Pathology and Nematology

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Section Editor

Evaluation of Fungicides and Biofungicides for Control of *Phytophthora cinnamomi* on Flood-stressed Flowering Dogwoods

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Index Words *Phytophthora cinnamomi*, *Cornus florida*, fungicides, biofungicides

Significance to Industry Trees in ornamental nurseries are often exposed to a variety of stressors including flooding. Flooding or high levels of soil moisture increase a tree's susceptibility to Phytophthora root rot. This research study will provide information to the nursery industry on the effectiveness of preventive and curative applications of fungicides and biofungicides for controlling Phytophthora root rot under flooding conditions.

Nature of Work *Phytophthora cinnamomi* is a soil-borne pathogen that causes major plant losses in nursery production. Numerous ornamental genera such as *Acer*, *Cornus*, *Juglans*, *Magnolia*, *Prunus*, *Rhododendron*, and *Quercus* are susceptible to *P. cinnamomi* (1). *Phytophthora* infects plant roots and infected plants can collapse suddenly. Many of the symptoms of Phytophthora root rot, including small, yellow, wilted foliage and limb dieback, can be confused with other plant diseases (1). Flooding predisposes trees to attack by Oomycetes as a result of creating hypoxic conditions in the soil, reducing root health, and increasing dispersal of zoospores in the soil (2). Even cultivars which are resistant to disease under normal growing conditions can become susceptible to disease during flood events (3).

The purpose of this research experiment was to evaluate the efficacy of fungicides and biofungicides for preventive and curative control of Phytophthora root rot on flooded dogwood seedlings.

Fungicides and biofungicides were evaluated for their ability to control *P. cinnamomi* on flooded dogwood seedlings under greenhouse conditions (Table 1). The experiment was conducted at the Tennessee State University Otis L. Floyd Nursery Research Center from July-August 2017 as a randomized complete block design (RCBD) with five replications. Flowering dogwood, *Cornus florida* L., seedlings were propagated from seeds and grown in 4 in. (10.2 cm) pots containing sterilized potting substrate (Morton's Grow Mix #2). Each fungicide was applied as a preventive drench treatment 7 d before flooding or as a curative drench treatment 24 hr after flooding, except Actigard, MBI-110, and Rootshield *Plus*$^{+}$, which were applied only as preventive drench treatments. All treatments were applied according to label directions. Non-fungicide-treated inoculated or non-inoculated plants served as controls. Plants were artificially inoculated by burying four *P. cinnamomi*-infested rice grains in the potting substrate on opposite sides of the seedling. Flooding conditions were imposed by enclosing pots in zip-top plastic bags and maintaining

standing water for 1, 3, or 7 d for each treatment. Plant morality was recorded during the experiment. The severity of Phytophthora root rot was assessed using a scale of 0-100% roots affected at the end of the experiment. Plant growth data (fresh weight, root weight, plant height, and plant width) also were recorded. The presence of *Phytophthora* was confirmed by plating selected root samples on PARPH-V8 selective medium. Root weight and disease severity were compared among preventive or curative treatments within each flood duration. Root weight data were analyzed with a one-way analysis of variance (ANOVA) using Proc GLM in SAS, and means were separated using Fisher's least significant difference (LSD) test (α=0.05). The percentage of roots affected data were analyzed using a generalized linear mixed model (GLMM) using Proc GLIMMIX in SAS with a logit link and assuming a beta distribution, and means were separated by least squares means (α=0.05).

Results and Discussion *Phytophthora*-inoculated dogwoods had significantly higher root weight at 7 d flooding with preventively-applied Pageant Intrinsic and 3 d flooding with curatively-applied Tartan Stressgard than the non-treated, inoculated control (Figs. 1, 2). No other fungicide treated plants had a higher root weight than the non-treated, inoculated control. Dogwoods preventively treated with Subdue MAXX effectively reduced the percentage of roots affected compared to the non-treated, inoculated control at all flooding durations, and dogwoods preventively treated with Orkestra Intrinsic reduced the percentage of affected roots relative to the non-treated, inoculated control at 1 and 3 d flooding durations (Fig. 3). The biofungicides, Rootshield *Plus*$^{+}$ and MBI-110, provided effective Phytophthora root rot control compared to the non-treated, inoculated control at 1 d of flooding, but not at 3 or 7 d flooding (Fig. 3). Curative applications of Aliette, Segovis, and Tartan Stressgard were effective at reducing the percentage of Phytophthora root rot on dogwoods compared to the non-treated, inoculated control at 1 d of flood duration, while curatively-applied Interface Stressgard was effective at 3 d of flood duration (Fig. 4). Dogwoods curatively treated with Empress Intrinsic and Orkestra Intrinsic had significantly less root rot than the non-treated, inoculated control plants in both the 1 and 3 d flooding durations (Fig. 4). Many fungicide treatments that were effective at 1 or 3 d flooding didn't show the same effectiveness at 7 d flooding. The reduction in fungicide efficacy with longer flooding duration may be due to increased disease pressure (inoculated plant mortality rates for the 1, 3, and 7 d flood durations were 2%, 16%, and 36%, respectively (data not shown)), drainage of curative fungicides, or reduced success of microbials in the biofungicide treatments.

Subdue MAXX and Orkestra Intrinsic were the most effective at providing preventive control of Phytophthora root rot, while Empress Intrinsic and Orkestra Intrinsic were the most effective at providing curative control. Many fungicides, especially when applied curatively, had reduced effectiveness with longer flooding duration. As a result, growers should apply curative fungicides as early as possible after flooding. Also, growers should use an integrated approach to manage *Phytophthora*, which includes proper irrigation to avoid high soil moisture levels that promote *Phytophthora* infection. Potentially, growers can use information from this study to manage Phytophthora root rot in times of flooding or in areas of the nursery that often experience high levels of soil moisture.

Acknowledgements This project was partially funded by USDA-NIFA Evans Allen and USDA Floriculture and Nursery Research Initiative Agreement No. 58-3607-3-984. We thank BASF, Bayer, BioWorks, Marrone, and Syngenta for donating the fungicide products used in this study. We thank Dr. Anthony Ejiofor and Dr. Dafeng Hui with help designing the project, Debbie Eskandarnia, Md Niamul Kabir, Joseph Lampley, and Terri Simmons for help with setting up the greenhouse experiment.

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Table 1. Fungicides and biofungicides

Treatment	Active Ingredients	Application Rate	Manufacturer
Empress Intrinsic	pyraclostrobin	6 fl oz/100 gal	BASF
Orkestra Intrinsic	pyraclostrobin + fluxapyroxad	10 fl oz/100 gal	BASF
Pageant Intrinsic	pyraclostrobin + boscalid	18 oz/100 gal	BASF
Interface Stressgard	trifloxystrobin + iprodione	80 fl oz/100 gal	Bayer
Tartan Stressgard	trifloxystrobin + triadimefon	40 fl oz/100 gal	Bayer
Signature Xtra Stressgard	aluminum tris (0-ethyl phosphanate)	80 oz/100 gal	Bayer
Aliette	aluminum tris (0-ethyl phosphanate)	50 oz/100 gal	Bayer
Rootshield *PLUS*$^{+}$	*Trichoderma harzianum* T-22 + *T. virens* G-41	8 oz/100 gal	BioWorks
MBI-110	*Bacillus amyloliquefaciens*	1%	Marrone
Actigard	acibenzolar-S-methyl	4 oz/100 gal	Syngenta
Segovis	oxathiapiprolin	3.2 fl oz/100 gal	Syngenta
Subdue MAXX	mefenoxam	2 fl oz/100 gal	Syngenta

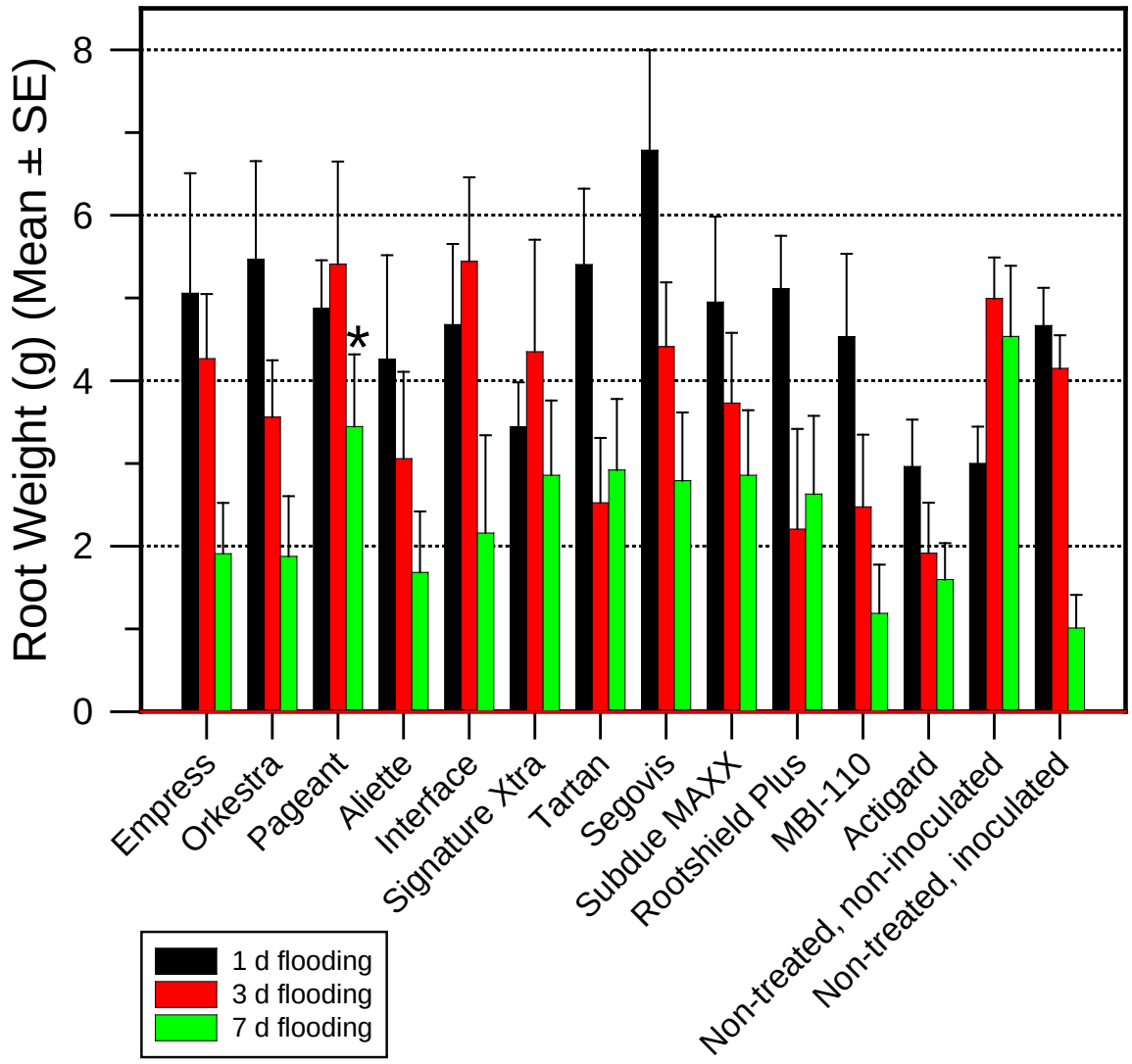

Figure 1. Mean root weight (± SE) of preventively treated dogwood plants at the end of the experiment for different fungicide or non-fungicide (controls) treatments and root flooding durations. Treatments were compared using a one-way ANOVA, and means were separated using Fisher's LSD test (α=0.05). Asterisks indicate the root weight of the fungicide treatment is significantly greater than the non-treated, inoculated control within the same flooding duration.

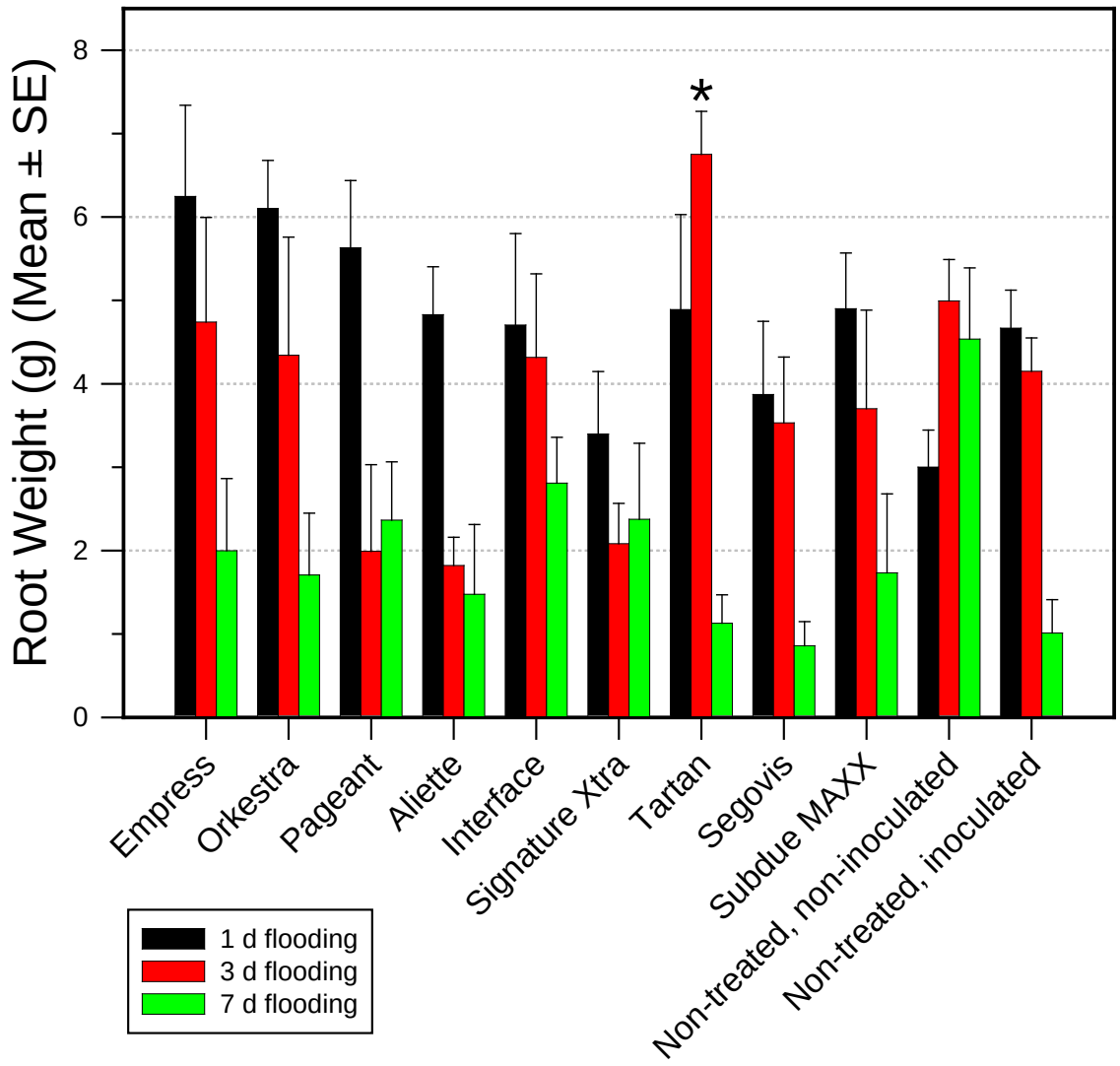

Figure 2. Root weight (± SE) of curatively treated dogwood plants at the end of experiment for different fungicide or non-fungicide (controls) treatments and root flooding durations. Treatments were compared using a one-way ANOVA, and means were separated using Fisher's LSD test (α=0.05). Asterisks indicate the root weight of the fungicide treatment is significantly greater than the non-treated, inoculated control within the same flooding duration.

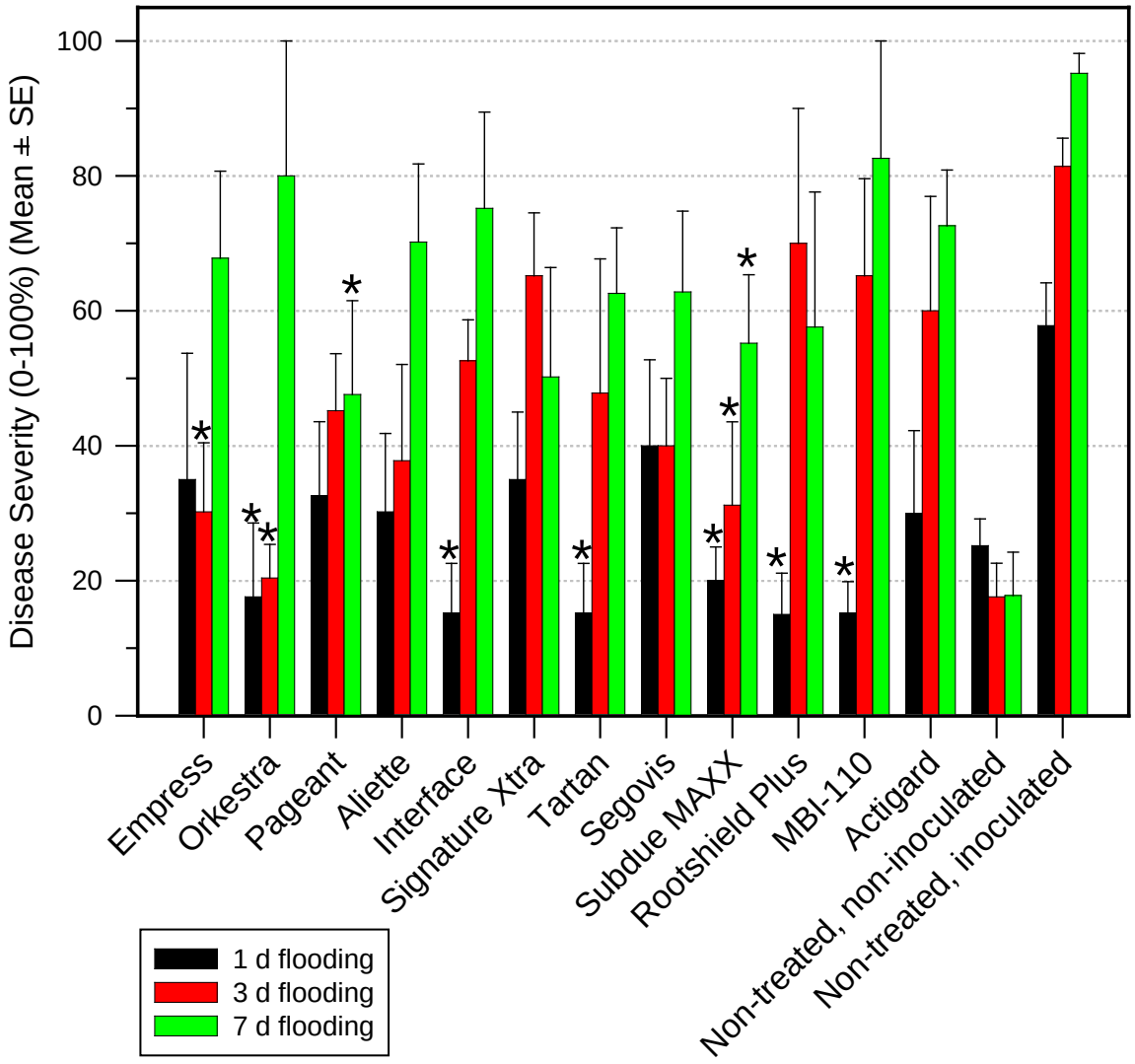

Figure 3. Percentage of roots affected (± SE) on preventively treated dogwood plants at the end of experiment for different fungicide or non-fungicide (controls) treatments and root flooding durations. Treatments were compared using a generalized linear mixed model with a logit link assuming a beta distribution, and means were separated by least squares means (α=0.05). Asterisks indicate the percentage of roots affected of the fungicide treatment is significantly less than the non-treated, inoculated control within the same flooding duration.

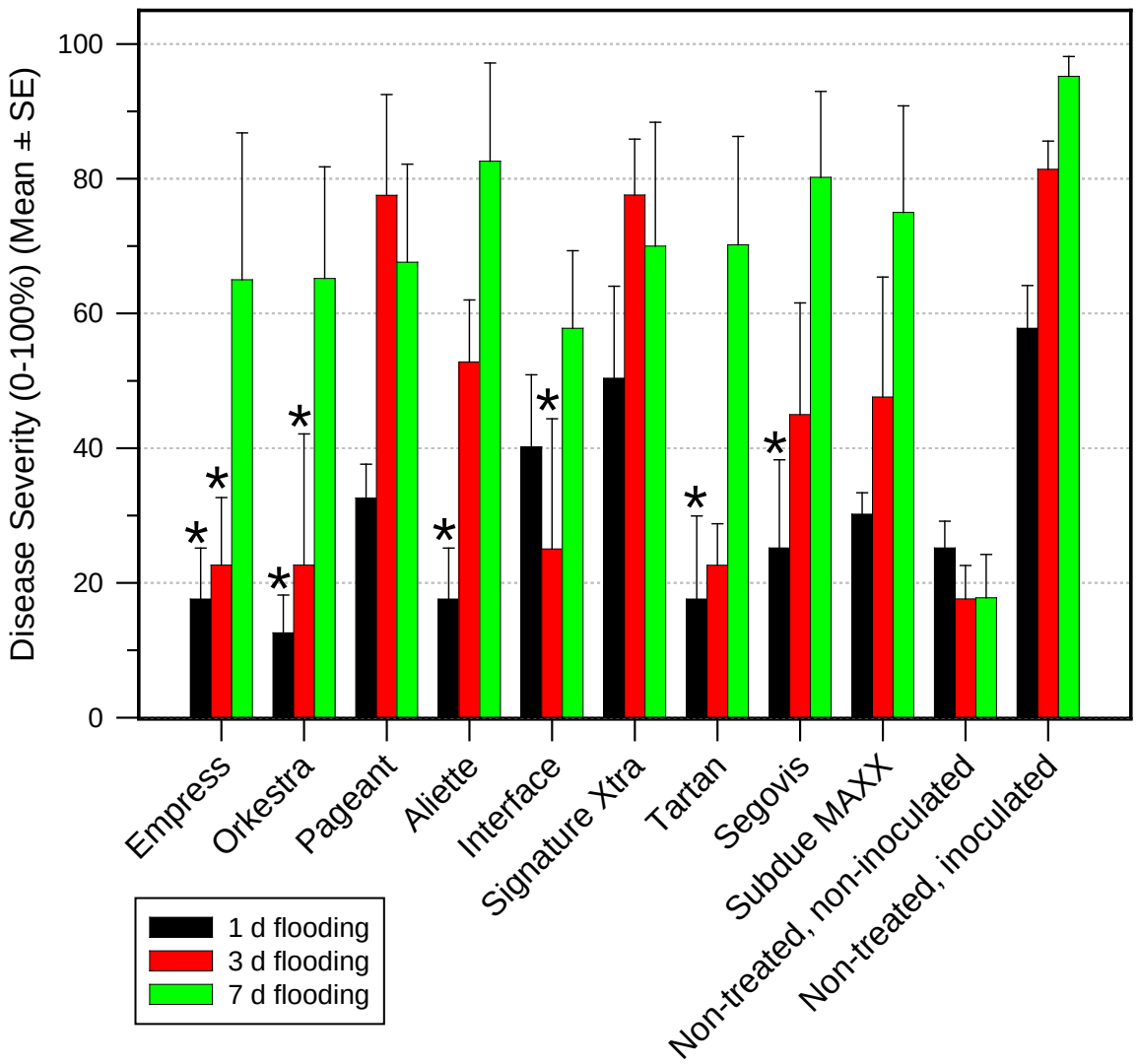

Figure 4. Percentage of roots affected (± SE) on curatively treated dogwood plants at the end of experiment for different fungicide or non-fungicide (controls) treatments and root flooding durations. Treatments were compared using a generalized linear mixed model with a logit link assuming a beta distribution, and means were separated by least squares means (α=0.05). Asterisks indicate the percentage of roots affected of the fungicide treatment is significantly less than the non-treated, inoculated control within the same flooding duration.

Response of Bigleaf Hydrangea Cultivars to Powdery Mildew Disease

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Index Words Bigleaf hydrangea, disease resistance, powdery mildew

Significance to Industry Although hydrangeas have a breeding history stretching back hundreds of years, improvement has focused on novel floral traits rather than disease resistance or environmental stress tolerance. Foliar diseases often have a detrimental impact on hydrangea plants in the landscape or on salability of container-grown plants. Powdery mildew caused by *Erysiphe polygoni* DC can limit hydrangea plant growth and affect plant quality, and preventative fungicide treatments are usually required to produce quality container-grown hydrangeas. Because of the prevalence of hydrangeas in nursery operations in the United States and globally, hydrangea varieties with improved resistance to pests, diseases, and drought would benefit the nursery crop industry by reducing the environmental and budgetary footprint associated with hydrangea production. The purpose of this study is to provide powdery mildew evaluation results for different bigleaf hydrangea cultivars and hybrids to help nursery producers make decisions about cultivar selection and management.

Nature of Work Hydrangea is one of the most economically important nursery crops in the United States, with sales topping $120 million in 2014 (1). Hydrangeas are popular ornamental plants in both home gardens and commercial settings. Some hydrangea species such as bigleaf hydrangea (*Hydrangea macrophylla* (Thunb.) Ser.) are more susceptible to powdery mildew while other species such as oakleaf hydrangea (*H. quercifolia* Bart.), appear to be more resistant (2). Variation in resistance to powdery mildew also has been reported among bigleaf hydrangea cultivars in previous studies (3, 4, 5, 6).

Producing hybrids between plant species offers potential for incorporating unique traits into popular ornamentals. For example, *Dichroa febrifuga* Lour. produces blue flowers in soilless media even when no supplemental aluminum is added, and produces blue fruit that persist throughout winter (7). F_1, F_2 and BC_1 progeny from *D. febrifuga* × *H. macrophylla* hybrids were produced and evaluated by USDA scientists in McMinnville, TN. In initial greenhouse evaluations, all F_2 and BC_1 had showy sepals and 80% produced blue or purple flowers in the absence of aluminum (8). Four promising genotypes were selected and propagated. The objective of this study was to determine the response of commonly available hydrangea cultivars and newly developed hydrangea hybrids to powdery mildew disease.

All plants in the experiments originated as rooted cuttings collected in mid-June from container plants grown under 60% shade in McMinnville, TN. Cuttings were rooted in pine bark under mist and overwintered in a greenhouse (55°F/45°F day/night and ambient light). In March, plants were potted in 3-gallon nursery containers filled with 100% pine bark substrate, which was amended with 0.48 lb of 19-5-9 Osmocote® Pro controlled release fertilizer, 0.06 lb of Micromax® micronutrient fertilizer, 0.04 lb iron sulfate and 0.01 lb Epsom salt per cubic feet of mix. Plants received additional 2.5 oz of 19-5-9 Osmocote® Pro in April and July. Five single-plant replications per treatment were arranged in a randomized complete block design in a greenhouse at the Otis L. Floyd Nursery Research Center in McMinnville, TN (75°F/65°F day/night and ambient light). Plants were irrigated for 3 minutes twice a day in June and for 4 minutes twice a day in July using micro bubbler emitters installed on short stakes.

Plants were evaluated weekly for powdery mildew infection. The severity of powdery mildew was evaluated using a scale of 0-100% foliage area affected. Area under the disease progress curve (AUDPC) values were calculated according to the formula: $\sum([(x_i+x_{i-1})/2](t_i-t_{i-1}))$ where x_i is the rating at each evaluation time and (t_i-t_{i-1}) is the number of days between evaluations. The experiment was repeated twice. Analysis of variance was performed using MINITAB statistical software and means were separated using Tukey method.

Results and Discussion Powdery mildew infection occurred naturally in both experiments. Final mean powdery mildew severity in the second experiment was significantly higher compared to the first experiment. There were significant differences among twelve cultivars in the severity of powdery mildew infection. Powdery mildew severity and disease progress (AUDPC) were highest in the *Hydrangea* cultivars Blaumeise and Kardinal by the end of the first experiment. Powdery mildew severity and disease progress (AUDPC) were highest in the *Hydrangea* cultivars Souvenir du President Doumer, Taube, Kardinal, Oakhill and Blaumeise by the end of the second experiment. Hybrids NA83993 (*Dichroa febrifuga* 'Yellow Wings' x *H*. *macrophylla* 'Nigra') and NA83991 [(*D*. *febrifuga* GUIZ48 x *H*. *macrophylla* 'Taube') x *H*. *macrophylla* 'Souv. Pres. Doumer'] were resistant to powdery mildew disease.

These results agree with previous reports that most *H. macrophylla* cultivars are moderately or highly susceptible to powdery mildew (3, 6). There was no difference in susceptibility between diploid cultivars (Decatur Blue, Endless Summer, Oakhill, Souvenir du President Doumer, and Veitchii) and triploid cultivars (Blaumeise, Kardinal, and Taube). The cultivars Decatur Blue, Endless Summer, and Veitchii were the most resistant to powdery mildew. Previous reports have characterized 'Veitchii' as the only powdery mildew-resistant *H*. *macrophylla* (3, 6). We found that in the second experiment where infection was most severe, 'Veitchii' had the least amount of infected tissues among *H*. *macrophylla* cultivars, though percent infected tissue was not statistically different from 'Decatur Blue' or 'Endless Summer'.

Most significantly, two hydrangea hybrids appeared completely resistant to powdery mildew disease (0% infected tissue) in both greenhouse experiments with moderate to

heavy disease pressure. Disease resistance appears to originate from the *Dichroa* cultivars, as all hydrangea parents of NA83991 and NA83993 are highly susceptible to powdery mildew (3, 6 and herein). Current powdery mildew resistance breeding uses only *H*. *macrophylla* 'Veitchii' and a few *H. macrophylla* ssp. serrata cultivars as sources of powdery mildew resistance (6). The identification of powdery mildew resistance in Dichroa x Hydrangea hybrids significantly broadens the sources of genetic resistance available for hydrangea breeding.

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Table 1. Response of hydrangea cultivars to powdery mildew disease.

Cultivar	First experiment		Second experiment	
	Powdery mildew (%)	AUDPC	Powdery mildew (%)	AUDPC
'Kardinal'	33.3[a*]	1206.9[a]	68.3[ab]	1318.3[a]
'Blaumeise'	30.0[a]	1206.9[a]	50.0[ab]	1025.0[a]
'Oakhill'	23.3[b]	767.1[b]	66.7[ab]	1293.3[a]
NA83990 (*Dichroa febrifuga* 'Yellow Wings' x 'Oakhill')	9.2[c]	288.8[cd]	5.0[bc]	74.1[bc]
'Veitchii'	9.2[c]	224.0[cd]	7.5[bc]	117.1[bc]
'Souvenir du President Doumer'	9.2[c]	326.1[c]	81.7[a]	1453.3[a]
'Taube'	8.3[c]	253.2[cd]	75.0[a]	1439.2[a]
'Decatur Blue'	6.7[c]	205.9[cd]	25.0[b]	431.7[b]
'Endless Summer'	6.7[c]	157.5[cd]	13.3[bc]	391.7[b]
NA83992 *(D. febrifuga* 'Yamaguchi Hardy' x 'Hamburg')	5.9[c]	121.3[cd]	10.0[bc]	207.1[b]
NA83993 (*D. febrifuga* 'Yellow Wings' x 'Nigra')	0.0[d]	0.0[d]	0.0[c]	0.0[c]
NA83991 [(*D. febrifuga* GUIZ48 x 'Taube') x 'Souv. Pres. Doumer']	0.0[d]	0.0[d]	0.0[c]	0.0[c]
P-value	≤0.0001	≤0.0001	≤0.0001	≤0.0001

[*]Values are the means of five replicate plants. Treatments followed by the same letter within a column are not significantly different at $P \leq 0.05$. All cultivars are *Hydrangea macrophylla* unless otherwise noted.

Phylogenetic Diversity of Endophytic Fungi in *Cornus florida* L.

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Significance to Industry Endophytic fungi are ubiquitous and phylogenetically diverse; they have wide plant host range including mosses, ferns, grasses, deciduous, coniferous (1, 2), aquatic, lichens, mangroves and bamboo (3). Endophytes can live inside host tissues without causing any external symptoms. There is considerable evidence suggesting that some endophytic fungi play a role in protecting plants against pathogens and promoting plant growth using various mechanisms. Some biodiversity studies have demonstrated that some endophytes are saprophytes, while others are latent or opportunistic pathogens that may be quite aggressive when the host plant is stressed. Thus, it is important to understand fungal populations colonizing dogwood plant tissues internally without external symptoms; this would facilitate early response to the disease outbreaks that may adversely impact dogwood production (1, 2, 4).

Nature of work Flowering dogwood (*Cornus florida* L.) is native to the southeastern region of the United States and it is of great economic importance (5). It is economically important in nursery production industry, but it also has been shown to have rich ethnobotanical history. A phytochemical study have shown that *C. florida* produce anti-parasitic compounds for the treatment of malaria (6). Several studies have shown that some endophytes produce chemicals for important pharmaceutical products, but endophytes associated with *C. florida* have not yet been investigated. Therefore, the aims of present study were to explore diversity of culturable endophytes of class Sordariomycetes and understand their phylogenetic relationship. Such study on dogwood endophytes may provide information on potential products of economic or pharmaceutical value.
Healthy stem samples of *C. florida* were collected from different location in middle Tennessee. For isolation of endophytic fungi, the plant material was thoroughly washed in running tap water followed by surface sterilization in 70% ethanol for 1 minute and 10% sodium hypochlorite for 3-5 min. Samples were subsequently rinsed 3 times in sterilized distilled water for 5 min. each. Using sterilized scalpel, 0.3-0.5mm vascular tissue segments were placed on acidified potato dextrose agar (APDA) in Petri plates. The Petri plates were sealed with parafilm and incubated at 28 ± 2°C until fungal growth was initiated. The tips of the fungal hyphae were sub-cultured on PDA (Sigma-Aldrich, St. Louis, MO). Repeated sub-culturing of fungal isolates on PDA was carried out until pure cultures were obtained.

Taxonomic identification of fungal endophytes was done using genomic DNA extracted using the FastDNA kit (MP Biomedicals, Santa Ana, CA) as per manufacturers'

instructions. Polymerase chain reaction (PCR) amplification of isolated DNA was performed with negative control using the universal primers internal transcribed spacer (ITS) 1 and ITS4 (7). The amplicon was purified using Exosap (USB-Affimetrix, Santa Clara, CA), and submitted to Eurofin Genomics (Louisville, KY) for sequencing. The resulting sequence was analyzed using BLASTN tool against the available sequences in Genbank National Centre of Biotechnology information (NCBI) database (https://www.ncbi.nlm.nih.gov). The identity of the organism was based on the closest similarity match in the GenBank database at ≥ 99-100% identity. The sequence alignment and phylogenetic tree was constructed by neighbor-joining method using MEGA 7.0 software. Multiple sequence alignments were performed using MUSCLE with reference ITS rDNA sequences obtained from GenBank.

Results and Discussion A total of 379 isolates were obtained from stem samples obtained from 70 plants. Isolates with same morphological features were grouped together and 164 representative groups of isolates were identified for DNA sequence analysis. PCR amplification of rDNA ITS region generated DNA fragment ranging from 450 to 650 bp in size. The analysis of ITS rDNA fragments using BLASTn showed that all 164 isolates belonged to species of phyla Ascomycota and Basidiomycota. Sordariomycetes (90 isolates, 54.87%) was the most frequently occurring class. These fungi were distributed over 5 orders comprising of 11 genus including Xylariales *(Hypoxylon, Whalleya, Nemania, Pestalotiopsis, Discotroma,* and *Xylaria),* Diaporthales (*Diaporthe* and *Cytospora*), Trichosphaeriales (*Nigrospora*), Glomerellales (*Colletotrichum)* and *Xylomelasma*). Many of these fungal isolates are important plant pathogens, endophytes, epiphytes, and saprophytes for other plant species. Xylariales (85.5%) was the most frequently predominant endophytes followed by *Diaporthales* (7.8%), *Trichosphaeriales* (3.3%), *Glomerellales* (2.2%), and *Xylomelasma* (1.1%) as shown in Figure 1.

To confirm identification, cluster analysis of isolated fungi was carried out using ITS rDNA and reference sequences obtained from GenBank using neighbor joining method. The fungus *Paludomyces mangrovei* of Chytridiomycota class was used as outgroup for cluster analysis. The isolates belonging to same family clustered together in the phylogenetic tree. The optimal tree with the sum of branch length (= 3.888) is shown in Figure 2 (A & B). The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Bootstrap values ≥50% are shown on branches. The phylogenetic tree represented diverse taxonomical affinities among identified taxa. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. Results from this study contribute to the understanding of species diversity in Sordariomycetes associated with *C. florida.*

Out of the organisms that reside in dogwood tissue without causing symptoms, *Hypoxylon* sp., *Diaporthe, Cytospora, Colletotrichum* sp. and *Pestalotiopsis* have been reported as plant pathogens associated with cankers, and dieback disease problems. In addition, some endophytes such as *Nigrospora* sp. have demonstrated potential as antagonists to plant pathogens. Thus, information from this study may facilitate early response to future

disease outbreak that may adversely impact dogwood production; it is also a resource in the identification of beneficial organisms that may help plant defense against pathogens (Mmbaga and Gurung, unpublished) as well as the identification of organisms that have pharmaceutical potential (Maheshwari et al., unpublished data).

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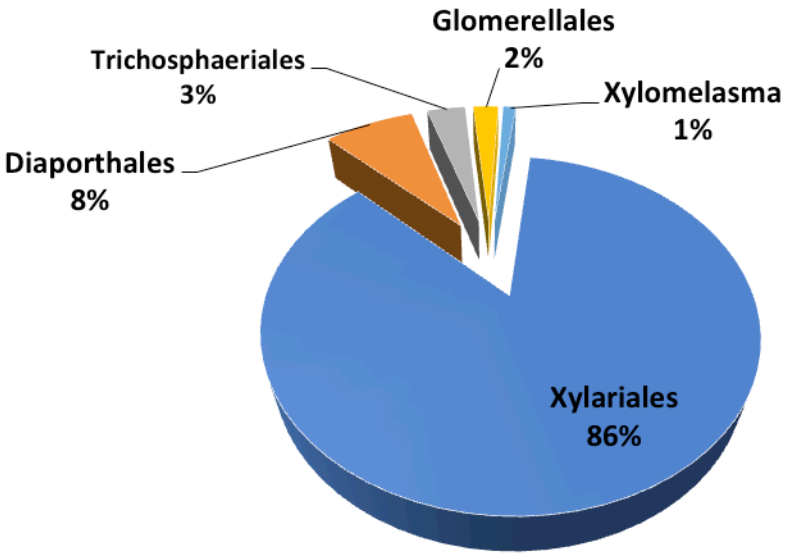

Figure 1. Relative abundance (%) of class sordariomycete at level of families isolated from *C. florida* stems. Xylomelasma belong to a large unsupported clade consisting of members the Ophiostomatales.

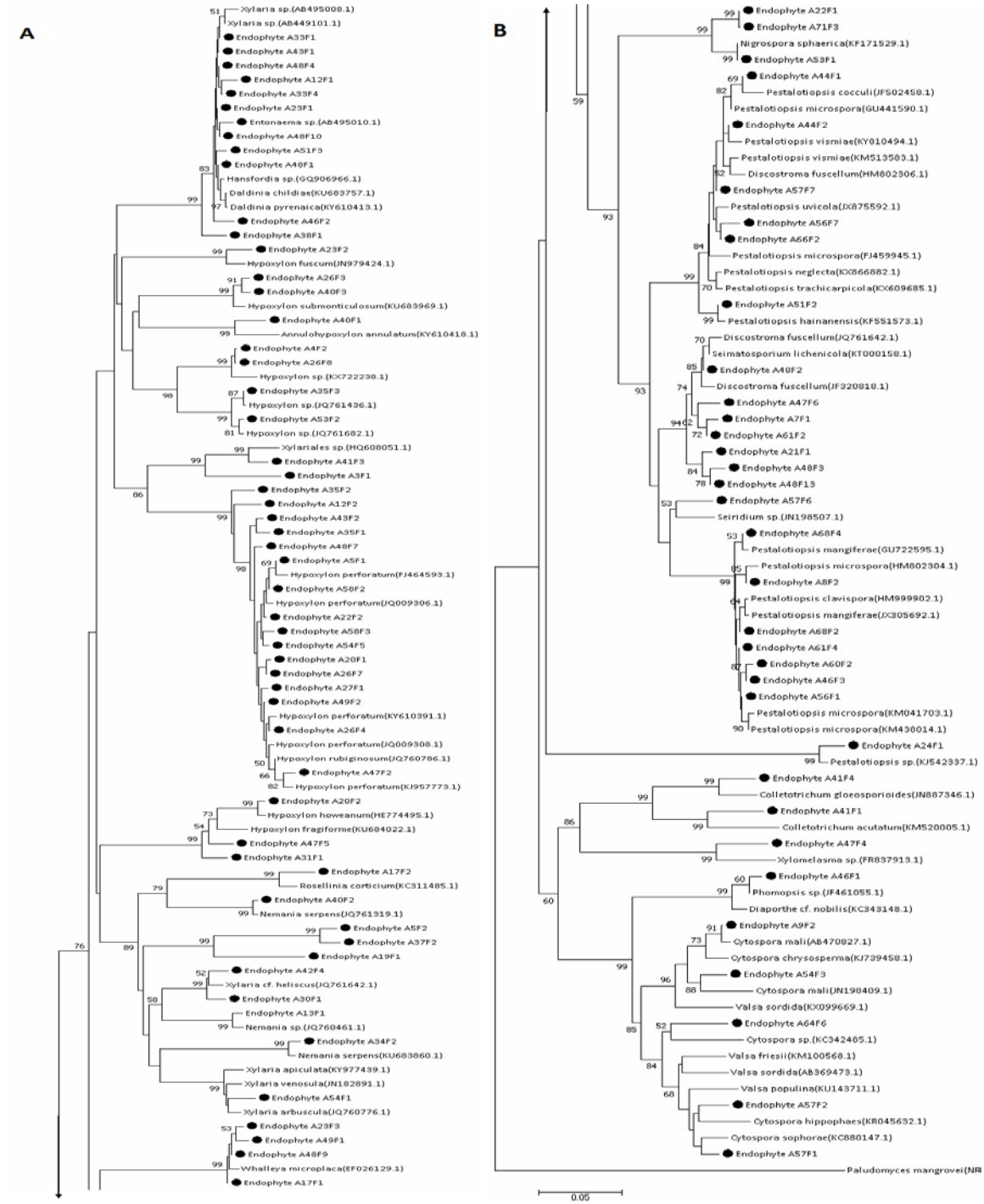

Figure 2. Dendogram (A & B) of phylogenetic analysis of endophytic fungi of class sordariomycetes associated with *C. florida*. This phylogenetic tree was constructed by using the neighbor-joining method (1,000 bootstrap replications). Bootstrap values (≥50%) are indicated at relevant nodes. The analysis involved 156 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 2678 positions in the final dataset. Evolutionary analyses were conducted in MEGA7. The fungus *Paludomyces mangrovei* was used as outgroup for clustering analysis.

Combating Rose Rosette Disease: The Disease, the Virus and the Development of New Virus Detection Technologies

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Index Words Rose rosette, virus diagnostics

Significance to Industry Over the past several decades rose rosette disease has become very serious and threatens to decimate the US rose industry [11]. The causal agent, *Rose rosette virus* (RRV, *Emaravirus*) [5, 10], is transmitted by wind-blown eriophyid mites (*Phyllocoptes fructiphilus*) [1, 2, 7] and can kill a rose within 2-3 years of infection (11). A recently-funded USDA, NIFA, Specialty Crops Research Initiative grant involves 17 scientists in 6 states working on a range of approaches to study this disease and determine how best to manage it. The only strategy currently available for disease management is early identification and eradication of the infected plants, thereby limiting its potential spread. Key to this effort is the development of efficient and affordable diagnostic tools to enable accurate detection of the virus. Molecular- and serological-based assays with potential for technology transfer and/or on-site implementation should be easy to use, offering visual detection, reliability and sensitivity to the end user. RRV-specific primers and probes (for nucleic acid-based tests) and monoclonal and polyclonal antibodies (for serology-based tests) have been developed. We report here a brief review of our recent research efforts in the development of accurate, rapid, efficient, easy-to-use and affordable rose rosette virus diagnostic tools.

Nature of Work When this NIFA, Specialty Crops Research Initiative project was initiated, *Rose rosette virus* had only recently been described and the nucleic acid-based diagnostic methods available included end-point reverse transcription-polymerase chain reaction (RT-PCR) and real-time quantitative RT-PCR (RT-qPCR). RT-PCR can be time consuming and requires skilled personnel and well-equipped laboratories. The real-time RT-qPCR assay is more sensitive for the detection of RRV, but it is also more expensive and requires even more specialized equipment. Currently, neither RT-PCR nor RT-qPCR can be used in field-based testing for RRV. The objective of this portion of the NIFA grant work is to develop one or more diagnostic techniques that are sensitive, discriminating, quick, and easy-to-run, for use both in the laboratory and the field [Table 1].

The Oklahoma State University (OSU) and University of Florida (UF) team members explored several variations of RT-PCR, including endpoint RT-PCR, TaqMan RT-qPCR, and SYBR Green RT-qPCR assays. They developed and validated an improved real-time PCR which is reliable, sensitive (with a detection limit of 1 fg viral RNA from infected plant tissue), and provides results in as little as 3 hours, thus reducing the time for analysis by 50% [3, 8]. In subsequent work, they developed a loop-mediated isothermal amplification (LAMP) assay, where the amplification reaction is carried out at a constant temperature (60–65 °C), which avoids the use of a thermal-cycler. The RT-LAMP results can be measured by turbidity, change of color, or by fluorescence using intercalating dyes that allows visualization of the reaction by the naked eye [12]. The method is friendly to lab-trained end-users and continues to be optimized for this use, but can be challenging for non-skilled operators.

The UF and OSU team members jointly developed a novel probe-based, isothermal reverse transcription-recombinase polymerase amplification (RT-exoRPA) assay. The assay is highly specific and sensitive, with a detection limit of 1 fg [5]. In addition, the assay could detect virus from leaves, stems, petals, pollen, primary roots and secondary roots [5]. A rapid (< 5 min) technique for the extraction of viral RNA was also developed and the entire assay, including the extraction, can be completed in ca. 25 min [4].

The USDA Maryland team members have focused on the serological approach. Virus-specific polyclonal and monoclonal antibodies have been developed to RRV nucleoprotein (NP) [9]. *In silico* consensus alignment of the NP proteins from >25 RRV isolates was used to identify conserved regions predicted to contain ‘good’ epitopes for analysis of the epitope-site specificity of these antibodies. To that end, twelve 25-mer synthetic peptides were produced for analysis and several NP surface-located regions were identified by these antibodies in ELISA-based assays. Known healthy and RRV-infected rose samples from a variety of geographical locations are currently being tested in a triple antibody sandwich (TAS)-based ELISA. The antibodies will subsequently be evaluated for usage in antibody-based lateral flow devices, either in-house, or in collaboration with Agdia, Inc., based on their proprietary Immunostrip platform. Another potential usage to be examined, in collaboration with the OSU and UF teams, will be to determine if using these as trapping antibodies in an Immunocapture-RT-PCR (IC-RT-PCR) assay can increase sensitivity and decrease sample preparation steps.

For the validation phase of this project, all three groups are preparing diagnostic reagent kits to be laboratory-tested by grant collaborators; including for example, by Jen Olson at the Plant Disease and Insect Diagnostic Laboratory (OSU) and by Kevin Ong at the Texas Plant Disease Diagnostic Laboratory (TAMU). As the validation process develops, the interaction between the developers of the diagnostics and the validators will lead to modifications to improve the robustness of the techniques.

Achieving the stated goals should result in availability of sensitive, accurate, reliable, and inexpensive molecular, serological, or in the case of IC-RT-PCR, combined serological/molecular tests, for diagnosing the occurrence of RRV – assays suitable for

use by high-volume commercial producers of roses, and in the case of Immunostrip lateral flow devices, even for plant collectors and gardeners.

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Table 1. Comparative analysis of the different potential diagnostic methods

Assay	Sensitivity	Skill required	Equipment needed	High throughput	Time required	Cost
Nucleic acid-based Assays						
RT-PCR	High	High	Yes	Low	6-8 hrs	High
RT-LAMP	High	Med	Yes	Med	1-3 hrs	Med
RT-exoRPA	High	Med/Low	Yes	Med	20-30 min	Med
Antibody-based Assays						
ELISA	Med	Med	Yes/No	High	4-18 hrs	Low
Immunostrip	Low/Med	Low	No	Med	10-30 min	Med

Susceptibility of *Sarcococca* Taxa to Boxwood Blight Caused by *Calonectria pseudonaviculata*

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Index Words *Sarcococca*, *Calonectria pseudonaviculata*, boxwood blight, disease resistance

Significance to Industry *Sarcococca*, or sweet box, is a genus of ornamental evergreen woody shrubs in the Buxaceae noted for their evergreen foliage, shade adaptability, insect and disease resistance, deer resistance, and fragrant winter flowers. With the recent emergence of boxwood blight, a fast-acting disease caused by *Calonectria pseudonaviculata* that affects members of the Buxaceae, questions remain regarding the susceptibility of *Sarcococca* to the blight. Boxwood blight was first reported in the United States in 2011 after being known in the United Kingdom since the 1990s (7). As of January 2017, it has since spread to at least 22 different states (6). Boxwood blight research has rightfully focused on disease susceptibility and control for *Buxus*, but other members of the boxwood family are susceptible including *Pachysandra* and *Sarcococca*. In 2014, *Sarcococca hookeriana* was reported to be infected by boxwood blight in a home landscape for the first time (8). However, no susceptibility screening for the disease has focused on *Sarcococca*, and only one unidentified *Sarcococca* species has been tested for susceptibility (5).

Nature of Work *Sarcococca* is poorly represented in American landscapes, with few species and cultivars commonly grown. If *Sarcococca* is ever to see increased landscape use or be incorporated into ornamental plant breeding programs, it is important to understand the susceptibility of the genus to boxwood blight caused by *C. pseudonaviculata*. The purpose of this study was to evaluate *Sarcococca* taxa for susceptibility to boxwood blight using a detached stem assay.

Pathogen. Eleven single-spore isolates of *C. pseudonaviculata* collected from a naturally-infected boxwood from a landscape in Dekalb County, GA (Cp1-1 to Cp1-11), were incubated on potato dextrose agar (PDA) (BD Difco, Sparks, MD) amended with ampicillin trihydrate (250 mg/L) (Sigma Aldrich; St. Louis, MO) for 2 weeks at room temperature in ambient light. Culture plates were flooded with sterilized deionized water (SDW) for 2 hr, decanted, scraped with a sterile metal spatula to remove aerial hyphae, and rinsed with SDW by swirling and decanting (1). Plates were incubated upside-down for 5 days at 23°C under 12 hr light/dark cycle. Spores were harvested by washing plates with a stream of SDW while being held over a beaker. The spore suspension was filtered through three layers of cheesecloth to remove clumps of hyphae. Spores were counted with a hemocytometer and adjusted to a final concentration of 20,000 spores/ml. This spore concentration was chosen based on the results of a stem spray inoculation assay in which

boxwood blight symptoms for *Buxus sempervirens* ‘Suffruticosa’ did not worsen at concentrations greater than 20,000 spores/ml (4).

Taxa. Sarcococca and *Buxus* taxa were acquired from several nurseries and gardens and grown at the University of Georgia Durham Horticulture Farm, Watkinsville, GA. Stems from 27 *Sarcococca* and 2 *Buxus* taxa (Table 1) were collected from plants in our collection and nearby gardens in June 2017. The collected stems were stored in moist plastic bags at 4°C. *Sarcococca* and boxwood stems were trimmed to have 10 leaves and approximately 15 leaves, respectively. The two *Buxus* taxa used in this study, *B. sempervirens* ‘Suffruticosa’ and *B. sinica* var. *insularis x B. sempervirens* ‘Green Gem’, have been previously tested for susceptibility to boxwood blight and thus served as positive controls (3).

Experiment. Four stems of each taxon were dipped in the spore suspension, with occasional swirling, for three to four minutes. Two stems of each taxon were dipped into deionized water as a non-inoculated control. Inoculated and non-inoculated stems were stuck into 50 ml plastic centrifuge tubes containing 1% water agar (10 g/l) (BD Bacto agar; Sparks, MD). Inoculated stems were randomly placed in cardboard holding trays and enclosed in clear plastic bags whose interiors were spritzed with water. Non-inoculated stems were incubated separately from inoculated stems. Following three days of incubation at room temperature on a counter in our lab, each leaf for each taxon stem was examined and the number of water-soaked leaf spots per leaf was recorded. The stems were then transferred to a growth chamber (23°C, 90% relative humidity, and 12 hr light/dark cycle) for 4 additional days.

Statistics. The differences in mean number of spots among taxa were evaluated using a one-way analysis of variance (ANOVA), and means were compared using Tukey’s Honest Significant Difference test. This was done in R (version 1.0.143), and means were grouped using the HSD.test function in the agricolae package (version 1.2-8) (9,2). Results were considered significant if $p \leq 0.05$ (Table 1).

Results and Discussion Overall, in a laboratory setting, every inoculated *Sarcococca* and *Buxus* taxon developed water-soaked spots, indicating susceptibility to boxwood blight (Table 1). No taxa were deemed immune. Nearly all controls had no spots three days after inoculation, except *S. hookeriana* ‘Purple Stem’ and *S. humilis* ‘Sarsid2’, which had 2 and 1 spots total, respectively (data not presented). These appeared to be caused by contamination during the inoculation process and not by some unknown factor.
Most *Sarcococca* taxa had a greater mean number of spots per leaf than the *Buxus* positive controls, though this is somewhat misleading because of smaller leaf size in the boxwood taxa. All *S. confusa* had significantly greater mean spots per leaf than any other plant tested. This is highly important to the green industry because *S. confusa* is among the most popular sweet boxes in cultivation.
Perhaps most interestingly, two sweet box taxa had almost no spots three days after inoculation. Both *S. ruscifolia* from Atlanta Botanical Garden and *S. ruscifolia* var. *chinensis* ‘Dragon Gate’ had a mean number of water soaked spots per leaf of 0.05 (Table

1). These plants may be valuable for breeding blight resistance into new cultivars and should be considered for increased landscape use. We are optimistic that these taxa may represent a source of resistance to boxwood blight in *Sarcococca*, though further testing is needed to confirm.

The results of this study indicate that susceptibility of *Sarcococca* to boxwood blight is variable, even within species. For example, for *S. hookeriana*, one taxon tested averaged 21.4 spots per leaf (HF source), while another only had 2.2 spots per leaf (FRF source). Knowing the susceptibility of cultivated *Sarcococca* will help guide landscape usage of sweet box and may inform breeders interested in introducing blight resistance into new releases. In the future, the experiment will be repeated and other work will be done to clarify boxwood blight susceptibility in *Sarcococca*.

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Table 1. Mean number of water-soaked spots per leaf of *Sarcococca* and *Buxus* taxa three days after being inoculated with Calonectria pseudonaviculata in June 2017.

Taxon	***Mean Spots per Leaf[z]***	***Group[y]***	***Source[x]***
Sarcococca confusa	37.7	a	SBG
S. confusa	33.2	ab	GPN
S. confusa	28.5	abc	HF
S. orientalis	24.8	a-d	HF
S. hookeriana	21.4	a-e	HF
S. vagans	21.3	a-e	FRF
S. humilis 'Sarsid2'	18.5	a-e	RFN
S. ruscifolia	16.1	a-e	SBG
S. sp.	15.0	a-e	ABG
S. saligna	13.7	b-e	JCN
S. wallichii	12.3	b-e	FRF
S. humilis 'Sarsid1'	11.2	b-e	NC
S. humilis	9.8	b-e	SBG
S. orientalis	8.9	c-e	HF
S. ruscifolia	8.1	c-e	JCN
S. ruscifolia	7.2	c-e	SBG
S. hookeriana 'Purple Stem'	7.0	c-e	PDN
S. humilis	6.0	c-e	WH
S. ruscifolia var. chinensis	5.9	c-e	PDN
S. orientalis	5.9	c-e	SBG
S. ruscifolia	5.4	c-e	WH
S. sp.	5.3	c-e	ABG
S. saligna	5.0	de	WH
S. sp.	4.4	de	HF
S. hookeriana	2.2	de	FRF
Buxus sempervirens 'Suffruticosa'	1.5	de	SFN
*B. '*Green Gem'	0.9	e	HF
S. ruscifolia	0.05	e	ABG
S. ruscifolia var. *chinensis* 'Dragon Gate'	0.05	e	FRF

[z]Means determined using four detached stem replicates per taxon with 10 leaves per stem for *Sarcococca* and 15 leaves per stem for *Buxus*.

[y]Treatments followed by the same letter within a column are not significantly different at $p \leq 0.05$. Means were separated using Tukey's Honest Significant Difference test.

[x]ABG = Atlanta Botanical Garden, Gainesville, GA; FRF = Far Reaches Farm, Port Townsend, WA; GPN = Griffith Propagation Nursery, Watkinsville, GA; HF = UGA Durham Horticulture Farm, Watkinsville, GA; JC = Joy Creek Nursery, Scappoose, OR; NC = Nurseries Caroliniana, North Augusta, SC; PDN = Plant Delights Nursery, Raleigh, NC; RFN = RareFind Nursery, Jackson, NJ; SBG = State Botanical Garden, Athens, GA; SFN = Silver Falls Nursery, Salem, OR; WH = Willis Harden, Commerce, GA.

Effect of Fungicides and Biocontrol Products on Phytophthora Root Rot of Hydrangea

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Index words *Hydrangea macrophylla*, *H. paniculata*, *H. quercifolia,* bigleaf hydrangea, panicle hydrangea, oakleaf hydrangea, Phytophthora root rot, fungicide and biocontrol products, *Phytophthora nicotianae*

Significance to Industry Hydrangeas are very popular summer–flowering deciduous shrubs in the landscape. With the recent advances in plant breeding, hydrangeas are now available in almost every size, color, and form imaginable. Many new cultivars also have the ability to rebloom, which further adds to their popularity among gardeners. Phytophthora root rot is one of the most important diseases, which affect wide range of nursery-grown woody ornamentals. Almost all hydrangeas are susceptible to Phytophthora root rot disease. Few hydrangea species such as the oakleaf hydrangeas (*Hydrangea quercifolia*) and panicle hydrangeas (*H. paniculata*) are more susceptible to this disease while other species such as the bigleaf hydrangea (*H. macrophylla*) come out to be more resistant. Through *Phytophthora*-contaminated container stock, rooted cuttings this pathogen can be introduced very easily into a nursery. Also water serves as an important means of introduction and spread of *Phytophthora* species in nursery production. The most effective management for controlling Phytophthora root rot is the proper application of fungicides (1, 2). Biocontrol products have gained increased attention of end-users for their environmental benefits and short worker re-entry interval. The rationale of this work is to present efficacy test results for hydrangea Phytophthora root rot management to help nursery growers to make proper management decisions about fungicides and biocontrol products to use on their production.

Nature of Work Hydrangeas are one of the most showiest and spectacular flowering woody ornamental plants in the landscape. In hydrangea productions the most important and commonly observed diseases are root diseases which can cause a continuing and permanent damage of the plant. Phytophthora root rot is considering one of the most economically important diseases in container and field grown production of hydrangeas. Sudden wilting, yellowing of the foliage, brown discoloration of infected roots are the main symptoms of the Phytophthora root rot and discoloration can be seen on crown at the soil line and stem above the soil line as well. Phytophthora root rot can be easily prevented with sanitation, cultural practices and chemical treatments (1, 3). Various products including fungicides and biocontrol products are available or in development that have the potential to contribute to the management of Phytophthora root rot.

The objective of this study is to test the efficacy of biocontrol products and fungicides to control Phytophthora root rot of hydrangea.

Biocontrol products and fungicides including RootShield *PLUS*, MBI110, IT-1503, OxiPhos, Grotab, Pageant Intrinsic, TerraClean 5.0 + TerraGrow program, Segovis, Empress Intrinsic, and Subdue Maxx were tested (Table 1). The efficacy of biorational products and fungicides against Phytophthora root rot of hydrangeas were assessed in greenhouse and field experiments. Uniform *H. quercifolia* 'Picnic Hill' and *H. macrophylla* 'Princess Juliana' were transplanted into a #1 nursery container in a greenhouse. A pine bark substrate was amended with Micromax (The Scotts Co., Marysville, OH) and AquaGro (Aquatrols, Paulsboro, NJ) and 19-5-9 (19N-2.2P-7.5K) Osmocote Pro 12 to 14 month controlled release fertilizer (Everris, Dublin, OH). Plants were watered daily using overhead irrigation system. Uniform *H. paniculata* were planted at the Otis L. Floyd Nursery Research Center in McMinnville, TN in field plot with Waynesboro loam soil. Plants were fertilized with 10 g of 18-6-8 Nutricote controlled-release fertilizer. Plants were watered as needed using drip irrigation system. Field plots and pots were inoculated with *P. nicotianae* infested rice grains. Treatments were applied as drench application. Plants were evaluated for Phytopthora root rot after 3 months of the first application. The experimental design was a randomized complete block design, and treatments were arranged with four replications of each plots and pots. Analysis of variance (ANOVA) was performed on the data set using the general linear model (GLM) procedure with SAS statistical software.

Results and Discussion In greenhouse experiments the severity of Phytophthora root rot were ~28 and 61% root affected, whereas in field study the severity was ~69% (Table 2). All of the treatments significantly reduced Phytophthora root rot severity compared to non-treated inoculated control. Plants treated with Empress Intrinsic, Segovis, Subdue Maxx, Pageant Intrinsic, MBI110 and Terraclean + TrerraGrow program had significantly less Phytophthora root rot than the other treatments in greenhouse experiments. The treatments most effective in reducing Phytophthora root rot severity were Segovis, Empress Intrinsic, Subdue Maxx and MBI110 in field experiment. Phytotoxicity and defoliation were not observed in any of the hydrangea plants. Bigleaf hydrangea (*H. macrophylla*) is more resistant than oakleaf hydrangea (*H. quercifolia*) against Phytophthora root rot.

An integrated approach should be used to control Phytophthora root rot in the nursery production. Results of both greenhouse and field experiments indicate that fungicides Segovis, Empress Intrinsic, Subdue Maxx provide control of Phytophthora root rot. Nursery producers could benefit from using MBI110 and Terraclean + TrerraGrow program alone or in a rotation of the fungicides typically drenched to control Phytophthora root rot in hydrangeas.

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Table 1. Treatments, active ingredients and rates.

Treatment	**Active ingredient**	**Rate**
Empress Intrinsic	Pyraclostrobin	3 fl oz/100 gal
Grotab	*Glomus* spp., *Trichoderma atrviride* and bacteria of the rhizosphere	2 tablets/plant
IT-5103	*Trichoderma* spp.	2 g/plant
MBI110	*Bacillus amyloliquefaciens*	1.0 %
OxiPhos	Mono- and di-potassium salts of phosphorus acid and hydrogen peroxide	0.2%
Pageant Intrinsic	Boscalid + pyraclostrobin	12 oz/100 gal
RootShield *PLUS*	*T. harzianum* Rifai strain T-22, *T. virens* strain G-41	8 oz/100 gal
Segovis	Oxathiapiprolin	3 fl oz/100 gal
SoilGard	*Gliocladium virens* strain GL-21	2 lb/100 gal
Subdue Maxx	Mefenoxam	2 fl oz/100 gal
TerraClean 5.0 + TerraGrow	Hydrogen dioxide and peroxyacetic acid, *Bacillus* spp. and *T. harzianum*	0.2% + 1 oz/10 gal 0.4 oz/10 gal 0.1 oz/10 gal

Table 2. Effect of biocontrol products and fungicides on severity of *Phytophthora* root rot disease on hydrangeas.

Treatment	Greenhouse experiments		Field experiment
	Phytophthora root rot severity on bigleaf hydrangea (%)[z]	*Phytophthora* root rot severity on oakleaf hydrangea (%)[z]	*Phytophthora* root rot severity on panicle hydrangea (%)[z]
Empress Intrinsic	4.5 b[y]	19.3 bc	14.9 ef
Grotab	10.9 ab	48.4 ab	-
IT-5103	-[x]	-	35.8 b
MBI110	6.8 b	10.9 c	17.1 def
OxiPhos	-	-	33.3 bc
Pageant Intrinsic	4.5 b	19.3 bc	-
RootShield *PLUS*	12.8 ab	31.8 abc	29.9 bcd
Segovis	4.5 b	27.6 bc	9.2 ef
Subdue Maxx	4.5 b	19.3 bc	15.6 ef
TerraClean 5.0 + TerraGrow	6.8 b	19.3 bc	21.4 cde
Non-treated, inoculated control	27.6 a	60.9 a	68.9 a
Non-treated, non-inoculated control	2.3 b	4.5 c	4.2 f
P value	0.0081	0.0002	≤0.0001

[z]Disease severity rating was based on percentage root affected.
[y]Values are the means of four replicate plots and containers. Treatments followed by the same letter within a column are not significantly different at $P \leq 0.05$.
[x]Treatment was not included into the trial.