee (1, 2). In the summer of 2008, a rust epidemic on these grasses was observed in landscapes and nurseries in Tennessee and North Carolina. In order to effectively manage the disease, characteristics of the pathogen and resistance of grasses to rust disease were investigated.

Diseased leaves of grasses were sampled and single-pustule isolates (SW-TN-03 from a landscape in Tennessee and SW-NC-05 from a nursery in North Carolina) were obtained and maintained on fresh switchgrass leaf segments by transferring urediniospores to new segments in 10 days intervals. Morphological characteristics of urediniospores and teliospores were observed using stereo- and light- microscopy with or without staining procedures. Experiments investigating urediniospore germination and appressorium formation were conducted on 1% agarose and healthy leaf surfaces, which were stained with trypan blue before examination using light microscopy. For the reaction of switchgrass and bitter grasses to rust infection, healthy leaf segments were inoculated with water suspension of urediniospores using a mini-sprayer to gauge the reaction of switchgrass and bitter grasses to rust challenges. Inoculated leaf segments were incubated on two layers of moist filter paper in petri dishes with a 16 h photoperiod. Symptoms and signs of rust disease were compared among grasses two weeks after inoculation.

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**Results and Discussion:** Leaf chlorosis was often the first disease symptom expressed and occurred when uredia were forming under epidermal cells. Powdery masses of urediniospores in pustules (uredia) on leaf surface were observed following the rupture of epidermal cells. Uredia were oval or fusiform and yellow to brown in color. Urediniospores were single-celled, globose or oval in shape about  $27 \times 25 \mu\text{m}$ . In late summer, dark-brown or black pustules (telia) were observed on diseased leaf surfaces. Teliospores had two cells and the cell wall of the top cell was thickened. On water agar substrate, urediniospores germinated 1 h after inoculation. Germ tubes elongated quickly from 2 to 3 h, branched several times, and differentiated appressoria. On leaf surfaces, appressoria formed over stomata and penetrated leaf surface through the pores of the stomata.

Variable susceptibilities and cultivar-isolate interactions were detected in switchgrasses and bitter panicgrass. 'Badland', 'Dewey blue', 'Hanse Herms', 'Huron Solstice', 'Rotstrahlbusch' and 'Shenandoah' were resistant to isolate SW-NC-05, but susceptible to SW-TN-03. In contrast, 'Alamo', 'Cloud Nine' and 'Dallas Blue' were resistant to SW-TN-03, but susceptible to SW-NC-05. Resistance reactions included brown specks with no or reduced sporulation. The results indicated that variation of pathogenicity exists in *P. emaculata* and susceptibility of grasses to rust disease could be variable in different geographic locations. After further investigation and standardization, cultivars with different responses to two rust isolates could be used for differentiating and identifying strains or races in *P. emaculata*.

Urediniospores are naturally disseminated in air currents (wind). Transportation of diseased potted grasses could be another important mean of causing pathogen population changes and could introduce new pathogenicity races into areas where agronomic switchgrass is grown.

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## 'No Spray' Rose Cultivars for the Mid South

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**Index Words:** disease resistance, black spot, *Cercospora* leaf spot, *Diplocarpon rosae*, *Cercospora rosicola*

**Significance to Industry:** Fifteen rose cultivars had acceptable levels of resistance to both black spot and *Cercospora* leaf spot. However, resistance claims in catalogs of companies selling roses could not be substantiated for many rose cultivars. Caution should be used in purchasing roses based on these claims.

**Nature of Work:** The popularity of roses as an ornamental plant is tarnished because of foliar diseases that often can not be controlled without preventive fungicidal sprays applied at weekly intervals. A large percentage of gardeners avoid roses due to an aversion of conforming to a spray routine or they do not want pesticides in their gardens or stored on their property. This aversion to pesticides contributes to the popularity of disease resistant roses, such as the cultivar 'Knock Out'. Rose companies have recognized the popularity of disease resistant roses and have used colorful terms to imply disease resistance. Some examples of terms used to describe "resistant" roses in wholesale catalogs are "resistant foliage", "remarkable disease resistance", "care free, trouble free", and "resists the dreaded fungus". Unfortunately, retail nurseries and their customers, desiring disease resistant roses; have been disappointed when disease resistant claims could not be substantiated at their locations.

In 2007, a no-spray rose trial was conducted at the West Tennessee Research and Education Center (WTREC) in Jackson, TN, the Plateau Research and Education Center (PREC) in Crossville, TN and the Thad Cochran Horticultural Research Laboratory (TCHRL) in Poplarville, MS. A total of 71 cultivars were evaluated in at least two of the three test locations. Most of these cultivars had been described as having resistance to disease in marketing descriptions of wholesale catalogs at least once in the last five years. The test included modern shrub roses and a number of *Rosa rugosa* hybrids. Hybrid tea, floribunda, grandiflora, polyantha, groundcover, and climbing rose cultivars were also represented in the evaluations.

The objectives of this study were to test the validity of marketing claims concerning disease resistance and to identify rose cultivars that exhibit superior levels of resistance to black spot and *Cercospora* leaf spot when inoculum levels of foliar pathogens are high and the environment is conducive for disease development.

Roses were transplanted in late spring/early summer in all plantings in a completely random design (replications = 4) with a spacing of 1.25m in a double row with 3.75m of grass between each double row, with the exception of the TCHRL planting where the

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roses were planted in single rows. After transplanting, plants were watered and mulched. A drip irrigation line was installed and plants were watered as needed during the summer. Plants were fertilized with a general 20-20-20 fertilizer once a month until late summer when plants were allowed to harden off. Pruning was used only to prevent one plant from overgrowing an adjacent plant.

No fungicides were used in the study. The cultivar 'Peace', known for being extremely susceptible to black spot, was planted in a grid with three plants in each replication. This cultivar served as a susceptible control and as an additional source of inoculum.

Plants were evaluated every two weeks from planting until frost for susceptibility to black spot and cercospora leaf spot using the following scale: 0 = no visible symptoms, 1 = < 2% of foliage diseased, 2 = < 10% of foliage diseased, 3 = < 25% of foliage diseased, 4 = < 50% of foliage diseased, 5 = > 50% of foliage diseased, and 6 = 100% of foliage diseased. Defoliation was rated on the same scale. This scale allows for the delineation between cultivars with low disease severity levels but placed very susceptible cultivars in the same category.

Data were analyzed by date and by year using the Proc GLM procedure of SAS. For both day and year variables, F-values were considered significant at the 0.05 level. When a significant F-value was detected, cultivars were separated using a LSD means separation test ( $p=0.05$ ). Data analyses are included by planting year.

Roses were considered to have superior resistance when disease and defoliation levels were all below 2% coverage. Roses with less than 10% coverage to all disease and defoliation were considered to be moderately resistant and may need limited spraying in a landscape setting to maintain clean foliage.

**Results and Discussion:** In all plantings and all years, both diseases were found in the late spring/early summer and progressed at a rapid rate reaching a logarithmic type growth by mid summer. The sole exception was the early summer of 2007 in the WTREC and PREC plantings where a drought occurred and delayed the onset of major infection until later in the summer. Data shown (Tables 1-3) represent the disease incidence and severity for each pathogen and percent defoliation once the epidemics had reached a logarithmic level of growth in each summer.

During the 2007 summer disease levels were overall lower than in 2008. This is due to several factors. The first major reason is the fact that the roses were just planted. New roses take approximately 6 weeks to develop lesions even among susceptible cultivars. This fact combined with the unseasonably late hard freeze and a record drought delayed the explosion of secondary infection until late summer. In 2008, the roses leafed out with the warm spring, extending the period of exposure to the wind borne pathogens significantly relative their planting in 2007. These factors led to 23 cultivars being labeled resistant or moderately resistant at the end of data collection in 2007, but only 8 of those displayed an acceptable level of resistance through both years.

Disease resistant claims in catalog descriptions were inconsistent our data. Among those with disease resistant billing which did not live up to their claims were: 'Bonica' and 'Champlain' (from the "trouble-free roses" shrub section -*Jackson and Perkins Roses for 2007*), 'Gold Medal' ("very resistant deep green foliage" – *Weeks Roses 2007*), 'Grandma's Blessing' ("disease-resistant foliage" – Bailey Nurseries' Easy Elegance publication 2007), 'Lovers Lane' ("disease-resistant" – *Jackson and Perkins Roses for 2007*), 'Sheer Magic' ("blackspot-resistant foliage" – *Jackson and Perkins Roses for 2007*), 'Tahitian Moon' ("excellent disease resistance" - Bailey Nurseries' Easy Elegance publication 2007), and 'Perfect Moment' ("good disease resistance" – *Weeks Roses 2007*).

Roses considered resistant through both years of the study were 'Golden Eye', 'Knock Out Rose', 'My Girl', and 'Pink Knock Out'. Among those with moderate resistance, 'Fiesta', 'Homerun', 'My Hero', and 'Super Hero', 'Homerun' and 'My Hero'. Moderate resistant roses were rated as resistant in year one and the variability between years may have been due to a buildup of inoculum from the first year, environmental factors, variation in populations of the pathogens, or a combination of any or all of these. Further study of this plant/pathogen system is required.

## Pathogenicity Tests for *Phytophthora* species Isolated From Tennessee Nurseries

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**Index words:** Phytophthora diseases, moist chamber, host plants

**Significant to Industry:** This work is part of a project to determine *Phytophthora* species and disease symptoms they cause in Tennessee nurseries. Results from this study will be useful in the development of integrated disease management strategies to reduce *Phytophthora* incidence and its economic impact on nurseries.

**Nature of Work:** *Phytophthora* spp. causes some of the most economically important diseases in ornamental crops in nursery production worldwide (Jeffers, 2003). Several species of *Phytophthora* infect ornamental crops causing aerial blight, stem dieback and root rots on nursery crops. After the devastating attack of *P. ramorum* in the coastal forests of California and Oregon, the nursery industry has become increasingly aware of the potential impact of *Phytophthora* species (Hansen et al 2005). Many host plants of *P. ramorum* are important nursery plants and are potential hosts for other species of *Phytophthora*. Symptoms caused by *P. ramorum* are similar to those caused by other species of *Phytophthora* already present in nurseries around the world (Jeffers, 2003). A survey of representative nurseries in different counties in Middle Tennessee was conducted between 2006 and 2008. This process has provided interesting data about the incidence and diversity of *Phytophthora* species in Tennessee nurseries. *Phytophthora* species were isolated from plant tissues, rhizosphere soil in field and container grown plants and from irrigation water including ponds, creeks, and rivers used in different nurseries. The objective of this study was to evaluate selected *Phytophthora* spp. for pathogenicity on different hosts, and determine the best method to obtain virulence data and the host plants that should be used to test all the remaining *Phytophthora* isolates.

A baiting technique was used to detect *Phytophthora* from irrigation water and soil samples. Baits consisted of leaf discs of rhododendron, pieris, azalea, and pieces of pears and apples. Baits were later transferred to PARPH, a selective media for *Phytophthora*. Pure cultures were initially characterized morphologically using colony features and microscopic characteristics of sporangia, mycelium and chlamydospores. Each morphological type was characterized by DNA sequence analysis of the internal transcribed spacer (ITS4) region. While some isolates were clearly of *Phytophthora* spp. and some were identified to specific species using DNA sequence analysis, some isolates were categorized as unclassified *Phytophthora* or unclassified fungi.

Ten different isolates of *Phytophthora* were tested for pathogenicity on 26 different hosts (Table 1). Six of the isolates were unclassified *Phytophthora* and four were identified species (Table 2). Plastic containers with folded paper towels drenched with 20 ml of sterile water were used as moist chambers for an in-vitro assay technique on detached leaves (Brooks 2008, Park et al. 2008). Four medium size leaves placed in each moist chamber were inoculated with plugs of 7-day old cultures of *Phytophthora* species. Plugs were placed in the middle of the leaf. Leaves for control were inoculated with a plug of sterile base media. A randomized complete block experimental design was used with a replication of three leaves for each potential pathogen and the control. The inoculated leaves were sprayed with a fine mist of sterile water, sealed in a plastic bag and incubated at room temperature. Leaves were monitored for disease symptoms and the pathogen was re-isolated. Confidence interval analysis was used to determine the most susceptible host and the most virulent isolate.

**Results and Discussion:** Seventeen nurseries from six counties in Middle Tennessee have been sampled during the period of 2006 -2008. Cultures with *Phytophthora* morphological characteristics were selected for DNA analysis. PCR results with >700 bp fragments suggested that the isolate was a potential *Phytophthora*. A total of 171 isolates with >700 bp fragments were further analyzed using DNA sequence analysis. Only 41 % corresponded to *Phytophthora* species, 44% corresponded to *Pythium* species, other species included *Absidia / Heterobasidion spp.* (3%), *Verticillium spp.* (1%), *Mortierella spp.* (1%), and others could not be identified. *Phytophthora* was represented by nine different species and 51 % were unclassified species (Figure 1).

Results from pathogenicity study showed that only six out of 26 hosts evaluated were susceptible to *Phytophthora* isolates causing necrotic leaf lesions (Table 1). Re-isolation of *Phytophthora* from infected leaf tissue confirmed that *Phytophthora* caused the damage. *Cornus florida* was susceptible to all ten isolates tested, *Pieris* was susceptible to nine and *Viburnum* was susceptible to eight isolates (Table 3). Silk dogwood, red maple and kousa dogwood were all susceptible to 4 and 5 *Phytophthora* isolates (Table 3). The most virulent isolate was of *P. citricola* which attacked all six hosts (Table 3). Leaf necrosis is associated with aerial blight, but some *Phytophthora* spp. cause stem dieback and root rots and requires a different bioassay technique. Other bioassay techniques include plugs attached to leaves, spray and drench on roots using zoospores. The use of such techniques will allow the evaluation for other diseases. These results show that in-vitro bioassay technique using detached leaves allows rapid evaluation of *Phytophthora* pathogenicity on a large number of hosts using limited space and inoculum resource. Other bioassay methods are being evaluated to evaluate other diseases.

High incidence of diverse *Phytophthora* species in irrigation water was most troubling in that irrigation water has the potential to spread *Phytophthora* diseases in nurseries and subsequently to landscapes and where ever the plants will be placed. Nine out of ten isolates tested were from irrigation water and all the isolates have the potential to infect foliage of some hosts (Table 3). Many investigators have

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demonstrated this problem and isolated *Phytophthora* from irrigation water (Werres, 2007). Maloney et al. (2002) and were able to bait *P. ramorum* from streams in California during the rainy season, suggesting that recycling irrigation water is a potential threat in *Phytophthora* incidence and spread. More isolates of *Phytophthora* were from water than any other source and can inoculate all susceptible hosts being irrigated. Results from this study show irrigation water is a potential threat to nursery production in Tennessee.

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Table 1. Host plants used to evaluate pathogenicity of 10 Phytophthora isolates using in-vitro technique on detached leaves.

Host code No.	Host Common name	Host Scientific name
H1	Willow Oak	<i>Quercus phellos</i>
H2	Pin Oak	<i>Quercus palustris</i>
H3	Water Oak	<i>Quercus nigra</i>
H4	White Oak	<i>Quercus alba</i>
H5*	Silky Dogwood	<i>Cornus amomum</i>
H6*	Dogwood	<i>Cornus florida</i>
H7	Camellia	<i>Camellia japonica</i>
H8*	Red Maple	<i>Acer rubrum</i>
H9	Maple	<i>Acer saccharum</i>
H10	Azalea	<i>Azalea japonica</i>
H11	Viburnum	<i>Viburnum dilatatum</i>
H12*	Pieris	<i>Pieris japonica</i>
H13	Viburnum	<i>Viburnum dentatum</i> 'Snow Ball'
H14	Rhododendron	<i>Rhododendron maximum</i>
H15	Camellia	<i>Camellia japonica</i> 'April Remembered'
H16	Camellia	<i>Camellia japonica</i> 'April Dawn'
H17	Camellia	<i>C. japonica</i> 'Bernice Bobby'
H18	Camellia	<i>C. japonica</i> 'Arctic snow'
H19	Sweetbay Magnolia	<i>Magnolia virginiana</i>
H20	Rhododendron	<i>Rhododendron maximum</i>
H21	Holly	<i>Ilex aquifolium</i>
H22*	Viburnum 'Erie'	<i>Viburnum dilatatum</i> 'Erie'
H23	Dogwood Selection R2'	<i>Cornus florida</i>
H24	Dogwood Selection R17	<i>Cornus florida</i>
H25	Maple	<i>Acer saccharinum</i>
H26*	Varigated Kousa Dogwood	<i>Cornus kousa</i> 'Wolfe's Eye'

\* Hosts that developed necrotic lesions from some of the Phytophthora on the detached leaf experiment

Table 2. List of *Phytophthora* isolates evaluated for pathogenicity on detached leaves.

Code No.	<i>Phytophthora</i> Isolates*	Nursery code	Source	Bait
T1	<i>P. citricola</i>	TSU-NRC Shadehouse	dogwood, leaves	
T2	<i>Phytophthora unclassified</i>	CLPond3, N	water	pine needles
T3	<i>Phytophthora unclassified</i>	BDPond2, P	water	pieris
T4	<i>P. megasperma</i>	BDPond1, P	water	pieris
T5	<i>P. hydrophatica</i>	CLPond1, P	water	pieris
T6	<i>P. hydrophatica</i>	SR3 B	soil	pear
T7	<i>Phytophthora unclassified</i>	BDPond2, P	water	pear
T8	<i>Phytophthora unclassified</i>	CLPond1, P	water	pear
T9	<i>Phytophthora unclassified</i>	CLPond1, A	water	apple
T10	<i>Phytophthora unclassified</i>	CLPond 3, P	water	pear

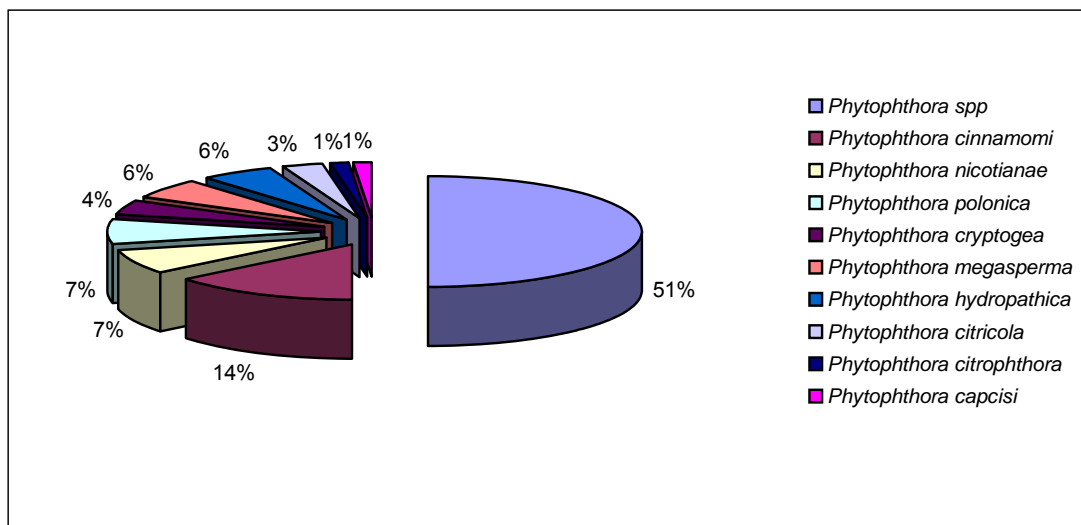
\* Identification based on DNA sequence results (ITS4) compared with other fungi in the Gen Bank

Table 3. Pathogenicity analysis for the six hosts that were susceptible for some of the Phytophthora isolates.

Phytophthora Isolate	<i>Phytophthora</i> Isolates*	Silky Dogwood*	Cornus florida	Red Maple*	Pieris	Viburnum 'Erie'	Variegated kousa Dogwood*
T1*	<i>P. citricola</i>	1	1	1	1	1	1
T2	<i>Phytophthora unclassified</i>	0	1	0	1	0	1
T3	<i>Phytophthora unclassified</i>	0	1	0	1	1	1
T4	<i>P. megasperma</i>	0	1	0	0	1	1
T5	<i>P. hydropathica</i>	0	1	1	1	1	0
T6	<i>P. hydropathica</i>	1	1	0	1	0	1
T7	<i>Phytophthora unclassified</i>	1	1	0	1	1	0
T8*	<i>Phytophthora unclassified</i>	1	1	1	1	1	0
T9	<i>Phytophthora unclassified</i>	0	1	1	1	1	0
T10	<i>Phytophthora unclassified</i>	1	1	0	1	1	0
		<b>5</b>	<b>10</b>	<b>4</b>	<b>9</b>	<b>8</b>	<b>5</b>

\* Silk dogwood, red maple and kousa dogwood were significantly different from others based on a Confidence Interval Analysis.

Figure 1. Phytophthora species isolated from Middle Tennessee Nurseries



## Efficacy and Growth Effects of Fungicide Spray of Growth Effects on Flowering Dogwood

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**Index words:** dogwood, powdery mildew, *Erysiphe pulchra*

**Significance to Industry:** Foliar fungicide sprays are still the primary means of managing powdery mildew on dogwood. In this study, azoxystrobin, chlorothalonil, propiconazole, a mixture of chlorothalonil+propiconazole+fludioxinil and the biorational products: copper octanoate and neem oil were efficacious against *Erysiphe pulchra* and increased growth of flowering dogwood as compared to the untreated dogwood seedlings.

**Nature of Work:** Powdery mildew on dogwood in Tennessee was a footnote, an after thought when discussing diseases found on *Cornus florida*, until 1994 (2,3). In that year, it exploded and could be found throughout the Eastern United States. In each subsequent year since, powdery mildew of flowering dogwood caused by *Erysiphe pulchra*, has become the major disease of flowering dogwood. While mildew-resistant dogwood cultivars are available, the majority of the dogwoods grown for the nursery industry are mildew-susceptible cultivars or seedlings. Fungicides have been shown to be efficacious against powdery mildew, and to enhance growth in some studies (1,2,3).

In this study, nine fungicide treatments were compared to an unsprayed set of controls on container grown, seedling, white flowering dogwoods. Fungicides evaluated in this study were: Instrata (a package pre-mix of chlorothalonil+propiconazole+fludioxinil), Heritage (azoxystrobin), Ortho Daconil (chlorothalonil), Propiconazole Pro (propiconazole), and biorational products: Bonide Liquid Copper Fungicide (copper octanoate), Monterey Neem Oil (neem oil) and Triple Action Plus (neem oil). Treatments were applied with a compressed air hand sprayer at 14 day intervals (fungicides) and 7 day intervals (biorationals) from June 29 to August 10. Dogwoods were sprayed to the point of runoff. Each week, trees were assessed for disease severity using the following scale: 0=healthy, 1<2%, 2<10%, 3<25%, 4<50%, 5>50% of foliage with powdery mildew. Tree height (cm) was measured at the beginning, mid-point and end of the study.

**Results and Discussion:** Mildew infection followed within a few days of the first fungicide application and infection levels were high throughout the test. All fungicide sprays were efficacious in the management of powdery mildew on dogwood foliage when compared to the untreated control (Table 1). Neem oil treatments were not as

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efficacious as the conventional fungicides; however the liquid copper fungicide had the same efficacy as most of the fungicides, with the exception of the Instrata treatments which were superior in preventing mildew. Fungicides did have a positive effect on vertical growth (Table 2). All dogwood seedlings treated with fungicides, produced a flush of growth, which was absent in the untreated control trees. Only the low rate of Instrata failed to have significantly more growth than the untreated trees. This research documents the benefit of fungicide sprays in the prevention of powdery mildew on flowering dogwood, documents the efficacy of a recently registered fungicide mixture (Instrata), and, as in similar studies documents an increase of vertical growth on trees without powdery mildew infection.

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Table 1. Efficacy of fungicide sprays for powdery mildew caused by *Erysiphe pulchra* on *Cornus florida* seedlings.

Treatment	ml/L	Spray Interval (Days)	Disease Severity*	LSD**
Untreated	-	-	5.0	A
Triple Action Plus	8	7	4.2	B
Monterey Neem Oil	8	14	4.0	B
Ortho Daconil	2.4	14	2.3	C
Heritage	0.15	14	2.2	C
Liquid Copper Fungicide	8.4	7	1.7	CD
Propiconazole Pro	0.6	14	1.2	D
Instrata	1.25	14	0.5	E
Instrata	2.6	14	0.4	E
Instrata	1.7	14	0.1	E

\*Disease severity scale: 0=healthy, 1= <2% diseased foliage, 2= <10% diseased foliage, 3= <25% diseased foliage, 4= <50% diseased foliage, 5= >50% diseased foliage

\*\*Mean separation within column by LSD (LSD = 0.67)

Table 2. Vertical growth of *Cornus florida* seedlings treated with fungicide sprays to prevent powdery mildew.

Treatment	ml/L	Spray Interval (Days)	Growth from June 29-August 10 (cm)	LSD*
Instrata	2.6	14	30.5	A
Liquid Copper Fungicide	8.4	7	29.5	AB
Ortho Chlorothalonil	2.4	14	27.7	AB
Propiconazole Pro	0.6	14	26.9	ABC
Instrata	1.7	14	25.4	ABC
Heritage L	0.15	14	23.1	BCD
Triple Action Plus	8	7	21.3	CD
Monterey Neem Oil	8	14	21.1	CD
Instrata	1.25	14	17.0	DE
Untreated	-	-	11.7	E

\*Mean separation within column by LSD (LSD=6.3)

## Symptomatology of Hosta Viruses

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**Index words:** hosta, hosta virus x, HVX, tobacco rattle virus, tomato spotted wilt virus

**Significance to Industry:** Big box stores, independent garden centers and wholesale nurseries were surveyed for the presence of virus infected hosta cultivars in Middle and West Tennessee. Twenty-nine symptomatic hosta cultivars were infected with one or more of the following viruses: hosta virus x, tomato spotted wilt virus and tobacco rattle virus.

**Nature of Work:** Hosta is an extremely popular perennial plant used in shade gardens in Tennessee. Unfortunately, it is susceptible to several virus diseases such as hosta virus x (HVX)(2), arabnis mosaic virus (ArMV), tomato spotted wilt virus (TSWV) and tobacco rattle virus (TRV). Recently, HVX was confirmed in hosta sold in Tennessee retail and wholesale outlets (1). The purpose of this survey was to determine the viruses present in hosta sold in big box stores, independent garden centers and wholesale nurseries in Middle and West Tennessee. Twenty retail outlets and nurseries were selected for the survey. Plants with symptoms of virus infection such as ringspots, mosaic, abnormal green streaks on gold hosta, and oak leaf patterns were collected for testing. Symptomatic hosta were taken to the UT Soil, Plant and Pest Center and tested for hosta virus x using Immunostrips for HVX from Agdia, Inc., Elkhart, IN. Also, leaves from symptomatic plants were shipped to Agdia, Inc. where they were assayed for hosta viruses including: arabnis mosaic virus, cucumber mosaic virus, impatiens necrotic spot virus, hosta virus x, tomato ringspot virus, tomato spotted wilt virus and tobacco rattle virus.

**Results and Discussion:** Virus infected hosta was recovered at fifteen of twenty locations visited in Middle and West Tennessee. Diseased hosta was collected at all big box stores surveyed, from all but one of the independent garden centers and from one of two wholesale nurseries surveyed. Twenty-nine hosta cultivars were determined to be infected with one or more of HVX, TSWV or TRV. Hosta virus x was the most common virus detected (Table 1), followed by tobacco rattle virus and the tomato spotted wilt virus. In most locations, virus infected plants made up 1% of the inventory. However, at one big box store, nearly 50% of *Hosta* 'Golden Tiara' were infected with HVX. Also, at one wholesale nursery, 15 cultivars of hosta infected with HVX and/or TRV. Symptoms were most easy spotted on gold hosta cultivars such as 'August Moon', 'Gold Standard' or 'Sum and Substance' due to abnormal green streaks

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(variegation) along veins (Image 1). Another common symptom was stunting. Hosta infected with HVX appeared to be less vigorous and stunted compared to healthy plants of the same cultivar (Image 2). One of the interesting aspects of this survey was the knowledge level of nursery and store personnel on hosta viruses and symptoms associated with virus infection. None of the personnel that we interviewed recognized common symptoms associated with virus infection. There appeared to be a general lack of knowledge of the viruses that infect hosta and the symptoms associated with virus laden hosta. To increase the awareness of the Green Industry on hosta virus symptoms, a web page was created on the Bugwood Network (3).

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Table 1. Hosta cultivars positive for plant viruses in retail stores, garden centers and nurseries in Middle and West Tennessee in 2008.

Hosta cultivar	Location*	Hosta Virus X**	Tomato Spotted Wilt Virus**	Tobacco Rattle Virus**
Albo Marginata	BBS, IGC	X		
Antioch	BBS	X		
August Moon	BBS, IGC, WN	X		
Aureomarginata	BBS	X		
Birchwood	WN	X		
Park's Gold				
Blue Cadet	WN	X		
Blue Diamond	WN	X		
Devon Green	WN		X	X
Elvis Lives	IGC		X	X
Francee	BBS			X
Gold Standard	BBS, WN	X		
Golden Tiara	BBS, WN, IGC	X		
Just So	WN	X		
Krossa Regal	WN			X
Lady Guinere	IGC	X		
Lancifolia	WN	X		
Marginata	BBS	X		
Superior				
Medio	BBS	X		
Variegata				
Paul's Glory	BBS		X	
Pilgrim	IGC	X		
Regal Splendor	WN	X		X
Revolution	IGC	X		
Royal Standard	WN	X		
Scooter	WN	X		
So Sweet	WN	X		
Spritzer	WN	X		
Sum and Substance	BBS, IGC, WN	X		
Sun Power	IGC			X
Wide Brim	IGC	X		
Wylde Green	IGC	X		
Cream				
Yellow Splash	BBS	X		

\*Location where hosta was collected : big box store (BBS), independent garden center (IGC), wholesale nursery (WN)

\*\* X indicates that the hosta cultivar was infected with either HVX, TSWV or TRV.

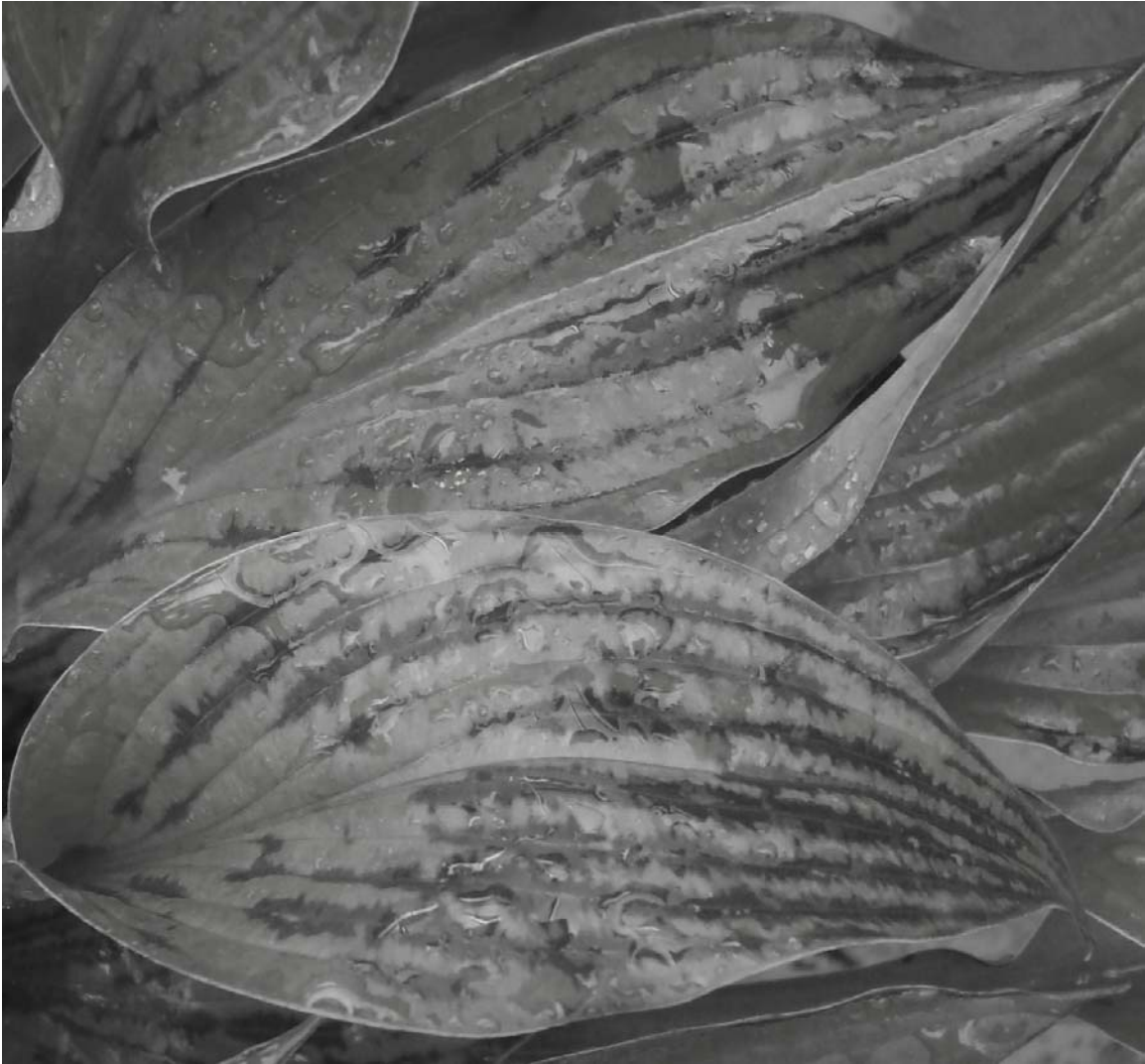


Image 1. Abnormal, dark green tissue along veins on *Hosta* 'August Moon' leaves infected with hosta virus x.



Image 2. Healthy *Hosta* 'Golden Tiara' leaf (lower left), diseased, stunted 'Golden Tiara' leaf with mosaic symptoms infected with HVX (upper right).