Pathology and Nematology

Alan Windham

Section Editor

Nuisance Fungi of Bark Mulches and Media

John Olive[1] and Alan Windham[2]

[1]Auburn University Ornamental Horticulture Research Center, Mobile, AL 36608
[2]University of Tennessee Extension Soil, Plant Pest Center, Nashville, TN 37211-5112

olivejw@auburn.edu

Index Words: bird's nest fungi, stinkhorn, artillery fungus, *Cyathus*, *Sphaerobolus*, *Leucocoprinus*, *Mutinus, Fuligo septica,*

Significance to Industry: Wood, bark and other forest by-products make up a significant portion of organic mulches in landscapes and potting media in commercial nurseries. Unfortunately, these substrates provide the perfect growing media for a number of nuisance fungi. Although these fungi are not harmful to the plants growing in the media, they are often unsightly and aesthetically unappealing, or malodorous and can be disagreeable to the final consumer. These fungi may speed decomposition of bark substrates and mulches.

Nature of Work: Various fungi that grow in landscape mulches and potting media are regular submissions to plant disease diagnostic labs and are often observed on field visits to nurseries and greenhouses. Most of these fungi are not harmful to the plants they are growing with so identifying these nuisance fungi is useful to growers and consumers alike to ease fears and prevent unnecessary chemical applications.

Results and Discussion: There are many fungi that are considered a nuisance in potting media, greenhouses, and landscape mulches. Only a few are covered here.

Bird's Nest Fungi are so named because they are vase shaped and resemble a tiny bird's nest complete with a cache of eggs. The fruiting structure varies in size depending on the species but is usually in the range of 5-12 mm (1/5 to ½ inches) across. The "eggs" are a group of spore sacs called peridioles. Dispersal of the spores occurs when a drop of water hits the cup and the eggs are splashed out as far as 7 feet (1). A number of genera and species make up this group of fungi with the most common being *Cyathus* and *Crucibulum spp.* These fungi can cover house siding with black specks which are difficult to wash off. On greenhouse and nursery plants the peridioles can look like insect frass and be so numerous on foliage that they cause the grower to believe they have an insect infestation. Peridioles of this group are easily recognized as they often have a length of white, hyphae attached called a funicular cord. They do not cause damage to the plants and there is no need to control them.

Artillery Fungi in the genus *Sphaerobolus* are much smaller (0.04-0.1 inches or 1-3 mm) than the bird's nest fungi and therefore are often not as visible and are difficult to detect. These "cannons" can send the single spore sac shot from the tiny fruiting body

as far as 17 feet (1). Like the bird's nest fungi, they do no damage to plants but the tiny projectiles can cover leaves, plant labels, and siding and are unsightly and often difficult to remove. Artillery fungus is commonly found on hardwood mulch. Choosing an alternative mulch can reduce the incidence of this fungus as can mixing mushroom compost with mulch (2).

Yellow Houseplant Mushroom, Yellow Parasol, or Flower Pot Parasol (*Leucocoprinus birnbaumii*) is commonly found growing in containers in greenhouses and houseplants. As the common name indicates, it is easily identified as a yellow mushroom 1-4 inches tall. The cap usually has a nipple in the center and is 1-3 inches across. There are reports of this mushroom causing gastrointestinal discomfort in some people so it is not advisable to eat this mushroom (3) but it is not damaging to the plant and there is no control recommended. They can be enjoyed as an unusual color surprise in houseplants. Containers infested with Leucocoprinus may have an extensive network of mycelium throughout the bark substrate which may affect water retention.

Stinkhorn is the common name given to a number of fungi which are common in landscape wood mulches, three of which are discussed here. Stinkhorns are often first noticed as a slimy, gelatinous white egg shaped mass buried in mulch. The stinkhorns emerge from this structure and are identified by their shape. The Elegant Stinkhorn, *Mutinus elegans* forms a single pink to red narrow column 4-7 inches tall. Sometimes the top portion will be covered with a brown slime. The Starfish Stinkhorn, *Aseroe rubra* emerges from the egg as a hollow white stalk topped with a crown of deeply divided arms radiating from the center like a flower. The Columnar Stinkhorn, *Linderia columnata* erupts from the egg as 5 to 7 spongy red to orange columns that are joined at the top. They can be 6 inches tall (4).

As indicated by the name, all are identifiable by the putrid smell they produce at maturity. When conditions are ideal, they can emerge in large numbers and be very malodorous. They are often detected by their odor long before they are observed. Flies and other insects are attracted to stinkhorns and disperse spores. They are usually short lived and do not last long but can be removed by hand if needed-rubber gloves recommended.

Dog Vomit Slime Mold is not a fungus but is included in this discussion because it looks like a fungus and is a nuisance in bark mulches. It is in the Kingdom Protista like the amoeba (3). This organism, *Fuligo septica* appears in early evening as a white-to-yellow gelatinous mass which slowly moves across mulch. In pre-dawn hours, this slime mold contracts to form soft crusty growth, which is sometimes mistaken for something a dog threw up. It can appear in mulch, lawns, or even bare ground. It is extremely common on hardwood mulch. As with the other fungi, this growth is not harmful, does not last long, and control is not required.

Other common fungi found in mulch include Coprinoid mushrooms (ink caps), *Leucoagaricus americanus*, *Gyromitra esculenta* (false morels) and puffballs such as *Geastrum, Lycoperdon,* and *Scleroderma.*

None of these fungi are harmful to the plants they grow adjacent to but are often the subject of frantic calls or emails to plant diagnostic labs and University Horticulture Departments. Being able to identify these nuisance fungi is useful in educating consumers as well as possibly preventing unnecessary chemical applications.

Literature Cited

1. Arora, David. 1979. Mushrooms Demystified. Ten Speed Press. 959pp.
2. Davis, DD., L.J. Kuhns and T. L. Harpster. 2005. Use of mushroom compost to suppress artillery fungi. J. of Envron. Hort. 24:212-214.
3. Volk, Tom. 2006 . Tom Volk's Fungi: Fungus of the Month. http://TomVolkFungi.net
4. Weber, Nancy Smith and Alexander H. Smith. 1985. A Field Guide to Southern Mushrooms. The University of Michigan Press. 280pp.

Nuisance Fungi of Bark Mulches and Media

A, D. *Fuligo septica*, B. *Mutinus elegans*, C. Mushrooms colonizing bark mulch, E. *Leucocoprinus birnbaumii*, F. *Aseroe rubra*, G. *Linderia columnata*, H. *Geastrum* sp., I. *Cyathus striatus*, J. *Lycoperdon* sp., K. *Sphaerobolus stellatus*

First Report of Boxwood Blight in Tennessee

Alan Windham[1], Mark Windham[2], Anni Self[3], Bruce Kauffman[4] and Tom Stebbins[5]

[1,4]UT Extension, Soil, Plant and Pest Center, Nashville, TN 37211
[2]Entomology and Plant Pathology Department, Knoxville, TN 37996
[3]Tennessee Department of Agriculture, Nashville, TN 37220
[4]UT Extension-Hamilton Co., Chattanooga, TN 37416

Index Words: boxwood blight, *Buxus*, *Calonectria pseudonaviculata*, *Cylindrocladium buxicola*

Significance to Industry: Boxwood is one of the most important woody ornamentals for the nursery and landscape industry in the Southeast. In October 2011, boxwood blight caused by *Calonectria pseudonaviculata syn Cylindrocladium buxicola* was identified in Western North Carolina and Connecticut (4). Boxwood blight has been reported in Western Europe since the 1990's, however, these represented the first reports for boxwood blight in the U.S. Since 2011, boxwood blight has been reported in many states and a few Canadian provinces. In June, 2014, boxwood blight was identified on boxwoods in a garden center in Chattanooga, TN. This represents the first report in Tennessee. Additional reports in Tennessee followed in September and November, 2014, and April and May 2015.

Nature of the Work: Boxwood blight caused by *Calonectria pseudonaviculata* represents the greatest threat to the culture of *Buxus* spp. in the United States. Boxwood blight can cause severe blight and leaf loss on susceptible cultivars (3). Also, additional plant taxa are susceptible to boxwood blight. *Pachysandra terminalis* and *Pachysandra procumbens* (6) are hosts of *C. pseudonaviculata*, as is *Sarcococca* spp. The biggest threat is the movement of infected plant material throughout the United States.

Results and Discussion: Boxwood blight was first identified in June 2014 in a garden center in Chattanooga, TN on boxwood. In September 2014, boxwood blight was identified on boxwood in a garden center in the Tri-Cities region of TN. In November, 2014, a landscape contractor in Nashville submitted boxwood specimens from a local landscape planting to the Soil, Plant and Pest Center which was positive for boxwood blight. In April and May, 2015, boxwood blight was found on boxwood in two garden centers in Metropolitan Nashville. In all cases, the infected boxwoods were traced to out-of-state wholesale nurseries. Infected plants were bagged and taken to local land fields.

Boxwood blight continues to spread. In 2015, boxwood blight was identified in garden centers in Alabama and at least one wholesale nursery in Florida. There is a real need to educate members of the green industry of the threat that boxwood blight poses; how to identify boxwood blight and how to manage boxwood blight.

Boxwood blight was confirmed by placing symptomatic plant material (leaves with leaf spots, and stems with black stem lesions) in moist chambers at room temperature. Plant

material was examined microscopically for sporulation, typical, cylindrical-rod shaped spores, and spear shaped vesicles.

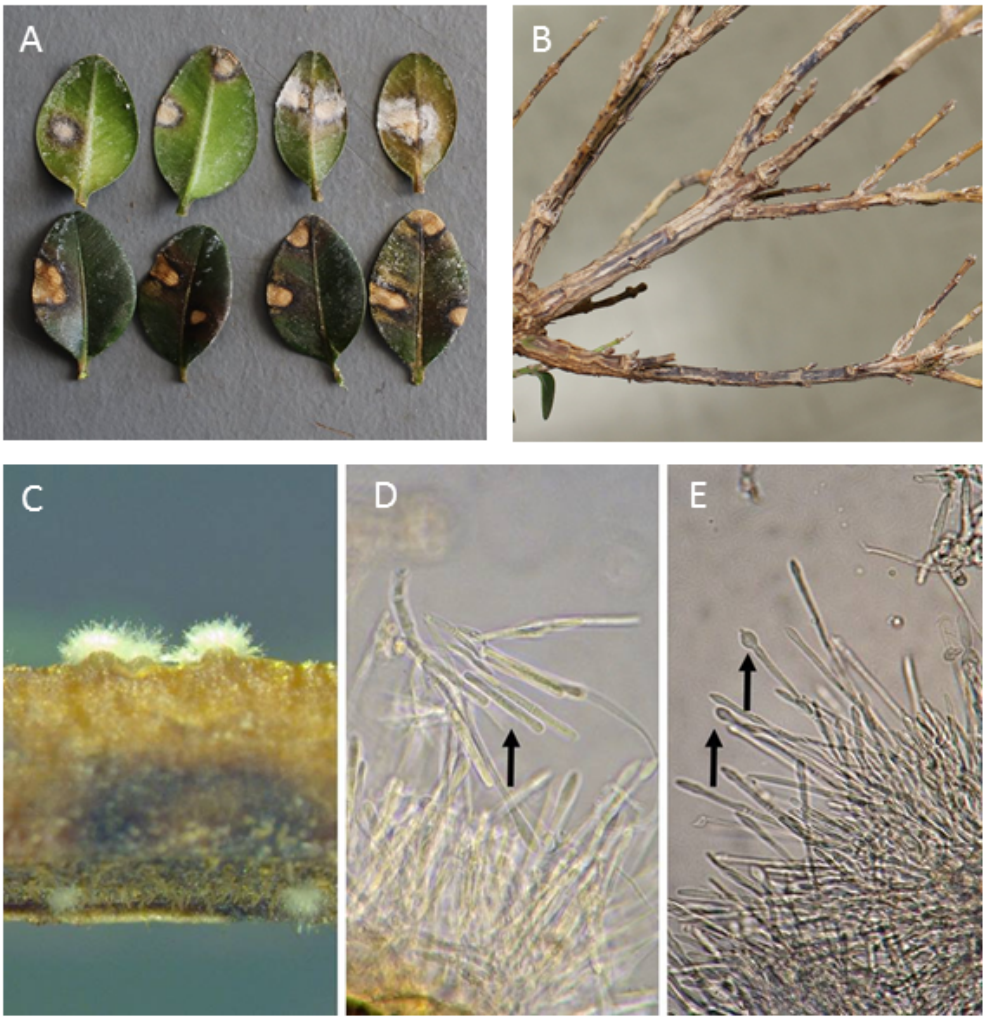
Plants with symptoms associated with boxwood blight should be reported to your state department of agriculture and/or to your local Extension agent. Best management practices for boxwood blight include:

- Being familiar with signs and symptoms of boxwood blight. All incoming and outgoing boxwood should be inspected.
- Quarantine newly acquired boxwood liners, plants, etc. for at least 1 month. Do not place newly acquired boxwood near existing stock as they may all have to be destroyed if boxwood blight is found.
- Follow a "systems approach" to managing plant diseases (1). This would include looking at critical control points: incoming plants, substrate storage, propagation areas, production areas, etc.
- Fungicides can protect boxwood from blight, but will not cure infected plants (5).
- Flaming has been used in areas where infected plants have been removed to kill the boxwood blight pathogen in leaf litter (2). It is not 100% effective.
- Infected plants should be bagged and taken to a local land field.
- Contact your state department of agriculture regarding a boxwood blight compliance agreement, which indicates your agreement to follow best management practices in the production of boxwood.

Literature Cited

1. Cochran, D., A. Fulcher, F. Hale, A. Windham. 2014. An overview of systems-based pest management for nursery production. UT Extension. https://extension.tennessee.edu/publications/Documents/PB1825.pdf
2. Dart, N. L., Arrington, S. M., and Weeda, S. M. 2012. Flaming to reduce inocula of the boxwood blight pathogen, Cylindrocladium pseudonaviculatum, in field soil. Online. Plant Health Progress doi:10.1094/PHP-2012-1026-01-BR.
3. Ganci, M., D. M. Bensonand K. Ivors. 2012. Susceptibility of commercial boxwood taxa to *Cylindrocladium buxicola*. Acta Hortic. (ISHS) 1014:369-370.
4. Ivors, K. L., et al. 2012. First report of Boxwood blight caused by *Cylindrocladium pseudonavicalatum* in the United States. Plant Disease. Vol. 96, No. 7. p. 1070.
5. Lamondia, J. A. 2014. Fungicide efficacy against, *Calonectria pseudonaviculata,* causal agent of boxwood blight. Plant Disease Vol. 98, No. 1, pp. 99-102
6. Lamondia, J. A., and S. M. Douglas. 2014. *Calonectria pseudonaviculata* can cause leaf spot and stem blight of *Pachysandra terminalis* and *Pachysandra procumbens*. Acta Hortic. 1085:205-206.

Boxwood Blight Signs and Symptoms



Symptoms: A, leafspots with sporulation, B. Black Stem lesions; **Signs**: C, *Calonectria pseudonaviculata* sporulating on a boxwood stem, D, Cylindrical rod shaped conidia, E, Spear shaped vesicles.

Impact of Mycorrhizae on Growth of *Echinacea purpurea*

David I. Yates[1] and Kimberly D. Gwinn[1]

[1]Department of Entomology & Plant Pathology
2505 E. J. Chapman Drive
370 Plant Biotechnology Building
Knoxville, TN 37996

yatesd@utk.edu

Index Words: Mycorrhizae fungi, *Echinacea*, Arbuscular, AM

Significance to Industry: Arbuscular mycorrhizal (AM) symbioses are integral components of root systems in most plants in natural settings. Their role in commercial agriculture is uncertain because the relationship between the plant and its symbiont can be, positive (mutualistic), neutral, or negative (parasitic); depending upon plant species, fungal species, soil chemistry, and available nutrients (1, 2). Several potting media and soil amendment products that contain AM are commercially available and are advertised to increase plant growth and drought resistance. Carefully controlled research supports the advertising claims of increased plant growth, drought resistance, and secondary metabolite production (1, 2, 3), but little is known about these benefits outside the research setting. There is generally increased expense associated with AM-augmented media and use of these products are only cost effective if production or marketability is increased.

Echinacea purpurea (Asteraceae) is a perennial plant marketed as an ornamental and as a medicinal plant. Dried roots and plant material serve as the base for dietary supplements associated primarily with stimulation of the immune systems (3, 4). Previous studies by our research group determined that *E. purpurea* plants treated with AM had a 13-fold greater biomass than untreated plants and required significantly few fertilizer inputs (most notably phosphorous) (3). Phytochemical profiles changed very slightly in response to AM symbiosis so the enhanced biomass would yield increased profits for the grower. The purpose of this study was to determine if the enhanced production achieved in carefully controlled experiments with plants grown in clay-based artificial medium (Turface^{TM}) and inoculated with AM cultures that are routinely monitored by research staff could be duplicated when relatively untrained personnel and resources available to producers were used.

Nature of Work: Research was conducted at two greenhouse locations, University of Tennessee (Knoxville, TN) (UT) and David Crockett High School greenhouses (Jonesborough, TN) (DC). Locations used differed in personnel caring for the plants, greenhouse types, water source, light, and amount of customer traffic in the greenhouse. Plants were planted, inoculated, watered, and fertilized by undergraduate (UT) and high school agriculture (DC) students with no specialized training in horticulture and no vested

interest in the research. Three treatments were used in both studies: 1) ready-to-use greenhouse growing medium with AM as purchased from a local vendor (none); 2) the media described above sterilized by autoclaving at 240°F for 60 minutes twice (24 hours apart) [autoclaved treatment (auto)]; 3) autoclaved treatment with commercially-available AM fungi added (auto + AM). *Echinacea purpurea* seed (Johnny's Selected Seed, Winslow, Maine, USA) were chilled (4 C) for 48 hours then planted in one of the three treatments (three seeds per container), watered, and fertilized (1/2 teaspoon Fertilome and 1/8 teaspoon Micromax per container). The experimental design was a completely random block design with three treatments and 45 replications repeated at two locations. Potted plants were separated into random blocks and placed on drip irrigation. Growth data (largest leaf and height measurements) were collected at 30, 60, 90, and 120 day intervals. At 120 days, plants were removed from the pots, and the roots were washed and cleaned. A root subsample (0.5 g fresh weight) was collected from each plant. Plants from each pot were combined and dried in a drying oven; mean dry weight/plant was calculated. Fungal colonization was determined by the method of McGonigle *et al*. (5).

Data were analyzed by PROC Mixed and analyzed for significance by a Tukey's Studentized Range Test at 5% confidence level for non-destructive measurements (largest leaf and plant height). For data collected only at 120 days (e.g., weight and AM colonization) means separation was by an F-protected ANOVA. All data was analyzed using SAS (SAS Institute, Cary, NC, USA). Significance level of $P < 0.05$ was used for all tests

Results and Discussion: There was a significant effect of treatment on fungal colonization percentages among all treatments at both growing locations ($P \leq 0.0001$) (Figure 1). Plants in the untreated media treatment (none) had approximately two-fold greater fungal colonization than those planted in the autoclaved medium (auto) and four-fold greater fungal colonization than the plants in the autoclaved medium with additional mycorrhizae (auto + AM) ($P \leq 0.0001$) (Figure 1). Increase in plant dry weight was consistent with increased mycorrhizal colonization at DC, but not UT (Figure 2). At DC, plants potted in autoclaved medium with added AM (auto + AM) weighed significantly less than plants in the other two treatments ($P \leq 0.0001$) (Figure 2).

There were significant effects of time on both largest leaf and plant height ($P \leq 0.0001$, for all times at both locations), but there was no effect of treatment or time x treatment interaction for the largest leaf measurements for plants grown at either UT or DC. There was no effect of treatment or time x treatment interaction on plant height at UT. At DC, heights of plants grown for 120 days in media that were autoclaved and to which mycorrhizae was added (auto + AM) were greater than those grown in autoclaved media (auto) for 120 days ($P = 0.0237$), but plants grown in media that was not autoclaved (none) were not different from any treatment at 120 days. Plants in the auto + AM treatment were taller at 120 days, but their dry weight was significantly lower (Figure 2). Crown measurements for the plants in auto + AM treatments were 20 - 25% less than those for the other two treatments (data not shown).

In this study, we attempted to duplicate results from a highly controlled research experiment with materials available to commercial growers; however, the percentage enhancement of growth in the previous study were not duplicated in this experiment. The clay media used in the research study provided essentially no nutrients for plant growth whereas in this study the plants were grown in a medium optimized for plant growth. Following initial experimental setup, plants were tended by untrained students in a commercial greenhouse setting (DC) and in an educational setting (UT). Subsequent watering, fertilizing, and insect/disease scouting and eradicating were conducted by students. Without trained personnel overseeing daily operations, the plants were relatively neglected. UT greenhouse provided better light, space, and temperature conditions, whereas the DC greenhouse provided a lower quality light source, less space due to other plants being grown, and greater fluctuation of temperature due to customer/student traffic. Seeds planted at DC germinated later and plants grew slower initially, possibly as a result of lower light intensity and duration. After additional lighting was provided plants grown at DC plants eventually accelerated in growth and surpassed the UT plants in weight. Better trained, more diligent, and more experienced growers might see greater effects than those that practice benign neglect, as in this study where plants were not properly or timely fertilized.

Even though *Echinacea purpurea* growers using ready-to-use greenhouse growing medium with AM may have mycorrhizal colonization, colonization in greenhouse growing media does not have the impact that would have been predicted from the previous research study. Growers have to consider both benefits and costs of growing media with added mycorrhizae. If *E. purpurea* is transplanted into the field as is typical for production of medicinal supplements, mycorrhizal colonization might enhance early growth of transplants. The increase in weight in this study may be a result of increased root production. Growers harvesting *Echinacea* roots for phytochemical extraction may benefit more from these findings than growers harvesting/selling *Echinacea* as landscape plants or cut flowers.

Literature Cited:

1. Smith, F.A. and S.E. Smith. 2011. What is the significance of the arbuscular mycorrhizal colonization of many economically important crop plants? Plant Soil 348:63-67.
2. Baum, C., W. El-Tohamy, and N. Gruda. 2015. Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal. Scientia Horticulturae 187:131-141.
3. Gualandi, R.J., R.M. Auge, D.A. Kopsell, B.H. Ownley F. Chen H.D. Toler, M.M. Dee, and K.D. Gwinn. 2014. Fungal mutualists enhance growth and phytochemical content in *Echinacea purpurea*. Symbiosis 63:111-121.
4. Barrett, B. 2003. Medicinal properties of *Echinacea*. Phytomedicine 10:66-86.
5. McGonigle, T.P., M. H. Miller, D.G. Evans, G.L. Fairchild, and J.A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115:495-501.

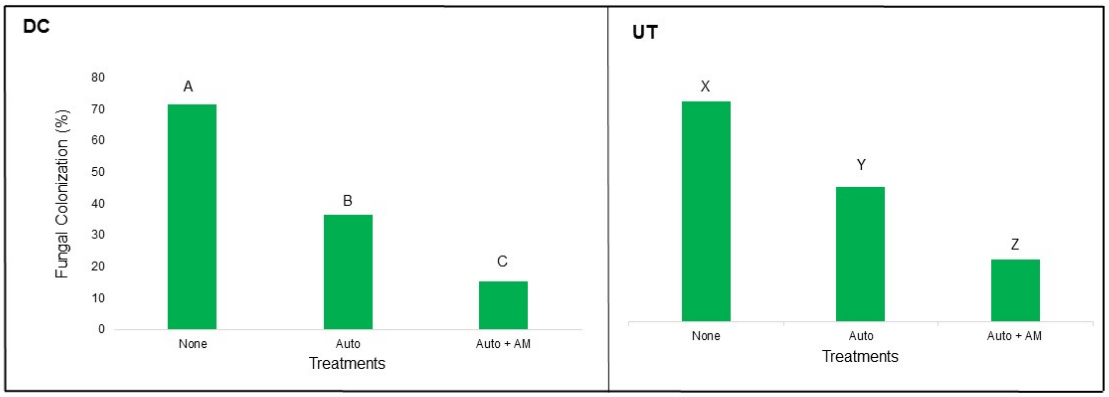


Figure 1. Impact of mycorrhizal treatments on colonization of roots of *Echinacea purpurea* in two greenhouse locations. Seed were planted in commercial greenhouse medium that contained mycorrhizae (none), in the same medium that had been autoclaved (auto), or in autoclaved media supplemented with a commercially available mycorrhizae inoculum (auto + AM). Plants were grown for 120 days; root subsamples were stained and colonization evaluated as described by McGonigle et al (5). Data for each location were analyzed separately. Means with the same letter are not significantly different at α = 0.05. DC = David Crockett High School; UT = University of Tennessee.

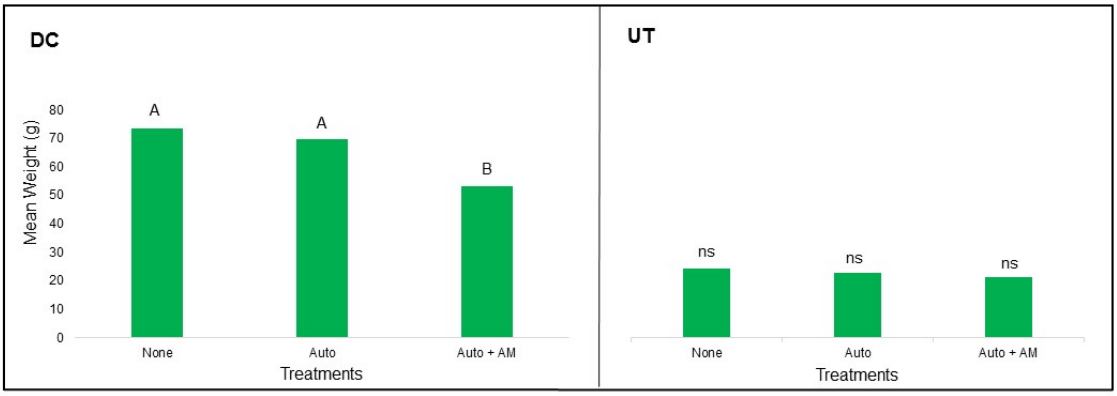


Figure 2. Impact of mycorrhizal treatments on biomass of *Echinacea purpurea*. Seed were planted in commercial greenhouse medium that contained mycorrhizae (none), in the same medium that had been autoclaved (auto), or in autoclaved media supplemented with a commercially available mycorrhizae inoculum (auto + AM). Plants were grown for 120 days; plants (root + shoot) were dried to a constant biomass. Data for each location were analyzed separately Means with the same letter are not significantly different at α = 0.05. DC = David Crockett High School; UT = University of Tennessee

Table 1. Impact of mycorrhizal treatments on plant height of *Echinacea purpurea* grown at David Crockett High School. Seed were planted in commercial greenhouse medium that contained mycorrhizae (none), in the same medium that had been autoclaved (auto), or in autoclaved media supplemented with a commercially available mycorrhizae inoculum (auto + AM). Plant height was measured every thirty days for 120 days. Tukey- Kramer adjusted P-values were used; interactions were categorized as not significant (ns) or significant at $P \leq 0.05$ (*) or $P \leq 0.0001$ (**).

		NONE				AUTO				AUTO + AM			
		30	***60***	***90***	***120***	***30***	***60***	***90***	***120***	***30***	***60***	***90***	***120***
NONE	***30***	-	ns	**	**	ns	ns	ns	ns	ns	ns	*	**
	60	ns	-	*	**	ns	ns	ns	ns	ns	ns	ns	**
	90	**	*	-	ns	ns	ns	ns	ns	ns	ns	ns	*
	120	**	**	ns	-	*	ns	ns	ns	ns	ns	ns	ns
AUTO	***30***	ns	ns	ns	*	-	ns	*	**	ns	*	*	**
	60	ns	ns	ns	ns	ns	-	ns	*	ns	ns	ns	**
	90	ns	ns	ns	ns	*	ns	-	ns	ns	ns	ns	*
	120	ns	ns	ns	ns	**	*	ns	-	ns	ns	ns	*
AUTO + AM	***30***	ns	ns	ns	ns	ns	ns	ns	ns	-	ns	ns	**
	60	ns	ns	ns	ns	*	ns	ns	ns	ns	-	ns	**
	90	*	ns	ns	ns	*	ns	ns	ns	ns	ns	-	**
	120	**	**	*	ns	**	**	*	*	**	**	**	-

A Survey for Soil-borne Pathogens of Woody Ornamentals in Mid-Tennessee Nurseries

L. A. Mackasmiel[1] and M. T. Mmbaga[2]

[1]Tennessee State University Otis Floyd Nursery Research Center, McMinnville, TN
[2]Tennessee State University Department of Agricultural and Environemntal Sciences, Nashville, TN

lmackasm@tnstate.edu

Index words: Nursery, ornamentals, pathogens, Middle Tennessee, soil-borne

Significance to Industry: Soil-borne pathogens form a large group of microorganisms causing losses in nurseries across Middle Tennessee (MTN), and nationwide that runs into hundreds of millions of dollars, annually. Monitoring soil-borne plant pathogens at regular intervals through field surveys in nurseries, will greatly help disease management for new disease outbreaks that may occur such as sudden oak death (SOD); stem/root rot and damping-off (Cook et al., 2007; De Boer, S. H., and Marıa M. Lopez, M. M. 2012). Plant pathogenic fungi in the genera *Cylindrocladium, Pythium, Phytophthora, Fusarium, Rhizoctonia*, and *Thielaviopsis*, are common soil inhabitants (Pal and Gardener, 2006). Consequently, they have a potential to cause huge problems in containerized production systems where contamination occurs during mixing, filling and planting. Plants brought into the region from other states or international sources without adequate phytosanitation and quarantines often increase the spread of these microorganisms. Simultaneously, water used for irrigation also acts as a suitable medium and may be source of many soil-borne pathogens.

Nature of Work: The survey was conducted during the 2014 growing season. Baiting and recovery of pathogens and other microorganisms was done as outlined by Mackasmiel and Mmbaga (2014). Three randomly selected creeks and a total of eight nurseries were surveyed in 2014. The chosen creeks form part of the water supply system and are often used for irrigation by a number of nurseries in the region.

Clean baits consisting of 6 leaves from each bait plant were placed in breathable (pollination) bags and floated on creek water for 3 days. The leaves were then collected, cleaned using sterile water, blotted dry using sterile tissue paper, then cut into 100 mm diameter discs; these were plated on Phytophthora specialized medium (PARPH-V8) for 48 hr. Colonies of *Phytophthora* spp. and other fungal spp., were sub-cultured on V8 agar using hyphal tips. Water samples from nursery irrigation ponds were also collected and placed in sterile 100 x 25 mm petri dishes to a depth of 15 mm; 100 mm diameter discs from *Rhododendron*, and *Pieris;* and *Pinus* needles were then placed on water surface for up to 72 h in a darkened laminar flow hood, at temperature of 20ºC. At the end of the 72 h., leaf baits were plated in PARPH-V8 agar for 48 h. Fungi growing around the edge of baits were then carefully lifted and plated on V8 agar and grown into pure

cultures. In the case of soil, the samples were first thoroughly mixed in a clean tray, then filled in 21.0 cm x 13.5 cm x 4.0 cm (L x W x H) sterilized plastic lunch boxes to a depth of 1.5 cm. Sterile water was then added up to a depth of 1.0 cm above the soil line and leaf discs were floated on the water to bait for *Phytophthora* species. A total of 6 leaf baits per plant (*Rhododendron*, *Pieris* and *Pinus*) per sample were used per container and replicated three times. After placing leaf baits on the water, the samples were maintained in the dark in a laminar flow hood at 12ºC as described above. Samples of plant materials for the isolation of *Phytophthora* and other pathogens were collected from the top 20cm soil, washed to remove soil particles and disinfected in 70% ethanol, rinsed in sterile water and blotted dry on sterile tissue paper before plating on PARPH-V8 for 3 days, as described above. A total of four pieces of plant materials per plate for each sample was replicated three times. Culturing and sub-culturing of fungal samples was done on V8 agar, as described above.

Fungi isolated were identified using DNA sequence analyses of the internal transcribed spacer (ITS) region, including the intervening 5.8S rRNA gene as described by White *et al*. (1990), (Davis Sequencing, Davis, CA). Qiagen DNeasy Plant mini Kit was used for DNA extraction of fungal isolates and DNA amplification was done using universal primer pairs ITS1 and ITS4. Each PCR reaction included 10X PCR buffer, 25 mM of MgCl2, 10mM of dNTPs, Taq polymerase, and sterile double distilled water (ddH2O) in 50-µL reaction mixes. The DNA was amplified using a Techne thermocycler and the PCR products were analyzed by electrophoresis in a 1.5% agarose gel in 0.5X TBE (Tris-Borate-EDTA) buffer. The gels were stained with ethidium bromide (0.5 µg/ml) and DNA was visualized using UV light. The PCR products were purified using the Quiagen PCR purification kit, DNA sequencing was done at Davis Sequencing Inc.(http://www.davissequencing.com). The sequences were compared with information available in the GenBank using Blast search; information was also compared with what is documented in literature.

Results and Discussion: Pathogenic and non-pathogenic fungi isolated included species of *Phytophthora*, *Pythium*, *Fusarium*, *Mortierela*, *Epicoccum*, *Trichoderma* and *Sordariomycetes*. Out of 129 samples submitted for DNA analysis in 2014, approximately 19.38% were *Phytophthora*, and 32.71% were *Pythium* spp. The proportions of individual species of Phytophthora are presented in Figure 1, and Pythium in Figure 2. Other microorganisms isolated are shown in Figure 3. The 2014 survey recovered a total of seven Phytophthora spp; compared to five in 2013. Our results show a number of similar spp., collected during a survey conducted in Eastern Tennesse **(Hulvey, et al. 2010)**. Out of Phytophthora isolates, only *P. hydropathica* was isolated in both 2013 and 2014, while all the remaining ones were different (Table 1). This is not surprising because different locations were surveyed.

On evaluating ten *Phytophthora* spp., isolated in 2014 on dogwood seedlings, we found that *P. parasitica* caused more damage on young dogwood than *P. cinnamomi*, a known root rot pathogen of woody ornamentals (data not shown).

Soil-borne pathogens can become a serious problem in containerized operation systems since they are easy to spread in local, interstate and international commerce (Erwin and Ribeiro. 1996). Water supply systems that go across state lines like creeks and rivers can spread pathogens to growers in other states **(Hong, et al., 2010).** Notorious pathogens like Phytophthora and Pythium usually gain higher priority in most disease control plans/strategies, however, other pathogens have cumulative effects that may have economic significance (Table 2).

Acknowledgements: The authors thank Terry Kirby and Terri Simmons for their technical support during the season.

Literature Cited:

Cook, D. E. L., Schena, L., and Cacciola, S. O. 2007. Tools to detect, identifyand monitor. *Phytophthora* species in natural ecosystems. J Plant Pathol 89:13.

De Boer, S. H., and Lopez, M. M. 2012. New grower-friendly methods for plant pathogen monitoring. Annu. Rev. Phytopathol. 2012. 50:197–218.

Erwin, D.C. and O.K. Ribeiro. 1996. *Phytophthora diseases worldwide*. St. Paul, Minnesota: APS Press.

Hong, C. X., Gallegly, M. E., Richardson, P. A., Kong, P., Moorman, G. W., Lea Cox,J. D., and Ross, D. S. 2010. *Phytophthora hydropathica*, a new pathogen identified from irrigation water, *Rhododenon catawbiense* and *Kalmia latifolia*. Plant pathol., 59: 913-921. Doi: 10.1111/j.1365-3059.2010.02323.x.

Hulvey, J., Gobena, D., Finley, L., and Lamour, K. 2010. Co-occurrence and genotypic distribution of *Phytophthora* species recovered from watersheds and plant nurseries of eastern Tennessee. Mycologia, 102(5), 2010, pp. 1127–1133. DOI: 10.3852/09-221

Mackasmiel, L. A., and Mmbaga, M. T. 2014. Sources of *Phytophthora* species and other pathogens in Middle Tennessee Nurseries. SNA bulletin.

Pal, K. K., and Gardener, B. M. 2006. Biological control of plant pathogens *ThePlant Health Instructor.* DOI: 10.1094/PHI-A-2006-1117-02.

White, J. T., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNS genes for phylogenetics. PCR protocols:Guides to methods and applications. Academic Press., 315-322.

Table 1: *Phytophtora* and related species (spp.) recovered during field survey in 2014 growing season. *P. hydropathica* was isolated in both 2013 and 2014 surveys.

Season	Pathogen
2014	*Phytophthora citricola*
2014	*Phytophthora citrophora*
2014	*Phytophthora hydropathica*
2014	*Phytophthora nicotianae*
2014	*Phytophthora parasitica*
2014	*Phytophthora syringae*
2014	*Phytophtora cactorum/ citricola*
2014	*Phytopythium helicoides*[*]

[*] *Phytopythium helicoides* was not classified as Phytophthora species.

Table 2: Other soil-borne microorganisms isolated along with *Phytophtora* and *Pythium*, species (spp.) during field surveys in 2014, and their economic importance.

Pathogen/Organism	Economic Importance	Reference
Diplodia pinea	Plant pathogen - Diplodia tip blight in Pines	Walis, C., et al. 2008
Epicoccum nigrum	Biocontrol agent; Flavipin production (Antibiotics)	de Lima Favaro, et al. 2012
Epicoccum spp	Biocontrol agents; Two spp are opportunistic pathogens	Bamford, P. C., 1961
Fungal Endophyte	Symbiotes	Rodriguez, R. J., et al. 2009
Fusarium oxysporum	Plant pathogen; saprophytes	Gordon, T. R., 1997
Fusarium solani	Plant pathogen - squash; human pathogens	Zhang, N., et al., 2015
Guignardia sp.	Plant pathogen - citrus/non citrus, ericaceous plants	CABI and EPPO (EU); Okane, I., et al. 2001
Mortierella echinosphaera	Soil inhabiting saprobic	Hibbett, D., and Glotzer, D. 2011
Mortierella lignicola	Soil inhabiting saprobic	Hibbett, D., and Glotzer, D. 2011
Mortierella wolfii	Soil inhabiting - Animal pathogen	Petkovits, T., et al. 2013
Nigrospora oryzae	Plant pathogen - Date palm	Abass, H. A., and Mohammed, N. H. 2014
Paraphaeosphaeria neglecta	May be a plant pathogen, or saprophyte	Verkley, G. J. M., et al. 2014
Paraphoma radicina	Produces Isochromenones, isobenzofuranone, and tetrahydronaphthalenes	El-Elimat T, et al., 2014
Phytopythium helicoides	Plant pathogen - Begonia	Yang, X., et al. 2013
Pseudonectria buxi	Plant pathogen - Leaf and stem blight in Buxus (Boxwood)	Shi, F., and Hsiang, T. 2014
Sordariomycetes sp.	Insect, human, and plant pathogens	Zhang, N., et al., 2006
Trichoderma hamatum	Biocontrol agent	Studholme D. J., et al. 2013
Uncultured *Mortierella*	Potential plant pathogens or saprophytes	Hibbett, D., and Glotzer, D. 2011

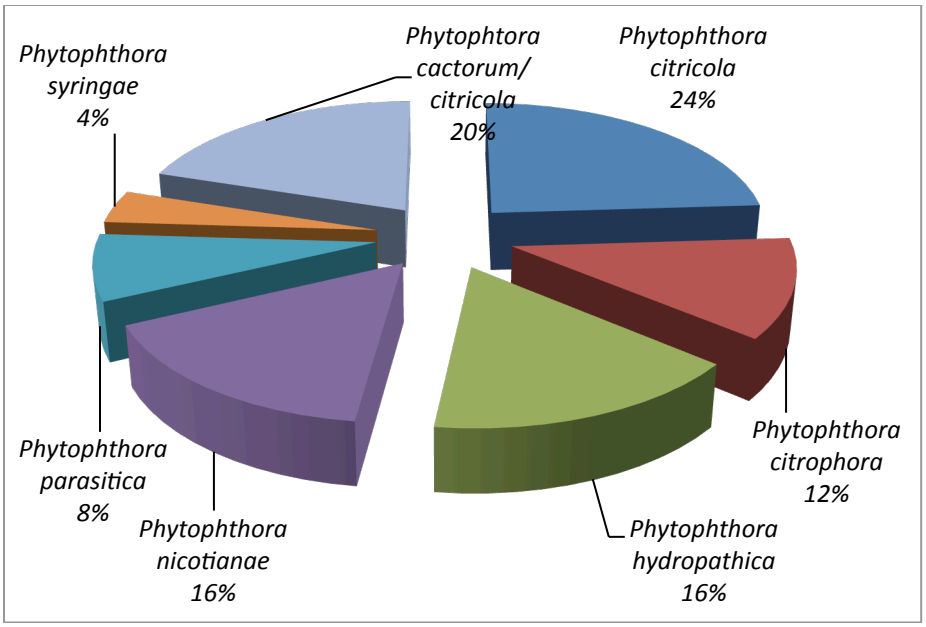


Figure 1: Proportion (%) of *Phytopthora* species that were analyzed and confirmed using standard DNA protocols during 2014 growing season.

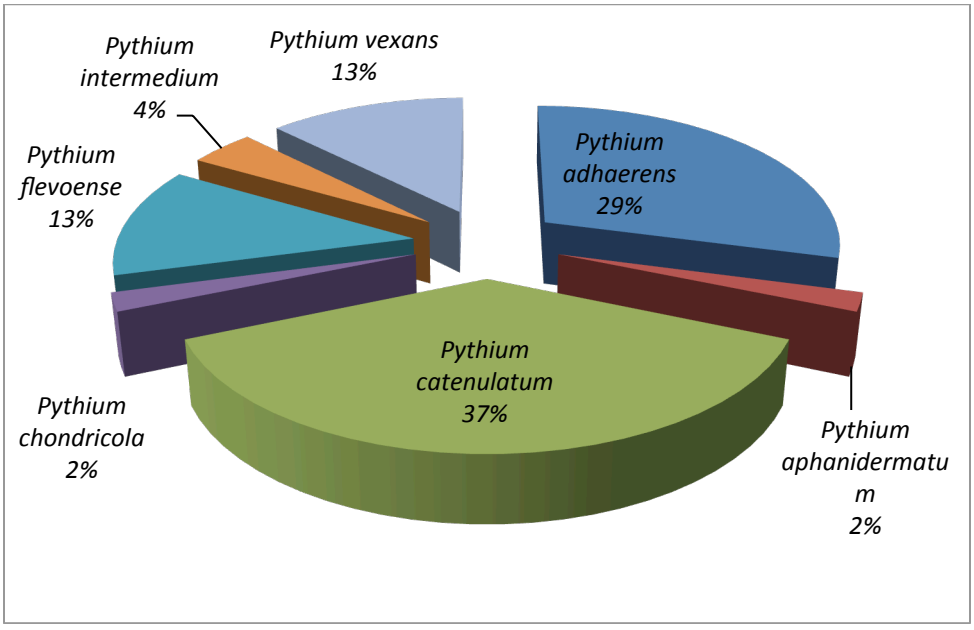


Figure 2: Proportion (%) of *Pythium* species that were analyzed and confirmed using standard DNA protocols during 2014 growing season.

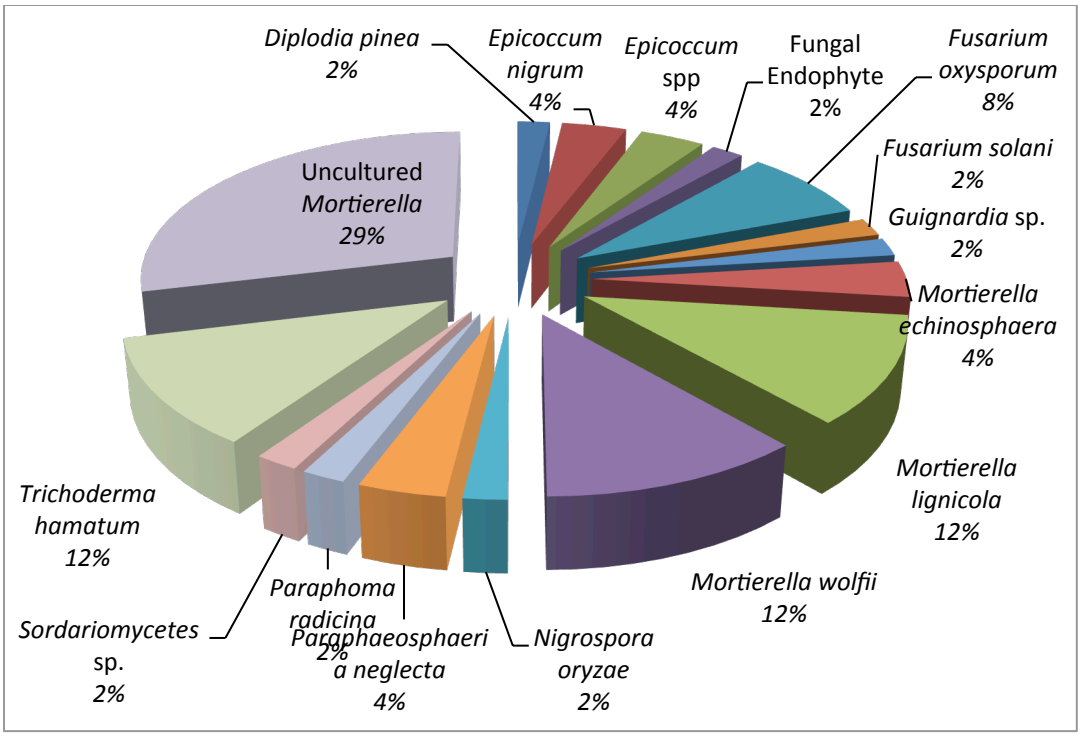


Figure. 3. Proportion (%) of other soil-borne species that were analyzed and confirmed using standard DNA protocols during 2014 growing season.

Diversity of Endophytes in Flowering Dogwoods and their Potential Applications

Asha Maheshwari, M. T. Mmbaga, J. Joshua, S. Gurung and A. Alyamani

Department of Agricultural and Environmental Sciences
College of Agriculture, Human and Natural Sciences
Tennessee State University
3500 John A. Merritt Blvd. Nashville TN-37209

amaheshw@my.tnstate.edu

Significance to Industry: Existence of endophytic microorganisms both bacteria and fungi has been known for over a century. They are ubiquitous, geographically and evolutionary diverse and have wide host range. However, they remain unexplored in most plant species (4, 9), consequently, they are underutilized. These endophytes reside internally in host vascular tissues and often remain asymptomatic and undetected for entire or part of their life cycle. While some endophytes have symbiotic or other relationships with their host plants, many endophytes are chemical synthesizer inside plants and produce many bioactive metabolites which enhance growth, development and host defensive mechanisms against diseases, pests and environmental stresses. These bioactive secondary metabolites are beneficial and possess wide array of biological applications in pharmaceutical use as anti-microbial, anti-cancer, anti-oxidant (3) and as sources of agro-chemicals (1,4, 9).

Endophytic microorganisms have potential as biological control agents (BCA) antagonistic to plant pathogens; they serve as important sources of natural and environmentally friendly alternatives to conventional chemical fungicides. Previous studies on bacterial BCA from dogwood endophytes show that they produce secondary metabolites that suppress fungal growth and powdery mildew spore germination, subsequently leading to plant disease control. Their role in conferring host defense by systemic induced resistance and other mechanisms has been implied (2,5) as a biological-based integrated disease management. The objective of this study is to identify the diversity of endophytic microorganisms that colonize dogwood and explore their potential application in biological control of fungal diseases and as a source of pharmaceutical compounds.

Nature of work: Flowering dogwood (*Cornus florida* L.) is native to southeastern region of United States (6). It is of great significance in horticulture due to its spectacular and showy blossom in spring; attractive foliage in summer and fall; brightly colored fruits and bark in fall. It is also a very important understory plant and valuable source of food and nutrients to more than 50 wildlife species. High Calcium content of foliage and oil content of berries facilitate rapid growth of feeding wildlife (2, 7, 8,10).

Flowering dogwoods has a rich ethno-botanical history which may be related to resident endophytes, but understanding the diversity of endophytes in them remain unanswered.

Therefore the aim of this study was to explore endophytic populations that inhabit flowering dogwoods and to screen them for their potential applications in agriculture and pharmaceutical industry.

In November 2014, a total of 72 flowering dogwoods were randomly selected from 8 different locations in middle Tennessee (Otis L. Floyd nursery Research Centre, McMinnville, Nashville and Murfreesboro). Healthy stem samples were collected in sterile plastic bags and stored at 4°C until processed. Endophytes colonizing the plant specimens internally were isolated from surface sterilized stem samples. Three sections of each stem sample were surface sterilized using 10% Clorox bleach (6.15% Sodium hypochlorite) for 3 minutes followed by three repeated washing with sterile water. Five segments of vascular tissue per section were transferred on acidified potato dextrose agar (APDA). A total of 1080 segments were plated. Plates were sealed and incubated at room temperature. Pure isolates of fungi and bacteria were obtained by at least two successive sub-culturing on potato dextrose agar (PDA) and nutrient agar (NA) respectively. All pure isolates were stored at -20 °C for further studies.

Taxonomic identification of 110 endophytic fungi was done on the basis of internal transcribed spacer (ITS) region which includes intervening 5.8s rDNA gene sequences as described by White *et al*., 1990. Fungal cultures for DNA extraction were grown on PDA for 15-21 days. About 150-200mg of fungal mycelium was scraped with sterile scalpel and DNA was extracted using FastDNA kit (MP Biomedicals, Santa Ana, CA) as per manufacturers' instructions; spectrophotometer (NanodropLite, Thermo Fisher Scientific, Wilmington, DE) was used for DNA quantification. PCR amplification of DNA was carried out using ITS1 and ITS4 universal primers; PCR products were checked for amplification using agarose gel. PCR products were purified using Exosap (USB-Affimetrix, Santa Clara, CA) in order to remove excess primers, dNTPs and non-specific amplified products and sent for DNA sequence analysis at Eurofin Genomics, Louisville, KY. The resulting sequences were analyzed using $BLAST_n$ tool against the Genbank NCBI database and identity of the organisms was based on closest match in Genebank database. DNA sequence similarities of ≥ 99-100% identity was used for species identification while similarities of ≤ 97% identity was used for genus identification (4). Identified endophytes plant colonization frequency (CF) was calculated as the number of segments colonized by fungi of particular taxon divided by the total number of segments investigated x 100, using formula $CF= N_{taxon}/N_{total} \times 100$ (9). Morphological and cultural characterization of fungal isolates will be done to support the molecular identification.

Few isolates were selected and tested for biological control activity (BCA) using in-vitro dual culture technique against known fungal pathogens *Fusarium oxysporium, Fusarium solani, Macrophomina phaseolina, Cercospora cornicola, Cercospora capsici, Phytophtophthora cinnamoni* and *Phytophtophthora capsici*.

Results and Discussion Present study suggested that fungal endophytes were abundant and diverse in fresh stems of flowering dogwoods. Out of 1080 segments

investigated for the presence of endophytic microorganisms, 61% did not have any growth and 39% had microbial growth (Fig. 1a). Out of the total microbial isolates obtained, 381 were fungi and 23 were bacteria which comprised of 197 (95.16%) different fungi and 10 (4.84%) different bacteria (Fig. 1b). Frequencies of isolation and mean number of fungal and bacterial endophytes per sampled tree at the eight different locations are presented in Table 1. Overall, there were differences in endophyte frequency of colonization between locations that may be influenced by environmental conditions, endophyte distribution or availability of endophyte inoculum. The source of endophyte inoculum may be from soil or seed, but more studies are needed to better understand factors that influence plant colonization with endophytes, such information will be useful in utilization of endophyte to boost plant growth or plant protection against diseases, insect pests and environmental stresses.

Taxonomic identification, GeneBank accession numbers and colonization frequency (%) of endophytic fungal isolates obtained from flowering dogwoods based on ITS rDNA sequence similarity using BLAST search is presented in Table 2. Out of the identifications, *Xylariales* sp. was most dominant endophyte in this study. The majority of fungal endophytes were under class of Dothideomycete and Sordariomycetes. Other prevalent endophytes were *Cytospora* sp., *Diaporthe* sp., *Dothideales* sp., *Phoma* sp. and several unidentified genera. Out of these endophytes some have been reported as pathogens in other plant species. Although source plant material was healthy-looking, some endophytes may be opportunistic and represent latent infections that may result in sporadic disease outbreaks when environmental conditions become conducive. Pathogenicity tests of the potential pathogens have not yet been done, but such tests are important to identify unknown pathogens that impact dogwood production. One of the bacterial endophytes and one fungus showed biological control activity against *Macrophomina phaseolina, Cercospora capsici, Fusarium solani* and *F. oxysporum* in *in-vitro* dual culture method. Previous studies suggest bacterial endophyte produce bioactive anti-fungal metabolites (2). Other studies are in progress to evaluate and confirm bioactivity of the isolated endophytes against known plant fungal pathogens. Selected fungal endophytes tested showed bio-activity against some fungal pathogens but not all. Therefore, it is reasonable to assume that these microbial agents are attractive alternative to chemical fungicides.

Endophytes play crucial roles in host plants physiology. Previously many studies were carried out in order to screen fungal endophytes for production of bioactive compounds (9). This study documents species richness and diversity of endophytic population in flowering dogwoods stems. Future work will investigate the isolated endophytes for the production of bioactive secondary metabolites; identify secondary metabolites for agricultural and pharmaceutical use.

Literature Cited

1. Silva-Hughes A. F., D. E. Wedge, C. L. Cantrell, C. R. Carvalho, Z. Pan, R. M. MoRaes, V. L. Madoxx and L. H. Rosa (2015). Diversity and antifungal activity of the endophytic fungi associated with the native medicinal cactus *Opuntia humifusa* (Cactaceae) from the United States. Microbiological Research, 175, 67-77.

2. Rotich E., M. T. Mmbaga, M. Zheng and L. Mackasmeil (2013). Determining the mechanism of action of bacterial biological control agents against powdery mildew in *Cornus florida*. SNA, 58, 208-212.
3. Garcia A., S. A. Rhoden, J. Bernardi-wenzel, R. C. Orlandelli, J. L. Azevedo, J.A. Pamphile (2012). Antimicrobial activity of crude extracts of endophytic fungi isolated from medicinal plant *Sapindus saponaria* L. Journal of Applied Pharmaceutical Science, 2(10), 35-40.
4. Raja H. A., A. Kaur, T. El-Elimat, M. Figueroa, R. Kumar, G. Deep, R. Agarwal, S. H. Faeth, N. B. Cech and N. H. Oberlies (2015). Phylogenetic and chemical diversity of fungal endophytes isolated from *Silybum marianum* (L) Gaertn. (milk thistle).Mycology, 6(1), 8-27.
5. Kiss L. (2003). A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Management Science, 59, 475-483.
6. Mmbaga M. T., R. J. Sauve and F. A. Mrema (2008). Identification of microorganism for biological control of powdery mildew in *Cornus florida*. Biological control, 44, 67-72.
7. Mmbaga M. T. and R. J. Sauve (2009). Epiphytic microbial communities on foliage of fungicide treated and non-treated flowering dogwoods. Biological Control, 49, 97-104.
8. Cheng Q., A. S. Windham, W. E. Klingeman, H. F. Sakhanokho, A. M. Saxton, Y. Li and M. T. Windham (2011). Histological investigation of infection processes of *Discula destructiva* on leaves of *Cornus florida*. Canadian Journal of Plant Pathology, 33(4), 525-531.
9. Khan R., S. Shahzad, M. I. Choudhary, S. Khan and A. Ahmad (2010). Communities of endophytic fungi in medicinal plant *Withania somnifera*. Pakistan Journal of Botany, 42(2), 1281-1287.
10. Li Y., M. T. Mmbaga, A. S. Windham, M. T. Windham and R. N. Trigiano (2009). Powdery mildew of dogwoods: current status and future prospects. Plant diseases, 93(11), 1084-1092.

Table 1: Isolation frequency of endophytic fungi and bacteria per tree from eight different locations. Samples were collected in November 2014.

Plot	A	B	C	D	E	F	G	H	Total Isolates
# of Trees	10	10	10	20	5	5	7	5	
# of Fungal Isolates	42	45	44	96	39	26	50	39	381
# of Bacterial Isolates	5	2	1	2	1	8	3	1	23
Mean # /Tree									
Fungi	4.2	4.5	4.4	4.8	7.8	5.2	7.1	7.8	
Bacteria	0.5	0.2	0.1	0.1	0.2	1.6	0.42	0.2	
Isolation Frequency/Tree									
Fungi	28%	30%	29.30%	32.00%	52%	34.70%	47.60%	52%	
Bacteria	3.30%	1.30%	0.60%	0.70%	1.30%	10.60%	2.80%	1.31%	

* Plot A-D=McMinnville, E-G=Nashville and H= Murfressboro

Table 2: Taxonomic identification, GeneBank accession numbers and colonization frequency (%) of endophytic fungal isolates obtained from flowering dogwoods based on ITS rDNA sequence similarity using BLAST search.

Species	GeneBank Accession Number	Colonization Frequency (%)
Acremoniula sarcinellae	KC806232	0.19%
Bjerkandera adusta	KF176334	0.093%
Botryosphaeria dothidea	KF293916	0.093%
Camarographium koreanum	JQ044432	0.093%
Colletotrichum gloeosporioides	JN887346	0.093%
***Coniozyma* sp.**	KF646090	0.093%
Cytospora sophorae	KC880147	0.093%
***Cytospora* sp.**	AY188991, KC342485	0.19%
Daldinia childiae	HM192905	0.19%
***Diaporthe* sp.**	KC145880, KC763092	0.19%
Diplodia seriata	KF465697, KJ463386, KM280101	0.37%
Discostroma fuscellum	JF320818, JF320818	0.46%
***Dothideales* sp.**	JX188157, JX188156	0.37%
***Dothideomycetes* sp.**	GQ153256, JX188157	0.74%
***Entonaema* sp.**	AB495010, AB495010	0.46%
Epicoccum nigrum	KF025954	0.093%
***Hansfordia* sp.**	GQ906966	0.093%
Hypoxylon fuscum	JN979424	0.093%
Hypoxylon howeanum	HE774495	0.093%
Hypoxylon perforatum	JQ009306, KJ957773	0.19%
Hypoxylon submonticulosum	KC968923, JQ009316	0.19%
***Leotiomycetes* sp.**	AB511812	0.093%
Marssonia populi	FJ238112	0.093%
***Microdiplodia* sp.**	KF010841, KM877481	0.37%
***Mycosphaerella* sp.**	HM751818	0.093%
***Nemania* sp.**	HQ846572	0.093%
Nigrospora sphaerica	KM999230	0.093%

***Nigrospora* sp.**	KM513620	0.093%
***Paraconiothyrium* sp.**	HQ999974	0.19%
***Peniophora* sp.**	KP050603	0.093%
Pestalotiopsis clavispora	HM999902	0.093%
Pestalotiopsis hainanensis	KF551573	0.093%
Pestalotiopsis mangiferae	JX305692	0.093%
Pestalotiopsis microspora	KM513583, KM041703, KM438014	0.46%
***Pestalotiopsis* sp.**	KP217176	0.19%
***Pleosporales* sp.**	KF636769	0.19%
Phoma fungicola	KF293764	0.74%
Phoma medicaginis	KF293990	0.27%
***Phoma* sp.**	HQ856051	0.093%
***Phyllosticta* sp.**	AB454329	0.093%
Rosellinia corticium	KC311485	0.093%
***Seiridium* sp.**	GQ153256	0.093%
***Sordariomycetes* sp.**	JQ761615, JQ760983, JQ761406 JQ761738, JQ760265	0.56%
Stereum sanguinolentum	AY089730	0.093%
***Stereum* sp.**	KJ831872	0.093%
Whalleya microplaca	EF026129	0.19%
Xylaria digitata	GU322456	0.093%
***Xylariales* sp.**	HQ608051, KM357308, KC952872, AB495008	0.46%
Uncultured Peniophora	KC785588.1	0.093%
Fungal endophyte	KF293764	0.093%
Uncultured fungus	GU174360, GQ999431, KF800392, KF800545	0.37%

*Isolates were grouped based on 99% sequence similarity.
* Frequency analysis based on 1080 segments plated.

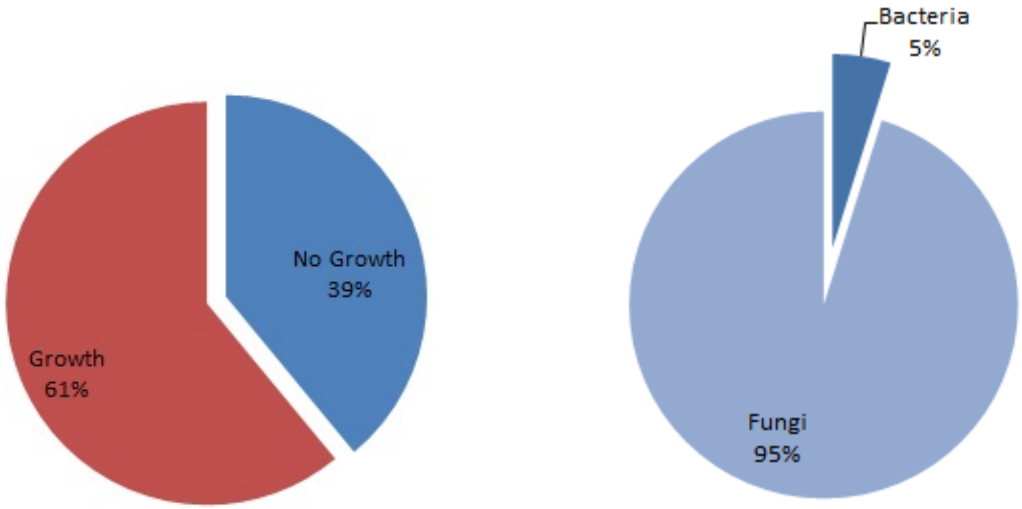


Figure 1: a) Showing the fraction of segments colonized by endophytic isolates.
b) Percentage of fungal and bacterial endophytes in total culturable isolates.

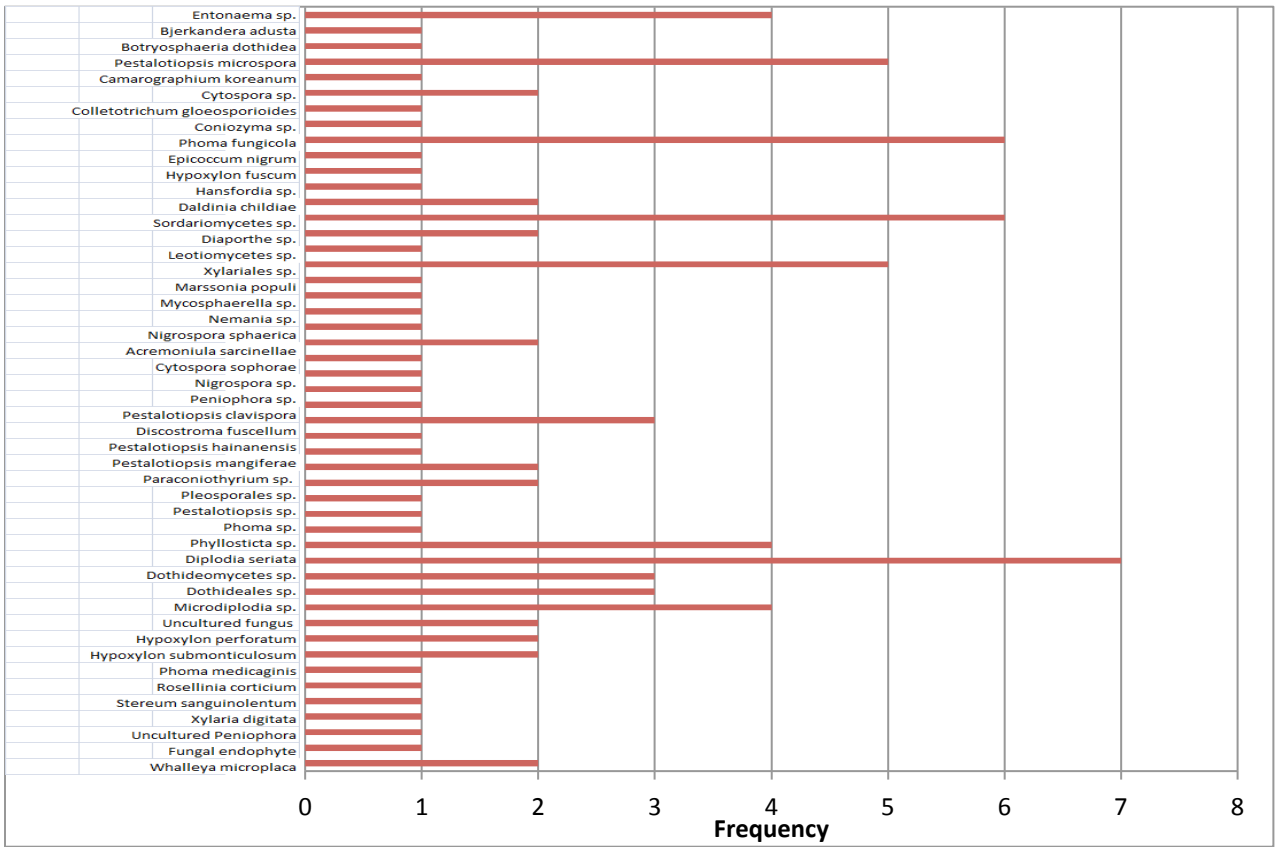


Figure 2: Various endophytic fungi isolated from flowering dogwoods and their frequency of occurrence in number of trees.

A Best Management Plan for Rose Rosette

M. Windham[1], F. Hale[2], A. Windham[2] and Q. Cheng[1]

[1]Entomology and Plant Pathology Department., Knoxville, TN 37996
[2]UT Extension, Soil, Plant and Pest Center, Nashville, TN 37211

Index words: rose, Rosa, Rose Rosette Virus, *Phyllocoptes fructiphilus*

Significance to Industry: Rose rosette has destroyed thousands of roses in retail garden stores, commercial plantings and private and public gardens across the Mid-South. Early detection of rose rosette and rouging of symptomatic plants is an effective strategy to reduce the rate of the epidemic within a rose garden. Plants should be inspected at least every two weeks to prevent the build-up of populations of the vector of Rose Rosette Virus, *Phyllocoptes fructiphilus*. Green barriers to the windward side of rose plantings may also reduce the rate of increase of Rose Rosette in those plantings.

Nature of Work: Rose rosette is a serious threat to roses and has spread across much of the Mid-South and North Central States. Recently the disease was detected in three counties in Connecticut (4) and Florida (2). The disease is caused by Rose Rosette Virus (3) and is transmitted by an eriophyid mite, *P. fructiphilus* (1). For the last six years, early detection of plants symptomatic for rose rosette and immediate rouging of symptomatic plants has been evaluated as a strategy for minimizing the effects of Rose Rosette in the Beall Family Rose Garden (n=250 roses) located within the UT Gardens in Knoxville. Early symptoms associated with rose rosette that were used to identify symptomatic plants have been previously described (5). Plants were rouged by covering the plant with a large garbage bag before cutting the plant away at the soil line. Once the plant was removed, the root ball was also dug and discarded. Seven days later, weather permitting, a new rose was transplanted into the same hole from which the rouged plant was removed. Data were kept of the incidence of Rose Rosette for the roses next to the removed plant and the newly transplanted plants.

At the Plateau Research and Education Center (Crossville, TN), roses were sprayed with one of the following treatments at industry recommended rates: Akari, Avid + horticultural oil, Forbid, horticultural oil, Kontos, Sevin, Talstar or water (control). A bed of roses with symptoms of rose rosette was planted to the windward side of the plots to serve as an inoculum source. Data were collected on incidence of rose rosette for two years. In adjacent plots, roses (n=16 per plot) were surrounded with a green barrier (*Miscanthus sinensis*). Roses were observed for two years for symptoms of Rose Rosette Virus.

Results and Discussion: In the Beall Rose Garden, no plant adjacent to a plant that had been rouged for rose rosette or a plant transplanted into the hole of the rouged plant has become symptomatic for rose rosette. From 2009-2014, 1-2% of the roses in the garden have been removed due to rose rosette annually. Since the master plan for the garden calls for up to 5% of the roses to be replaced each year, loss of roses in the

garden has been minimal and not noticed by the general public. In similar gardens in the Knoxville area, where inspections for early symptoms and rouging have not been done, gardens have been destroyed by rose rosette.

In year 1 of the miticide study, no roses were detected with rose rosette. In year 2 roses with rose rosette symptoms were limited to the water (control) and Sevin treatments. However, incidence was low in both treatments and not statistically different from the other treatments. In the barrier treatment, a green barrier reduced the rate of spread of rose rosette symptomatic plants into the rose plots (Figure 1). It is still too early to determine if miticides are effective in reducing incidence of rose rosette. However, a green barrier to the windward side of the plants that intercepts ballooning mites may help in reducing the rate of increase of rose rosette in a garden.

Literature Cited:

1. Amrine, J., Hindal, D., Stasny, T., Williams, R., and Coffman, C. 1988. Transmission of the Rose Rosette Disease agent to *Rosa multiflora* Thunb by *Phyllocoptes fructiphilus* Keifer (Acari:Eriophyidae). Entomological News 99:239-252.
2. Anonymous. 2014. Rose Rosette Disease confirmed in Florida. Nursery Management Magazine. http://nurserymag.com/ros-rosette-confirmed-in-florida.aspx
3. Laney, A., Keller, K., Martin, R., and Tzanetakis, J. 2011. A discovery 70 years in the making: characterization of the Rose Rosette Virus. J. Gen. Virol. 92:1727-1732.
4. Rowlands, W. 2015. New test confirms Rose Rosette Disease. Connecticut Gardner Magazine. May/June: 1-4.
5. Windham, M., Windham, A., Hale, F., and Hitch, W. 2014. Rose Rosette: identification and management. South. Nurs. Assoc. Proc. Res. Conf. 54:143-146.

Figure 1. The effect of a green barrier, *Miscanthus sinensis*, on rate of Rose Rosette symptomatic plant increase in beds of roses (n=12).