Plant Pathology and Nematology

Fulya Baysal-Gurel

Section Editor

Comparative Performance of Fungicides and Biorational Products in Managing *Botrytis cinerea* in Hydrangea Cut Flower

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Index Words *Botrytis cinerea*, cut flowers, hydrangea, fungicides, biorational products

Significance to Industry The cut flower sector is an economically important group within the ornamental crop production industry. The cut flower market depends upon ornamental characteristics such as longevity, shape, form, size, color and texture of the flowers. However, incidence of *Botrytis cinerea*, in both pre and post-harvest, can greatly reduce the quality of ornamental flowers, making them unsalable and a huge economic burden to growers. This research will provide information to the cut flower industry on the effectiveness of preventive dip applications of fungicides and biorational products for managing Botrytis blight in postharvest conditions.

Nature of Work *Botrytis cinerea* is the most problematic and notorious post-harvest pathogen of the cut flower industry (1). It is an ubiquitous necrotrophic fungus which can affect a wide variety of plant species (more than 200 species) including ornamental and nursery plants, vegetables, fruits etc. (2, 3*). B. cinerea* is easily dispersed by wind, water, and mechanical tools. The fungus colonizes flower petals, anther or stigma tissues (4) and ultimately causes the flower head to drop. Disease infection usually starts in preharvest during flower growth and development, but *B. cinerea* remains inactive and quiescent (5). Eventually the pathogen will become aggressive when physiological and physical changes occur in the host under certain environmental conditions. With a wide range of hosts, various attack modes, and both sexual and asexual stages to survive in times of unfavorable conditions, *B. cinerea* is very difficult to manage (6).

The objective of this research was to evaluate the efficacy of dip applications of fungicides and biorational products for preventive control of *B. cinerea* on hydrangea cut flowers.

Dip applications of fungicides and biorational products were evaluated for their ability to control *B. cinerea* on hydrangea cut flowers (Table 1). This experiment was conducted at Tennessee State University, Otis L. Floyd Nursery Research Center. Hydrangea (*Hydrangea macrophylla*) ‘Nikko Blue’ plants were potted in no. 5 nursery containers filled with 100% pine bark substrate, which was amended with 7 kg of 19-5-9 Osmocote® Pro controlled release fertilizer, 898 g of Micromax® micronutrient fertilizer,

597 g iron sulfate and 148.5 g Epsom salt per cubic meter of mix. 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 in a greenhouse. Hydrangea flowers were harvested at normal maturity dates. Six single-flower replications per treatment were arranged in a completely randomized design. Dip applications of fungicides and biorational products were applied as preventive treatments to the newly harvested hydrangea flowers. All treatments were applied according to according to IR4 protocol recommended rates (Table 1). When all flowers were dried out after dip treatment, they were artificially inoculated with *B. cinerea* spore suspension. Conidial suspension was prepared by flooding 10-14-day-old cultures of *B. cinerea* with sterilized distilled water; spore concentration was determined with a hemocytometer and adjusted to 10^6 conidia/ml. Non-treated, inoculated and non-treated, non-inoculated plants served as controls. Flowers were stored in cold storage for 3 days at 4ºC. Then, flowers were displayed in conditions similar to retail stores (22ºC, 60% RH, 13 µmol $m^{-2}s^{-1}$ light for 24 hrs.). Evaluations were made on disease severity using 1-5 ordinal scale (1= no symptom; 2= 1-25% petals affected; 3= 26-50% petals affected; 4= 51-75% petals affected; 5= 76-100% petals affected), marketability using 1-5 ordinal scale (1= dead; 2= poor quality; 3= commercially acceptable; 4= good quality; 5= healthy), phytotoxicity (same as disease scale), and application residue (same as disease scale). The longevity was the period (days) between harvest and time when flowers were still in 3 or higher on marketability scale. Evaluations were made after removal from cold storage every 1 or 2 days until no more meaningful data could be collected. The ordinal disease scale was change to percentage by taking the mid value and area under disease progress curve (AUDPC) was calculated using formula AUDPC = $\sum [(Xi+1 + Xi)/2] (ti+1–ti)$. Disease severity, AUDPC and longevity were analyzed with one-way analysis of variance (ANOVA) using Proc GLM in SAS 9.4, and means were separated using Tukey test (α=0.05).

Results and Discussion Non-treated, non-inoculated and non-treated, inoculated control hydrangea flowers had lowest (0%) and highest (88%) Botrytis blight disease severity, respectively (Figure 1). Dip application of Astun (2.4%), Medallion (25%), Broadfoam (34%) and Orkestra intrinsic (40%) significantly reduced Botrytis blight disease severity compared to other treatments and non-treated, inoculated control. Dip application of Astun, Medallion, Broadfoam, Orkestra Intrinsic, Botector and SP 2480 significantly reduced disease progress (AUDPC) compared to other treatments and non-treated, inoculated control (Figure 2). Even though treatments such as BW165N, Chipco, OxiPhos, and Regalia reduced disease progress, they were not statistically different than non-treated, inoculated controls.

The vase life of hydrangea cut flowers was the period between harvest and time until the flower maintains marketability rating of 3 or higher. All treatments except for Regalia, OxiPhos, Chipco, BW165N maintained the longer vase life of cut hydrangea flowers compared to non-treated, inoculated control (Figure 3). Highest vase life was observed in non-treated, non-inoculated control (13 days). The treatments that significantly maintained postharvest shelf life of hydrangea cut flowers were Astun (12

days) and Medallion (11 days), which were not significantly different than non-treated, non-inoculated control. No phytotoxicity was observed in any of the treated hydrangea flowers. However, application residue of BW165N (dark residue on flower petals) and Regalia (red or pink residue on flower petals) were observed on hydrangea flowers.

Dip application of Astun (Isofetamid, FRAC 7) and Medallion (fludioxinil, FRAC 12) demonstrated low disease severity and area under disease progress curve and maintained a higher postharvest shelf life of cut hydrangea flowers despite inoculation. Isofetamid is a succinate dehydrogenase inhibitors (SDHI) fungicide with single-site of action that inhibits the cellular respiration; whereas fludioxinil inhibits transport-associated phosphorylation of glucose as well as prevent glycerol synthesis. The biorational products such as Botector and SP2480 did not effectively reduced the disease severity however they were able to significantly suppress the progression of *Botrytis* infection over the trial period. Biorational products may suppress disease by competitive exclusion of pathogen for space, nutrients, and induce disease resistance. This implies that such biorational products can be used by growers in combination with chemical fungicides for effective management of *B. cinerea* in cut flowers. Information from this research can assist growers in management of Botrytis blight in cut flowers in post-harvest condition.

Acknowledgements This project was funded by the IR-4 Environmental Horticulture Program. We thank BASF, Bayer, BioSafe, BioWorks, Marrone Bio Innovations OHP, Syngenta, and Westbridge Agriculture Products for donating the fungicide and biorational products used in this study.

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Table 1. Fungicides and biorational products used in this experiment.

Product Name	Company	Application rate (per 100 gal)	FRAC code
Astun (isofentamid) aka IKF-5411	OHP	13.5 fl oz	7
Broadform (fluopyram + trifloxystrobin)	Bayer	8 fl oz	7+11
Chipco 26019 (iprodione)	Bayer	16 fl oz	2
Orkestra Instrinsic (fluxapyroxad + pyraclostrobin)	BASF	8 fl oz	7+11
OxiPhos (mono and di potassium salts of phosphorus acid + hydrogen peroxide)	BioSafe	1 gal	P 07
Medallion (fludioxinil)	Syngenta	4 fl oz	12
SP 2480 + Capsil	SePRO	30 fl oz + 4 oz	
Botector (*Aureobasidium pullulans* strains DSM 14940 and DSM 14941)	Westbridge Agriculture Products	10 fl oz	
BW165N (*Ulocladium oudemansii* strain U3) + Brandt Organics Ag Aide (surfactant)	BioWorks	3 lb + 8 fl oz	
Regalia (extract of *Reynoutria sachalinensis)*	Marrone Bio Innovations	1 gal	P 05

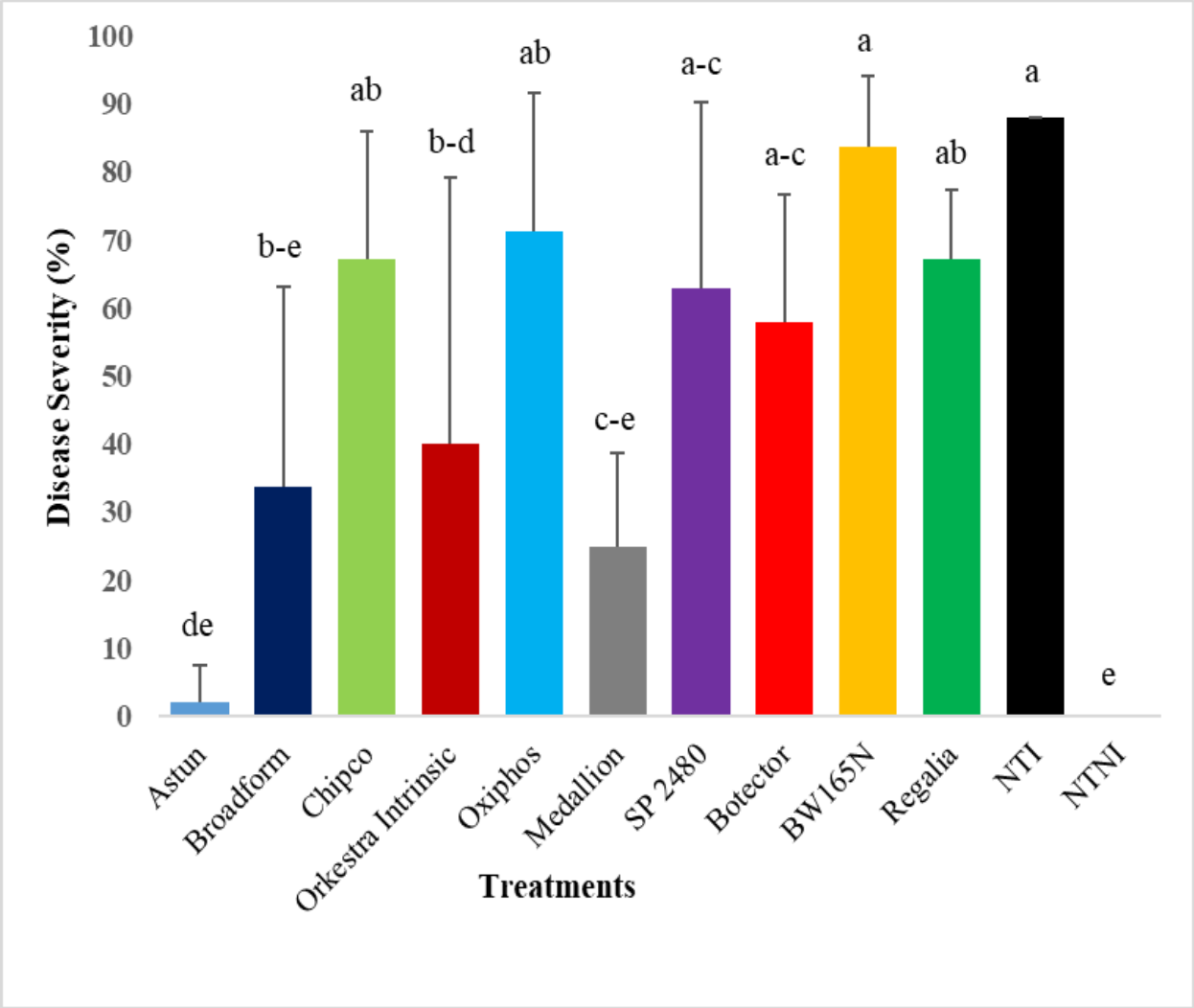

Figure 1. Botrytis blight disease severity (± SE) of preventively treated hydrangea flowers with different fungicides, biorational products when non-treated, inoculated control group (NTI) were at 5 on disease severity scale. NTNI is non-treated, non-inoculated control. Data is recorded on 06/26/2019. Treatments were compared using a one-way ANOVA, and means were separated using Tukey test. Treatments followed by same letters are not statistically significant at $P \leq 0.05$.

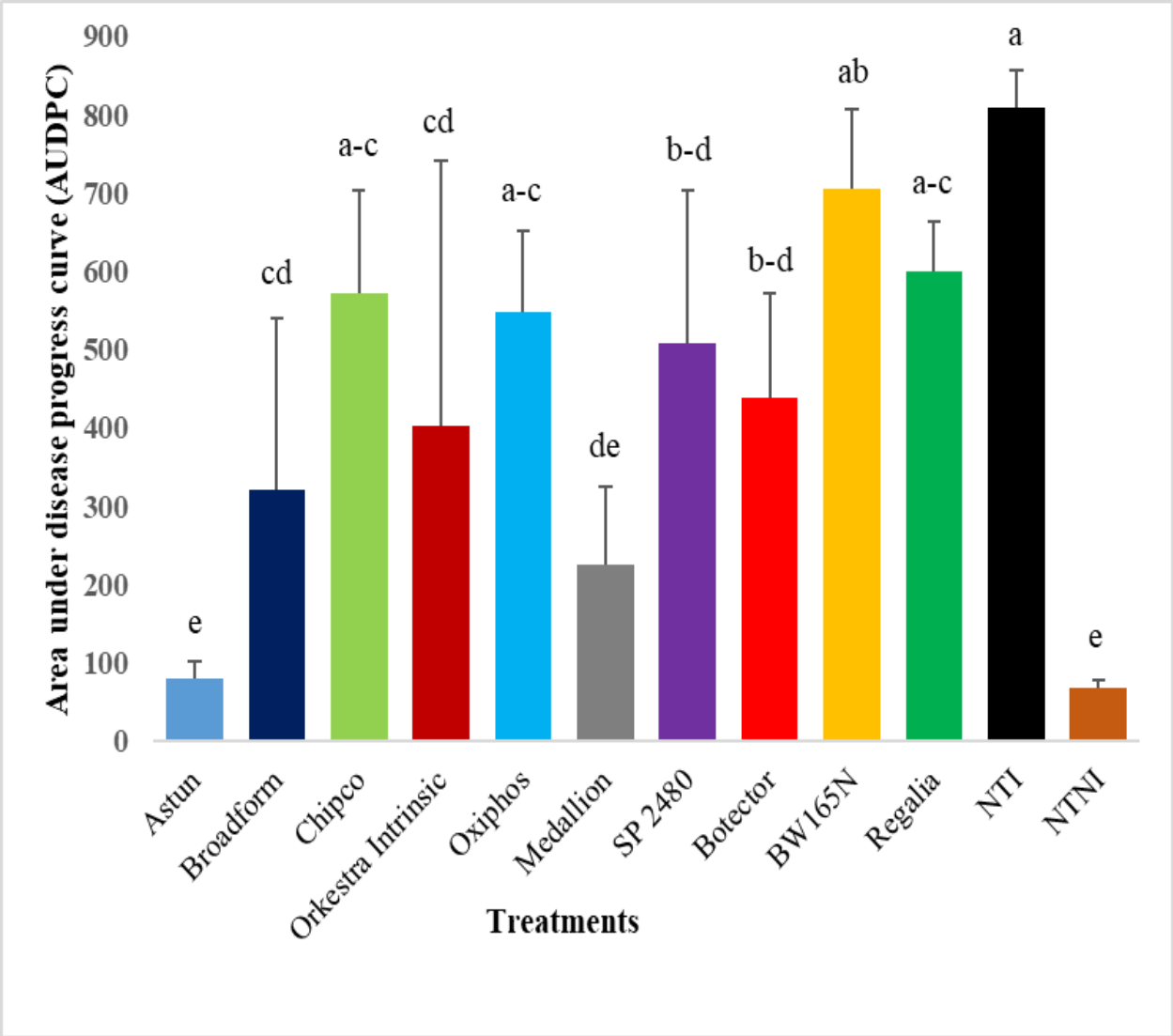

Figure 2. Area under disease progress curve (AUDPC) (± SE) of preventively treated hydrangea flowers with different fungicides, biorational products at the end of trial compared to two control groups (NTI: Non-treated, inoculated control; NTNI: Non-treated, non-inoculated control). Observation on AUDPC was made from 06/15/2019 to 06/30/2019. Treatments were compared using a one-way ANOVA, and means were separated using Tukey test. Treatments followed by same letters are not statistically significant at $P \leq 0.05$.

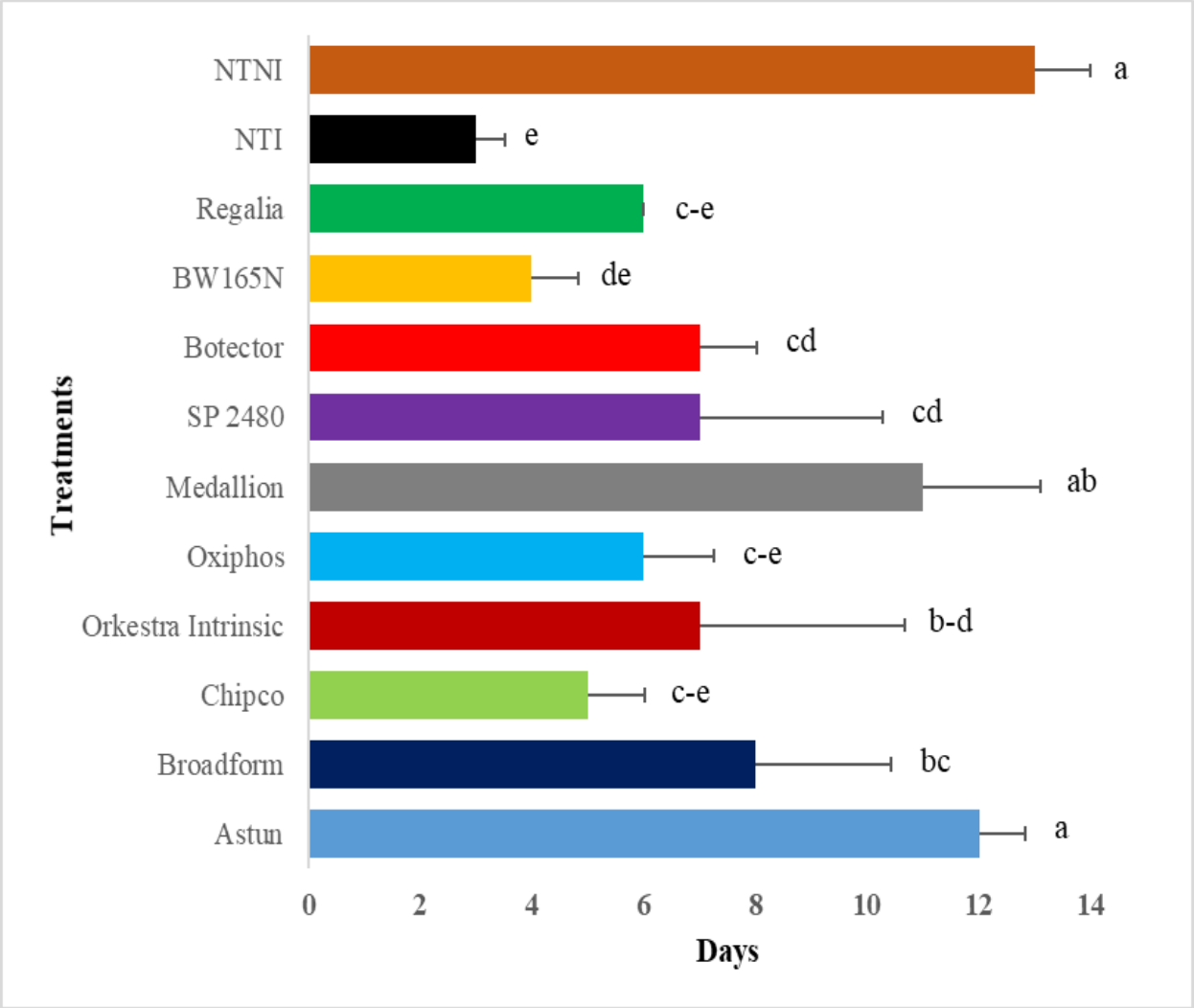

Figure 3. Longevity (± SE) of preventively treated hydrangea flowers with different fungicides, biorational products at the end of trial compared to two control groups (NTI: Non-treated, inoculated control; NTNI: Non-treated, non-inoculated control). Data on longevity of flowers were recorded from 06/15/2019 to 06/30/2019. Treatments were compared using a one-way ANOVA, and means were separated using Tukey test. Treatments followed by same letters are not statistically significant at $P \leq 0.05$.

Impact of Cover Crop Usage on Soilborne Diseases in Field Nursery Production

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Index Words *Phytophthora nicotianae*, cover crop, soilborne disease, field production

Significance to Industry The purpose of this project is to improve the production and profitability of nursery production through the suppression of soilborne diseases with minimum ecosystem impacts and promotion of plant growth using cover crops. This project will evaluate the impacts of cover crop use in field-grown woody ornamental production, with disease management as the primary focus. Mainly confined to row crop, vegetable, fruit and flower production, there is little knowledge about the impact or management needs of cover crops in perennial woody ornamental production. Natural Resources Conservation Service and extension agents have recommended cover crops in between rows of woody ornamentals for some time [1-2] but in many areas of the Southeast, the adoption of cover crops has been slow or reduced following the economic crisis of 2008.

Nature of Work *Phytophthora nicotianae* is a soilborne plant pathogen which can infect 255 genera in 90 families [3] and is one of the most devastating soilborne pathogens in the south-eastern United States [4, 5]. This pathogen can affect a diverse group of plants, including woody ornamentals, causing wilting and chlorosis of leaves, stem and crown necrosis, while below ground symptoms comprise root necrosis.

The objective of this research experiment was to evaluate the impact of cover crops on the soilborne diseases in field nursery production.

Winter cover crops (triticale or crimson clover) were seeded at the manufacturer recommended rates in September - October (optimal timing for each cover crop species) in 2.4 x 14.6 m field plots with four replicates per treatment at Pleasant cove nursery, Rock Island, TN USA (Warren Co.). Plots were prepared by disk harrow and cover crop seeds broadcast, followed by a cultipacker to incorporate seed into the soil. Plots with no cover crop (bare soil) were used as control. A preemergent herbicide (Sureguard (Valent BioSciences LLC., Libertyville, IL USA)) was applied post-transplant within tree rows to prevent weed/cover crop competition at the base of the trees. Each plot was sampled randomly at four locations each within rows and within middles, mixed in situ with a spade, and placed in a plastic bucket. The soil was stored for one week, at an ambient temperature in a greenhouse before use. The greenhouse bioassays were conducted at the Tennessee State University Otis L. Floyd Nursery Research Center (TSUNRC) in McMinnville, TN, USA. The soil sample from each field treatment -1)

cover crop- triticale, 2) cover crop- crimson clover, and 3) bare soil (control); and replication was divided into round plastic containers (top diameter-16 cm, bottom diameter-13.5 cm and height-16 cm) with 3 kg of soil per container. Those soils were then used as either inoculated (with *P. nicotianae*, the rice grain method [6]) or non-inoculated. Isolate FBG201507 of *P. nicotianae* was obtained from the culture collection of Dr. Fulya Baysal-Gurel at the TSUNRC. For each bioassay, ten single-pot replications per treatment were arranged in a randomized complete block design. Rooted cuttings of red maple were transplanted into the containerized field soil, and disease severity was assessed 2 months later. Drip irrigation system was used once per day for 1 min during the experiment. The severity of root rot was assessed using a scale of 0-100% at the end of the experiment. Plant width and height were recorded at the beginning and end of the experiment to be able to calculate the difference. Total plant fresh weight and root weight were recorded at the end of the experiment. The presence of *Phytophthora* was confirmed by plating root samples on PARPH-V8 selective medium. Disease severity, pathogen recovery, total plant weight, root weight and increase in plant height were analyzed with a one-way analysis of variance (ANOVA) using Proc GLM in SAS, and means were separated using Tukey test (α=0.05).

Results and Discussion In the greenhouse bioassay without the addition of pathogen inoculum, *Phytophthora* disease severity was significantly lower in soil collected from the cover crop treatments compared to the bare soil treatment (Figure 1). But there were no significant differences between triticale and crimson clover cover crops in disease severity. The pathogen recovery was significantly lower in triticale cover crop treatment compared to the bare soil treatment. There were no significant differences between crimson clover cover crop treatment and the bare soil treatment in pathogen recovery. There were no significant differences between the cover crop treatment and the bare soil treatment in total plant weight and height increase with no pathogen inoculum introduction (Figure 2 and Figure 3).

In the greenhouse bioassay with the addition of *P. nicotianae* inoculum, *Phytophthora* root rot severity and pathogen recovery was significantly lower in soil collected from the cover crop treatments compared to the bare soil treatment. Disease severity was lower in triticale cover crop treatment compared to crimson clover cover crop treatment (Figure 4). There were no significant differences between triticale cover crop treatment and crimson clover cover crop treatment in *Phytophthora* pathogen recovery. Total plant weight was significantly greater in the cover crop used soil compared to the bare soil (Figure 5). Increase in maple plant height was significantly greater when the crimson clover cover crop was used compared to the bare soil (Figure 6).

Overall, the cover crops were effective in reducing *Phytophthora* pressure in maple production system, however, longer period of cover cropping might be required to see the prolonged effect of cover crops. Growers can get benefit of incorporating these cover crops into production by reducing the need for synthetic crop protection materials.

Acknowledgements This project was funded by National Institute of Food and Agriculture (NIFA), United States Department of Agriculture (USDA) Evans-Allen grant, under award numbers TENX-1520-CCOCP, TENX-1926-CCOCP and TENX-S-1083, and the NIFA, USDA, Southern Sustainable Agriculture Research and Education program. We thank Terri Simmons for help with setting up the greenhouse experiment.

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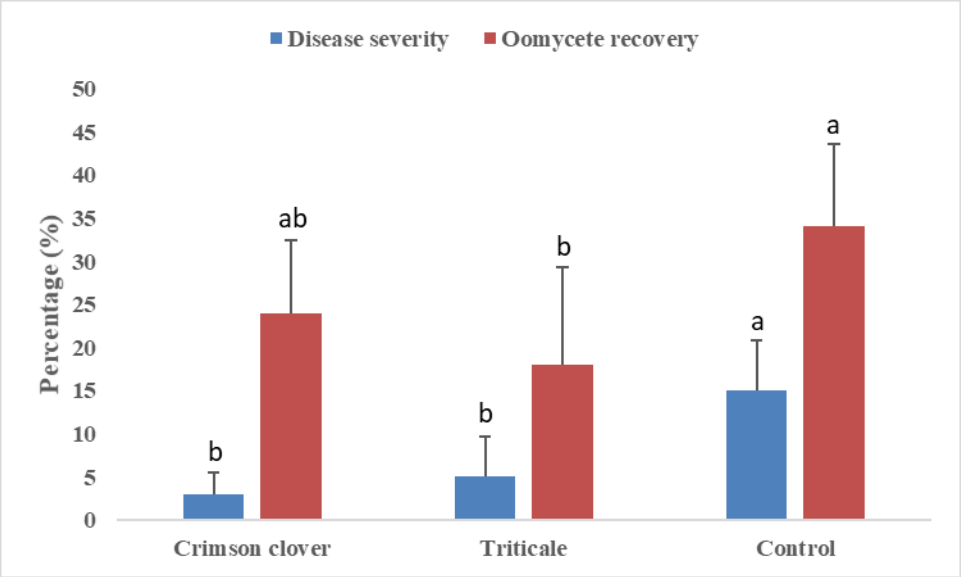

Figure 1. Mean disease severity (± SE) (blue) and pathogen recovery (red) of red maple at the end of the experiment for natural pathogen pressure in a greenhouse bioassay. Treatments were compared using a one-way ANOVA, and means were separated using Tukey test (α=0.05). Different letter indicates the significant difference between the treatments.

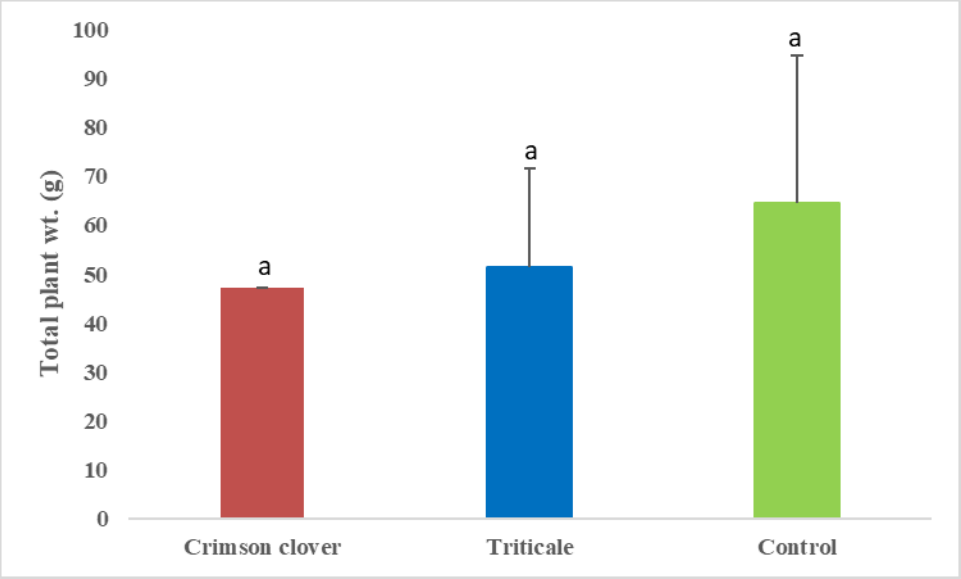

Figure 2. Mean total plant weight (± SE) of red maple at the end of the experiment for natural pathogen pressure. Treatments were compared using a one-way ANOVA, and means were separated using Tukey test (α=0.05). Different letter indicates the significant difference between the treatments.

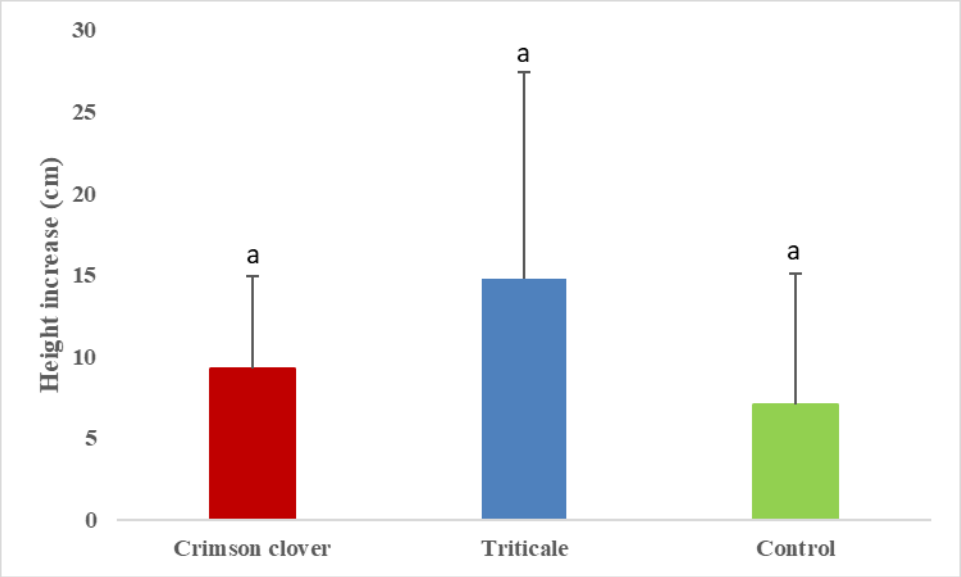

Figure 3. Mean height increase (± SE) of red maple at the end of the experiment for natural pathogen pressure. Treatments were compared using a one-way ANOVA, and means were separated using Tukey test (α=0.05). Different letter indicates the significant difference between the treatments.

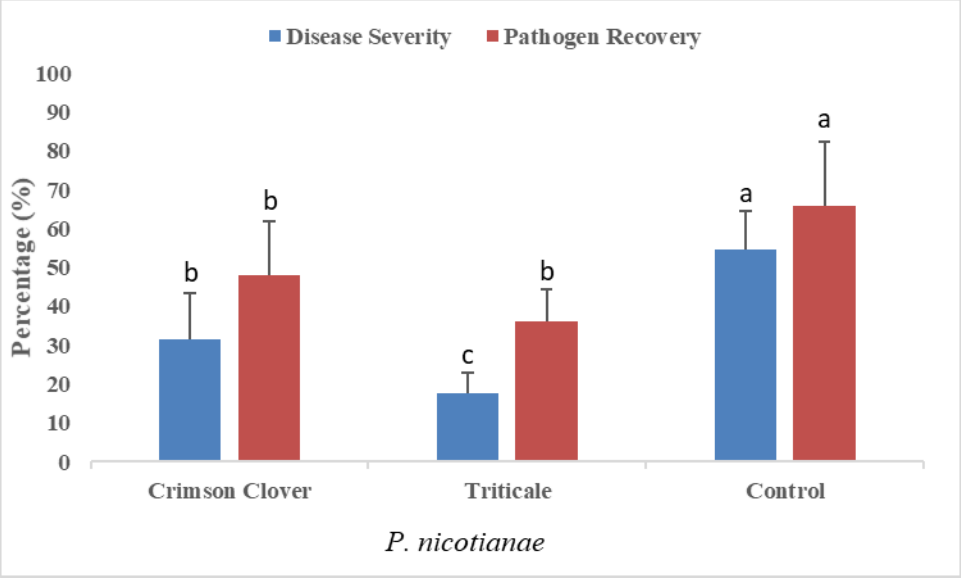

Figure 4. Mean disease severity (± SE) (blue) and pathogen recovery (red) of red maple at the end of the experiment when *Phytophthora nicotianae* was inoculated into the soil in a greenhouse bioassay. Treatments were compared using a one-way ANOVA, and means were separated using Tukey test (α=0.05). Different letter indicates the significant difference between the treatments.

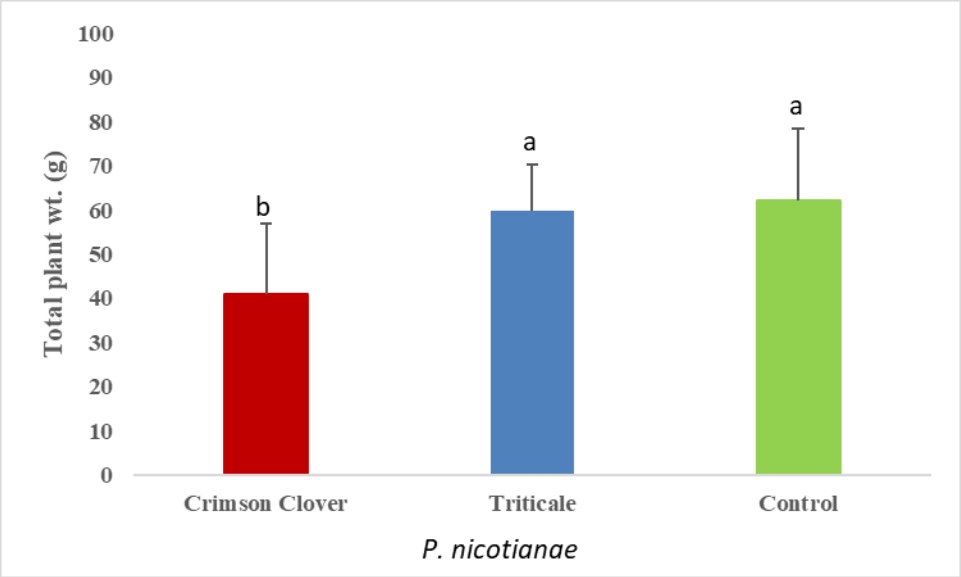

Figure 5. Mean plant weight (± SE) of red maple at the end of the experiment when *Phytophthora nicotianae* was inoculated into the soil in a greenhouse bioassay. Treatments were compared using a one-way ANOVA, and means were separated using Tukey test (α=0.05). Different letter indicates the significant difference between the treatments.

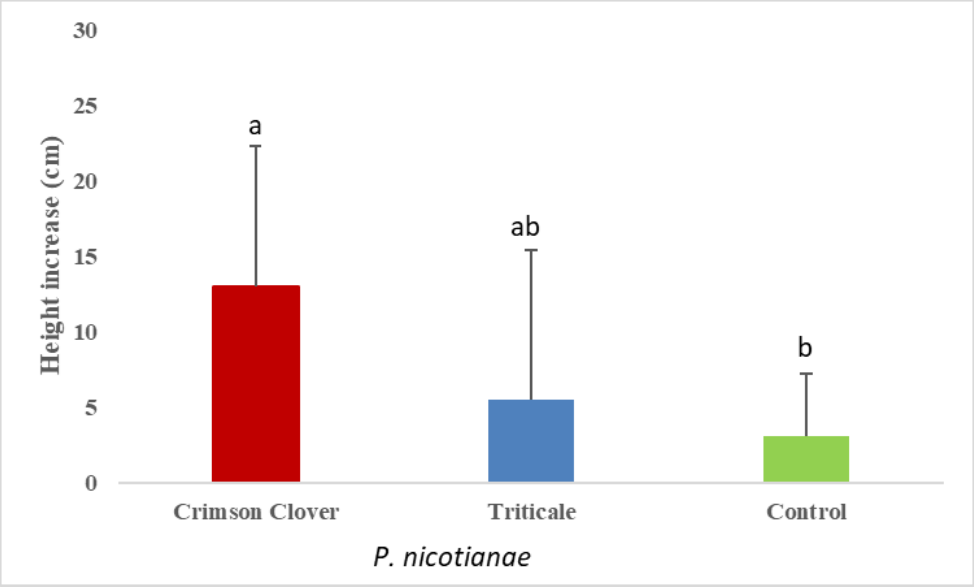

Figure 6. Mean height increase (± SE) of red maple at the end of the experiment when *Phytophthora nicotianae* was inoculated into the soil in a greenhouse bioassay. Treatments were compared using a one-way ANOVA, and means were separated using Tukey test (α=0.05).

Investigation of Copper-resistance in *Pseudomonas syringae* pv. *syringae* strains from Cherry

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Index words Cherry, bacterial canker, copper, *Pseudomonas syringae* pv. *syringae*

Significance to Industry Cherry (*Prunus* spp.) is an important crop for the horticulture industry. Bacterial canker and blast are caused by *Pseudomonas syringae* pv. *syringae. P. syringae* pv. *syringae* is a major problem in cherry orchards and nurseries. The canker on branches or trunks causes the most severe damage. Due to the lack of resistant cultivars and failure of cultural practices, bacterial canker reduces marketability and yield of cherry. Copper-based bactericides are used to control bacterial diseases. However, control of bacterial diseases by copper-based bactericides has been inconsistent. The purpose of this study is to isolate and identify the causal agents of bacterial canker of cherry in Marmara region of Turkey, to characterize them by morphological, physiological, biochemical and molecular test, and to also determine copper sensitivity of the bacterial strains.

Nature or Work *P. syringae* and its close relatives cause diseases of monocots, herbaceous dicots, and woody dicots, and these pathogens utilize an impressive array of virulence factors such as effectors, toxins, and phytohormones to incite disease symptoms (2, 3). Bacterial canker is most common on sweet cherry, wild cherry and ornamental flowering cherries (4, 5, 6). *Pseudomonas syringae* pv. *syringae* is a causal agent of bacterial canker and blast, which has the ability to kill both young and old cherry trees. Systemic infection and death of young trees is a recurring problem in nurseries, and canker development leading to the girdling and death of scaffold limbs and entire trees is a common situation that can lead to the rapid loss of older cherry orchards (1). The objective of this study was to identifiy and characterize the bacterial canker causal agents and determine the susceptibility of bacterial strains to copper bactericides.

The samples were randomly obtained by collecting young infected stems and branches of sweet cherry according to Lazarow and Grigorav (7). The surveyed orchards with and without symptom were recorded to determine the disease prevalence in the Marmara region.

All samples were placed in paper bags and transported to the laboratory for isolation and identification of the causal organism. Surface-sterilized small pieces of lesion area were macerated in 1 mL of sterilized distilled water. A loopful of suspension was

streaked on the King's B medium (KB) and incubated at 25^{o}C for 2-3 days. Single fluorescent or non-fluorescent representative colonies of the predominant morphological types of bacterial isolates were re-streaked on the new KB plates and incubated at 25^{o}C for 2 days for obtaining pure cultures. The pure single colonies with greyish-white appearance on KB medium (8) were then stored at 4^{o}C or in 20% glycerol at -80^{o}C until further identification.

Pathogenicity tests were carried out as described by Lelliot and Stead (8). Immature cherry fruits and bean pods (as control) were inoculated using bacterial strains. The immature cherry fruits and bean pods were disinfected in 0.5% sodium hypochlorite for 1 min, rinsed in sterilized distilled water, were inoculated by placing 20µl of a bacterial suspension on the wounded surface using sterile needles at three different sites. After inoculation, bean pods and cherry fruits were incubated at RH 82% and 22 ± 2°C for 15-20 days. The presence of necrosis or water-soaked lesions was recorded as positive pathogenic reaction.

All bacterial isolates were tested for Gram reaction, levan production on 5% saccharose nutrient agar (SNA), oxidase and pectolytic activity, arginine dehydrolase reaction, and HR test on tobacco leaves (LOPAT) (9), gelatine and aesculin hydrolase and used of tyrosine and tartaric acid (GATTa) (10).

PCR studies were performed in a thermal cycler using primers, B1 (5'–CTTTCCGTGGTCTTGATGAGG- 3')-B2 (5' –TCGATTTTGCCGTGATGAGTC- 3') specific for *P. syringae* pv. *syringae* for amplification of 752 bp *syrB* gens (11).

Copper sensitivity of *P. syringae* pv. *syringae strains* was tested on SPA medium (20.0 g of sucrose, 5.0 g of peptone, 0.5 g of dibasic potassium phosphate, 0.25 g of magnesium sulfate and 15.0 g of agar in 1,000 mL of distilled water) amended with copper sulphate as described by Ritchie and Dittapongpitch (12). Fresh solutions of copper or streptomycin were prepared in sterilized distilled water and then filter-sterilized. Different concentrations of copper (30, 100 and 200 mg/mL) were added to SPA before pouring into the petri dishes. *P. syringae* pv. *syringae* strains were streaked on the amended SPA plates and incubated for 48 h at 25^{o}C and the presence or absence of growth was recorded.

Results and Discussion Disease prevalence recorded between 25 to 40% in Marmara region of Turkey. Eighty-two strains from naturally infected plants were isolated and subjected to biochemical and pathogenicity tests. Forty-five strains out of 82 were positive for levan production; hypersensitive reaction on tabacco, gelatin hydrolysis, aesculine hydrolysis; and negative for oxidase reaction, pectolitic activity, arginine dehydrolase, tyrosine, and tartaric acid.

According to LOPAT test results, bacterial canker pathogen determined as *P. syringae* and all isolates were allocated in LOPAT 1a group and based on GATTa test results, all of them were classified GATTa^{+} (gelatine and aesculin positive) resulting causal agent as *Pseudomoas syringae* pv. *syringae*.

The identification of the isolates was confirmed as *P. syringae* pv. *syringae* by amplification of species-specific sequences that yield amplicons of expected size 752 bp (11).

Based on *in vitro* copper sensitivity test, five out of 45 strains grew on SPA medium amended with copper sulfate (30, 100 and 200 mg/mL). Eleven percent of all strains showed resistance to copper at all tested rates. Eighty-nine percent of all *P. syringae* pv. *syringae* strains were sensitive to copper. There is evidence that some copper-based bactericides are superior to others in controlling bacterial diseases, but results have not been consistent among plant bacterial pathogens. Future studies will be conducted for determine copper resistant of *P. syringae* pv. *syringae* in *in vivo* conditions.

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Management of Phytophthora Root and Crown Rot Using Biofumigation on Field Grown Boxwood

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Index Words *Phytophthora nicotianae*, boxwood, biofumigation, field nursery production

Significance to Industry Boxwood (*Buxus* spp.) is the aristocrat of formal gardens widely used as edging, hedges and foundation plants (1). It is a broadleaf evergreen plant sold in the U.S., with an annual revenue of $126 million according to the 2012 USDA Census of Agriculture. In Tennessee, boxwood crops account for a significant portion of nursery production with $4 million in sales annually (2). Boxwood is susceptible to several root diseases. Phytophthora root and crown rot, caused by *Phytophthora nicotianae*, is one of the major limitations to boxwood production. This disease is a leading cause of premature decline and death of boxwood. Root rot develops primarily on wet, poorly drained soils which aids infection by the pathogen. This research aims to provide information on the effectiveness of biofumigant usage to manage Phytophthora root and crown rot in the field condition.

Nature of Work Phytophthora root and crown rot is caused by several species of Oomycetes (known as water molds). The *Phytophthora* species are *P. cinnamomi, P. nicotianae, P. citrophthora* and *P. occultans*. American (common), Japanese and English boxwood species are susceptible to this disease. The pathogen can infect all growth stages of field or container grown boxwood plants. Warm, humid and rainy conditions favor disease development. *Phytophthora* can survive in soil or potting mix as resting spores (oospores, chlamydospores), or inside infected plant tissues as mycelium for long periods, and can infect other healthy boxwoods or other host plants. This disease may result in complete crop loss, reduce plant growth and increase costs to the nursery producer. The maintenance, environment, and production chain to speed up the boxwood production cycle (from propagation beds to field; from field to container) are increasing the chances of crop losses due to Phytophthora root and crown rot. The objective of this experiment was to manage Phytophthora root and crown rot of boxwood by using the bio-fumigants in the field.

Soil incorporation of cover crops in the family of Brassicaceae has the ability to suppress soil microorganisms through the hydrolysis of glucosinolates (GSL) into isothiocyanate, a natural bio-fumigants (4). GSL content and concentration differs among *Brassica* cultivars, the development stage of the plant (5), and the end product formed by hydrolysis of the GSL, so that different *Brassica* cultivars may have different levels of potential to control pathogens (6).

This experiment was conducted in field plots with Waynesboro loam soil at the Otis L. Floyd Nursery Research Center in McMinnville, TN (USDA Hardiness Zone 7a). The field was fertilized with nitrogen, phosphorus and potassium based on University of Tennessee soil test recommendations. Treatments were arugula ‘Astro’; brown mustard ‘Kodiak’; mighty mustard ‘Pacific Gold’; mustard green ‘Amara’; rape ‘Dwarf essex’; turnip ‘Purple Top Forage’; yellow mustard ‘White Gold’; mustard meal ‘Pescardo Gold Mustard Meal’ (Farm Fuel Inco, Freedom, CA, USA); biofumigant Dominus (allyl isothiocyanate) (at 170 and 340 lb/A) (ISAGRO company, Durham, NC, USA) and only solarization (Table 1). Plots were in a completely randomized design with four replications per treatment. Plots were inoculated with *P. nicotianae* infested rice grains by placing rice grain 2 in. below the surface every 12 in. Biofumigant cover crops were planted with a seed rate provided by manufacturer in April. Flowering biofumigants were rototilled 6 in. deep into the soil. After incorporation, plots were covered with polyethylene film for 30 days. Biofumigant Dominus were applied in dedicated plots same day. Those plots and solarization-only plots were covered with polyethylene film for 30 days as well. Inoculated/non-biofumigated, non-solarized, non-inoculated/non-biofumigated, non-solarized plots were used as controls. Soil temperature in the plots was

monitored using a WatchDog. After the 30-day period, the polyethylene film was removed and uniform boxwood seedlings (*B. sempervirens* 'Green Velvet') were planted into the plots, with five plants per plot (5 plants per plot x 4 replications). Plant growth data (fresh weight and root weight) were recorded and roots were assessed for disease severity using a scale of 0-100% roots affected at the end of experiment. Statistical analyses were conducted using the mixed models procedure with SAS statistical software and means were separated using Tukey test (α=0.05).

Results and Discussion Average soil temperature of solarized beds was 91.58°F between August 8 and September 7, 2018; average soil temperature of cover crops incorporated solarized beds was 97.5°F; average soil temperature of control non-treated, non-solarized beds was 78.6°F (data not shown). All treatments except both rates of Dominus and turnip 'Purple top forage' significantly reduced Phytophthora root and crown rot disease severity compared to the inoculated, non-biofumigated, non-solarized control (Table 1). Mustard green 'Amara' incorporation with solarization significantly increased the total fresh weight compared to other treatments and controls. There was no significant difference between treatments considering the root weight. Phytotoxicity were not observed in any of the boxwood plants.

Boxwood growers can use information from this study and can be benefited by using biofumigant crops to manage Phytophthora root and crown rot in their field production.

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Table 1. Efficacy of biofumigants with solarization in suppression of Phytophthora root and crown rot in field grown boxwood.

Treatment	Seed rate or application rate	Phytophthora root and crown rot severity (%)*	Total fresh weight (oz)	Root weight (oz)
Inoculated control	N/A	60.5 a**	0.5 b	0.3 a
Non- inoculated control	N/A	0.0 e	0.7 b	0.4 a
Arugula 'Astro'	30-50 seeds/ft^2	44.3 cd	0.6 b	0.3 a
Brown mustard 'Kodiak'	15 seeds/ft^2	41.8 d	0.6 b	0.4 a
Mighty mustard 'Pacific Gold'	15-20 lb/A	37.3 d	0.9 b	0.3 a
Mustard green 'Amara'	15 seeds/ft^2	44.3 cd	1.8 a	0.3 a
Rape 'Dwarf essex'	5-10 lb/A	46.8 bcd	0.6 b	0.3 a
Turnip 'Purple Top Forage'	5-8 lb/A	53.0 abc	0.6 b	0.3 a
Yellow mustard 'White Gold'	15-20 lb/A	39.3 d	0.7 b	0.4 a
Dominus	170 lb/A	56.8 ab	0.6 b	0.4 a
Dominus	340 lb/A	54.3 abc	0.6 b	0.3 a
Mustard meal	968 lb/A	49.3 bcd	0.7 b	0.4 a
Solarization	N/A	46.8 bcd	0.6 b	0.3 a
P-value		<0.0001	<0.0001	<0.0001

*Disease severity based on percentage of roots affected.

**Values are the means of 20 plants; treatment followed by the same letter within a column are not significantly different at $P \leq 0.05$.

Evaluation of Fungicides for the Control of Rose Black Spot Caused by *Diplocarpon rosae*

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Index Words Rose black spot, *Diplocarpon rosae*, fungicides

Significance to Industry Black spot (*Diplocarpon rosae*), the most common disease on roses, is a destructive disease of rose in landscape and nursery production. Black spot is easily identified by dark spots on the leaves. The leaf spots may also have yellow halos surrounding them. Black spot of rose can be easily prevented with planting resistant cultivars, cultural practices and chemical treatments. Various products are available or in development that have the potential to contribute to the management of black spot of rose. The objective of this study is to test the efficacy of fungicides to control black spot of rose.

Nature of Work Black spot will cause the overall health of rose plants to decrease through defoliation, bloom quantity and quality, and exposes the plant to an increased risk for other stressors. The disease typically presents itself in lower leaves first, before moving up the plant as time progresses. Black spot symptoms can also display on the canes of the plant. Lesions with infection and fallen leaves provide black spot with the means to overwinter. Disease can also thrive in overgrown or crowded conditions.

The efficacy of fungicides against black spot were assessed on rose ‘Queen Elizabeth' and ‘Louis Philippe’. Fungicide treatments used include Broadform SC (4 fl oz/100 gal and 8 fl oz/100 gal), Eagle 20 EW (8 fl oz/100 gal), Mural 45WG (7 oz/100 gal), and Orkestra Intrinsic SC (8 fl oz/100 gal). Six single-plant replications per treatment were arranged in a randomized complete block design in a hoophouse at the Otis L. Floyd Nursery Research Center in McMinnville, TN. Black spot of rose infections occurred naturally under 45% shade. The initial fungicide application was made after observing the first symptoms of black spot. Fungicides were applied as a foliar spray to run-off using a backpack CO_2-pressurized sprayer at 40 psi for a total of 3 applications.

The severity of black spot was evaluated using a scale of 0-100% foliage affected. Area under the disease progress curve (AUDPC) values were also calculated. Phytotoxicity (scores ranging between 0 = no phytotoxicity and 10 = complete kill), chlorosis, defoliation, discoloration and stunting (scores ranging between 0 = no effect and 10 = complete plant affected) were also evaluated on the same dates disease was assessed. Plant quality/acceptability was evaluated on the same dates diseased was assessed using a scale of 1-9 where 1 is dead, 6 is commercially acceptable and 9 is a perfect

plant. Plant height was measured at the end of the experiment. Statistical analyses were conducted using the mixed models procedure with SAS statistical software.

Results and Discussion Black spot infection occurred naturally, and disease pressure was moderate to high on 'Queen Elizabeth' plants with non-treated control plants showing 37.5% foliar disease severity (Table 1); disease pressure was low on 'Louise Philippe' plants with non-treated control plants showing 12.9% foliar disease severity by 3 Jul (Table 2). All of the treatments significantly reduced final severity (3 Jul) and AUDPC compared to the non-treated control in both trials. The treatments that most effectively reduced black spot severity were the high and low rates of Broadform, Eagle 20EW and Mural for 'Queen Elizabeth' trial and the treatments that most effectively reduced black spot severity were the high rate of Broadform, Eagle 20EW and Mural for 'Louise Philippe' trial. There were no significant differences in disease progress (AUDPC) between fungicide treatments for 'Queen Elizabeth' trial. Treatment with the high and low rates of Broadform, Mural and Eagle 20EW were more effective in reducing AUDPC in 'Louis Philippe' trial than Orkestra Intrinsic. There were no significant differences in plant height between treated and non-treated plants at the end of both 'Queen Elizabeth' and Louise Philippe' trials. Non-treated control 'Queen Elizabeth' plants were not commercially acceptable due to the level of disease at the end of the trial (data not shown). Phytotoxicity, chlorosis, defoliation, discoloration and stunting were not observed in any of the treated rose plants.

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Table 1. Efficacy of fungicides for the control of black spot of rose cultivars 'Queen Elizabeth'.

Treatment and rate/100 gal	Cultivar	Disease severity (%)* (3 Jul)	AUDPC*	Plant height (cm) (3 Jul)
Non-treated control	Queen Elizabeth	37.5 a**	886.1 a	87.8 a
Broadform SC 4 fl oz	Queen Elizabeth	7.1 bc	217.9 b	89.5 a
Broadform SC 8 fl oz	Queen Elizabeth	3.0 c	75.3 b	79.5 a
Eagle 20EW 8 fl oz	Queen Elizabeth	3.2 c	108.5 b	83.5 a
Mural 45WG 7 oz	Queen Elizabeth	6.3 bc	163.6 b	90.3 a
Orkestra Intrinsic SC 8 fl oz	Queen Elizabeth	9.6 b	188.7 b	91.5 a
P-value		$\leq$ 0.0001	$\leq$ 0.0001	0.7625

*Disease severity ratings and area under the disease progress curve (AUDPC) were based on percentage foliage affected.
**Treatments followed by the same letter within a column are not significantly different at $P \leq 0.05$.

Table 2. Efficacy of fungicides for the control of black spot of rose cultivars 'Louis Philippe'.

Treatment and rate/100 gal	Cultivar	Disease severity (%)* (3 Jul)	AUDPC*	Plant height (cm) (3 Jul)
Non-treated control	Louis Philippe	12.9 a**	203.3 a	38.8 a
Broadform SC 4 fl oz	Louis Philippe	3.7 c	61.3 bc	39.2 a
Broadform SC 8 fl oz	Louis Philippe	1.4 d	28.3 c	37.3 a
Eagle 20EW 8 fl oz	Louis Philippe	1.8 d	20.7 c	41.2 a
Mural 45WG 7 oz	Louis Philippe	1.6 d	32.9 c	39.8 a
Orkestra Intrinsic SC 8 fl oz	Louis Philippe	5.8 b	106.2 b	38.7 a
P-value		$\leq$ 0.0001	$\leq$ 0.0001	0.9746

*Disease severity ratings and area under the disease progress curve (AUDPC) were based on percentage foliage affected.
**Treatments followed by the same letter within a column are not significantly different at $P \leq 0.05$.

Evaluating Boxwood Endophyte for Control of Phytophthora Diseases

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Index Words Boxwood endophyte SSG, biocontrol, horticultural crops

Significance to Industry *Phytophthora* species are fungus-like plant pathogens responsible for huge losses of many agriculturally and economically important plants worldwide (1). Diseases caused by both aerial and soil-borne *Phytophthora* species are common in plant nurseries (2-4). In addition to affecting crop quality at production, they impact everyone in the horticultural chain from retailers to landscapers and consumers (5, 6). These pathogens must be well managed at nurseries. Chemical control remains a most effective measure in Phytophthora disease management and a key component in Best Management Practices (http://phytosphere.com/BMPsnursery/Index.htm). However, this measure is not sustainable due to increasing cost and development of oomyceticide resistance. Alternative methods are urgently needed to improve sustainability. Here we report on a bacterial endophyte SSG for control of Phytophthora diseases. This endophyte was recently isolated from boxwood and has been shown to provide excellent control of boxwood blight (7).

Nature of Work In this study, SSG, a new member of *Burkholderia cepacia*, was evaluated for control of three important Phytophthora diseases using both dual culture assay and *in-planta* inoculation.
A 0.5-cm culture plug of a target species was placed in the center of 9-cm potato dextrose agar (PDA) plates with a streak of 48-h SSG broth culture on its left and right sides. Control plates were streaked with nutrient broth in place of SSG. These plates were placed at 25°C. *Phytophthora* colony diameter was measured after 4 weeks. This assay was repeated twice with triplicate plates per treatment in each run. SSG suppression was calculated by dividing the difference in colony diameter between SSG streaked plates and control plates by the colony diameter of control plates times 100. Annual vinca (*Catharanthus roseus* 'Little Bright Eye', 8-week old) – *P. nicotianae*, pepper (*Capsicum annuum* L. 'California Wonder', 4-week old) – *P. capsici,* and rhododendron (*Rhododendron catawbiense* 'Boursault', 1-gallon) – *P. ramorum* were used in *in-planta* assay. Plants were used after grew in 6-inch or 1-gallon pots with pine bark-based potting mix for at least 4 weeks in a greenhouse. Host plants were cover sprayed with 48-h SSG broth culture at 10^9 cells/ml or nutrient broth only without SSG as control. After 24 h, the pretreated plants were inoculated with a *Phytophthora* species at 10^4 zoospores/ml which was prepared using mycelia mates in V8 broth as described previously (8) and kept in a moist plastic box for 2 days. Blighted leaves and total leaves were counted for each plant 7 days after inoculation. This experiment was

done three times with triplicate plants per treatment in each run. Disease incidence data were analyzed using Excel. T-test was conducted to determine difference of significance between SSG and the control at *P* = 0.05.

Results and Discussion SSG strongly inhibited growth of all 3 *Phytophthora* species tested (Table 1). Mycelia growth of test *Phytophthora* species placed between SSG culture streaks was restricted. SSG suppression of *P. ramorum* and *P. nicotianae* were greater than 90% while that of *P. capsici* was greater than 80% when compared to the control.

In plant protection experiments, SSG efficacy varied with phytopathosystem (Fig. 1). Best result was obtained in annual vinca – *P. nicotianae* with 98% blight reduction (*P* = 0.0141). The second best was in rhododendron – *P. ramorum* with 60% blight control (*P* = 0.0018). SSG also provided moderate protection of pepper from infection by *P. capsici* (55%, *P* = 0.0169). These levels of Phytophthora blight control in general are at least as good as those recently reported biocontrol by many agents including strains of *B. cepacia* (9-15).

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Table 1. Suppression of *Phytophthora* growth by SSG

Species	Isolate	Host	Suppression (%)	SE
P. capsici	22H3	*Cucurbita* sp.	80.9	0.8
P. nicotianae	1B11	*Catharanthus roseus*	81.5	1.8
P. ramorum	32G2	*Camellia* sp.	91.3	0.5

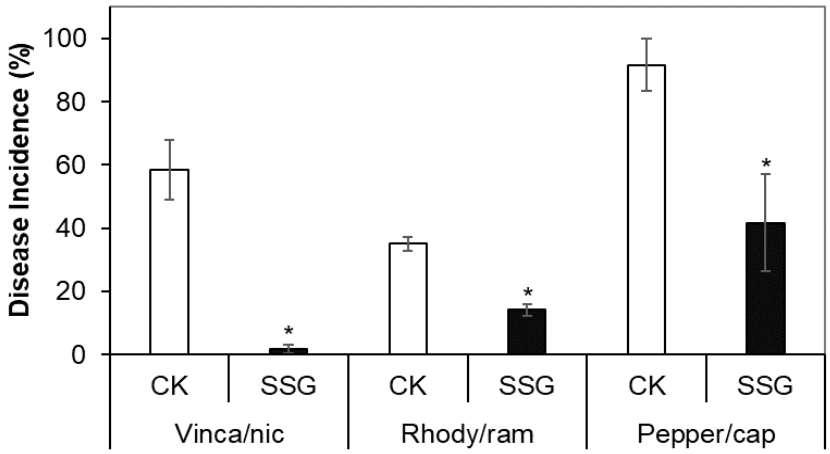

Figure 1. Disease incidences of plants between treatments with SSG and the control (CK) in three phytopathosystems. nic= *P. nicotianae*, ram =*P. ramorum*, cap= *P. capsici.* Foliage blight was assessed at 7 days post inoculation (dpi). Each column is an average of 9 plant pots in three experiments. Bars indicate standard errors. * on the top indicates the difference significance from the control by t-test at $P = 0.05$.