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# Comparative Performance of Fungicides, Biofungicides, Host-plant Defense Inducers, and Fertilizer in Management of Phytophthora Root Rot on Boxwood

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***Abstract.* Phytophthora root rot, caused by *Phytophthora nicotianae* Breda de Haan, is one of the destructive diseases of boxwood (*Buxus sempervirens* L.) and can affect all growth stages of field- and container-grown boxwood plants. Management is a problem and is only possible through an integrated approach. In this study, the efficacy of fungicides, biofungicides, host-plant defense inducers, and fertilizer were evaluated to manage Phytophthora root rot of boxwood. The objective of this experiment was to develop fungicide and biofungicide recommendations for Phytophthora root rot management in boxwood production. Field and greenhouse experiments were conducted in 2019 (Trial 1) and 2020 (Trial 2). The field experiment was arranged in a completely randomized design with four plots per treatment with five single 'Green Velvet' boxwood plants per plot. The greenhouse experiment was arranged in a completely randomized design with five single 'Green Velvet' container-grown boxwood plants per treatment. Plots/containers were inoculated with *P. nicotianae* grown on rice grains. Plant growth data such as height and average width were recorded at the beginning and end of the experiments. Total plant fresh weight and root fresh weight were recorded at the end of the experiments. Roots were assessed for root rot disease severity using a scale of 0% to 100% roots affected. Treatments used in both experiments were fungicides—ametoctradin + dimethomorph, fluzapyroxad, mefenoxam, oxathiapiprolin, pyraclostrobin, pyraclostrobin + boscalid; host-plant defense inducers—aluminum tris-drench, aluminum tris-foliar, potassium salts of phosphoric acid; biofungicides—*Trichoderma harzianum* Rifai strain T-22 + *Trichoderma virens* strain G-41, *Bacillus amyloliquefaciens* Priest; fertilizer—water-soluble nitrogen (nitrogen 5%) and soluble potash; and combination of water-soluble nitrogen, soluble potash, and *T. harzianum* Rifai strain T-22 + *T. virens* strain G-41. All treatments were drench applied except one of the aluminum tris, which was applied as foliar. The controls were nontreated, inoculated and nontreated, and noninoculated boxwood plants. In the greenhouse experiments, treatments that effectively reduced disease severity were pyraclostrobin, ametoctradin + dimethomorph, and oxathiapiprolin. In the field experiments, treatments such as pyraclostrobin, oxathiapiprolin, mefenoxam, fluzapyroxad, and combination of water-soluble nitrogen (nitrogen 5%), soluble potash, and *T. harzianum* Rifai strain T-22 + *T. virens* strain G-41 effectively reduced Phytophthora root rot severity. Oxathiapiprolin and pyraclostrobin are the chemical fungicides that were effective in both field and greenhouse experiments.**

The nursery sector, one of the leading agricultural industries in the United States, contributes $6 billion to the national economy with an area of 344,946 acres comprising both the field and greenhouse for production. Tennessee alone contributes $59 million revenue with a total of 19,678 acres of land for production (USDA-NASS, 2020). As an important woody ornamental sold in the United States, the annual sales value of boxwood was ≈$140.9 million for the United States and $2.162 million in Tennessee (USDA-NASS, 2020). Soilborne diseases caused by oomycetes, such as species of *Pythium, Phytophthora,* and *Phytopythium*, and fungal pathogens, including species of *Fusarium, Rhizoctonia, Verticillium,* and *Sclerotinia,* are detected in U.S. nurseries, limiting production of woody ornamentals (Donahoo and Lamour, 2008; Erwin and Ribeiro, 1996; Panth et al., 2020). Root rot caused by *Phytophthora* and *Pythium* spp. was ranked as the top disease of importance by growers and university representatives during the Southern Region Integrated Pest Management Meeting in 2009, and during the IR4 Environmental Horticulture Workshop priority-setting session in 2021; *Phytophthora* and *Pythium* efficacy studies were top ranked as a national project by meeting attendees under the pathology section (Interregional Research Project Number-4, 2021). *Phytophthora* is a threat to agriculture and is ever-increasing since the discovery of the causal organism of potato late blight in the 19th century (Kroon et al., 2012). *Phytophthora nicotianae* Breda de Haan, one of the most common plant pathogens, is ranked eighth in 10 devastating oomycetes and attacks more than 255 species, including many ornamental plant species (Kamoun et al., 2015; Moralejo et al., 2009; Pane et al., 2005). In ornamental nursery production, *P. nicotianae* can attack the plants several times in a year because of its repeated production cycle (Hu et al., 2008; Moralejo et al., 2009). The pathogen infects stems, leaves, and roots by oospores and zoospores and results in symptoms such as wilting, rot, and chlorosis (Gallup et al., 2018). Phytophthora root rot, caused by *P. nicotianae*, is one of the major destructive soilborne diseases to boxwood production and was first reported in 1933 (Andrus, 1933; Erwin and Ribeiro, 1996; Irwin and Armour, 2015).

Moreover, based on nursery inspections and disease samples received in the Ornamental Plant Pathology Laboratory at Tennessee State University Otis L. Floyd Nursery Research Center, McMinnville, TN (TSUNRC), Phytophthora root rot is documented as one of the most economically important boxwood diseases in Tennessee. The maintenance, environment, and production chain to speed up the boxwood production cycle (from propagation beds to field; field to container) are increasing the chances of crop losses due to Phytophthora root rot. Containerized boxwood plants are placed in large blocks close together under overhead irrigation systems, which allows easy movement of pathogen.

Root and crown rot disease management is difficult because most plants are asymptomatic in initial stages (Madriz-Ordeñana et al., 2019). Disease-resistant varieties and crop rotations alone are not sufficient for management of soilborne diseases and should be followed by usage of fungicides and biocontrol products (Baysal-Gurel et al., 2012). Use of chemicals is the principal control method against *Phytophthora,* and it depends on the ability of chemicals to suppress sporangia formation and restrict germination or motility of zoospores (Hausbeck and Lamour, 2004; Matheron and Porchas, 2000). Field and greenhouse nursery production of woody ornamentals relies on conventional fungicides and insecticides for managing pests and diseases, which are fairly expensive and few alternatives are available (Daughtrey and Hagan, 2001; Klittich, 2008). Widely used oomycete fungicides for Phytophthora disease management include pyraclostrobin, dimethomorph, fluopicolide, flumorph, cymoxanil, kresoxim-methyl, mancozeb, zineb, and metalaxyl (Luo et al., 2020; Yang et al., 2018). In addition, fungicides recommended for management of *P. nicotianae* include metalaxyl-M, ametoctradin + dimethomorph, fludioxonil + metalaxyl-M against root and crown disease of *Solanum lycopersicum* L. (Altõn et al., 2018), fosetyl-Al, captan, dimethomorph, mancozeb,

Table 1. Different chemical fungicides, biofungicides, host-plant defense inducers, and fertilizer used in the study with their active ingredient, rate, method, interval and number of applications.

| Treatment (active ingredient) | Trade name | Rate of application | Type | Application method | Application interval and number | FRAC code | Manufacturer |
|---|---|---|---|---|---|---|---|
| Aluminum tris (80%) | Aliette WDG | 3.74 g·L$^{-1}$ | Host-plant defense inducer | Drench | 3 wk/4 | 33 | Bayer CropScience LP, St. Louis, MO |
| Aluminum tris (80%) | Areca | 5.99 g·L$^{-1}$ | Host-plant defense inducer | Foliar | 4 wk/3 | 33 | OHP Inc., Mainland, PA |
| Ametoctradin (26.9%) + dimethomorph (20.2%) | Orvego | 1.09 mL·L$^{-1}$ | Fungicide | Drench | 2 wk/6 | 45 + 40 | BASF Corporation, Research Triangle Park, NC |
| *Bacillus amyloliquefaciens* (96.4%) | Stargus | 10 mL·L$^{-1}$ | Biofungicide | Drench | 1 wk/11 | N/A | Marrone Bio Innovations, Davis, CA |
| Fluzapyroxad (21.26%) | Orkestra Intrinsic | 0.78 mL·L$^{-1}$ | Fungicide | Drench | 3 wk/4 | 11 + 7 | BASF |
| Mefenoxam (22%) | Subdue MAXX | 0.16 mL·L$^{-1}$ | Fungicide | Drench | 10 wk/2 | 4 | Syngenta Crop Protection, Greensboro, NC |
| Potassium salts of phosphorous acid (60.56%) | Agri-Fos Plus | 0.64 mL·L$^{-1}$ | Host-plant defense inducer | Drench | 6 wk/1 | 33 | Vivid Life Sciences, Minneapolis, MN |
| Oxathiapiprolin (18.7%) | Segovis | 0.25 mL·L$^{-1}$ | Fungicide | Drench | 3 wk/4 | 49 | Syngenta |
| Pyraclostrobin (12.8%) + boscalid (25.2%) | Pageant Intrinsic | 1.35 g·L$^{-1}$ | Fungicide | Drench | 3 wk/4 | 11 + 7 | BASF |
| Pyraclostrobin (23.3%) | Empress Intrinsic | 0.47 mL·L$^{-1}$ | Fungicide | | | | BASF |
| *Trichoderma harzianum* Rifai strain T-22 (1.15%) + *Trichoderma virens* strain G-41 (0.61%) | RootShield Plus WP | 0.60 g·L$^{-1}$ | Biofungicide | | | | BioWorks Inc., Victor, NY |
| Water-soluble nitrogen (total nitrogen 5%), soluble potash | ON-Gard | 5 mL.L$^{-1}$ | Fertilizer | Drench | 3 wk/4 | N/A | BioWorks |
| Water-soluble nitrogen (total nitrogen 5%), soluble potash + *T. harzianum* Rifai strain T-22 (1.15%) + *T. virens* strain G-41 (0.61%) | ON-Gard + RootShield PLUS WP | 5 mL·L$^{-1}$ + 0.6 g·L$^{-1}$ | Biofungicide + fertilizer | Drench | 3 wk/4 | N/A | BioWorks |

N/A = not applicable.

hexaconazole, azyclostrobin, chlorothalonil, and metalaxyl against root rot of *Crossandra undulaefolia* Salisb. (Sonavane and Sriram, 2021). Phenylamide fungicides such as mefenoxam or metalaxyl are widely used against *P. nicotianae* and as a result of repeated applications, different studies have reported high resistance against these fungicides (Hausbeck and Lamour, 2004; Matheron and Porchas, 2000; Parra and Ristaino, 2001). Although environmentally friendly chemical and nonchemical plant disease management methods have been developed, their results are still inconsistent and less effective than the previous standard (Gerik and Hanson, 2011).

Biological fungicides, which are derived from beneficial fungal or bacterial species, can attack causal plant pathogens and control plant diseases and are less toxic and harmful to nontarget microorganisms. Commonly used biocontrol agents against soilborne pathogens are *Trichoderma, Bacillus, Coniothyrium minitans*, species of *Gliocladium*, and *Streptomyces* (Paulitz and Bélanger, 2001). Microbial inoculants to promote plant growth and enhance resistance have been developed and field-tested for Phytophthora root rot management (Baysal-Gurel et al., 2012). In addition, suppression of *P. nicotianae* by using biofungicides in several plants are reported, such as *Burkholderia* sp. on *Catharanthus roseus*

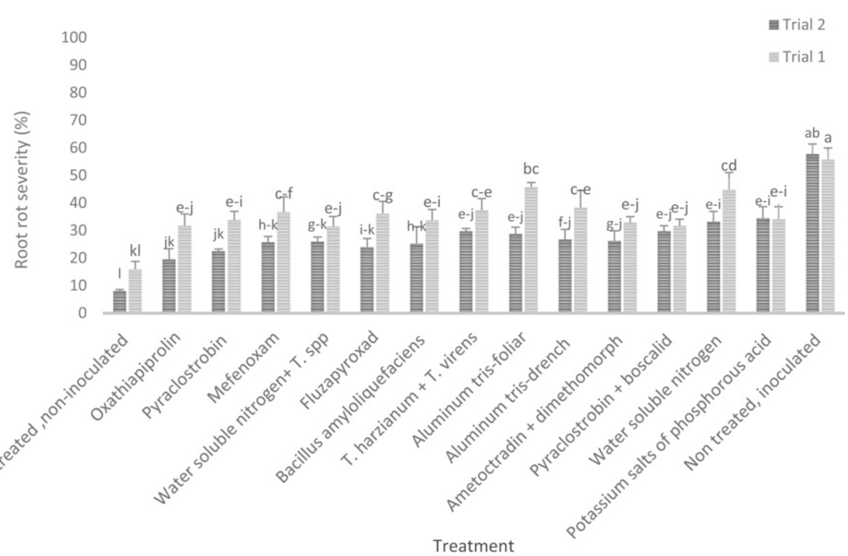


Fig. 1. Phytophthora root rot severity (mean ± SE) of boxwood plants after being treated with different chemical fungicides, biofungicides, host-plant defense inducers, and fertilizer in field conditions (Trials 1and 2). Plants were evaluated at 0% to 100% scale based on roots affected. Differences in letters beside the bars indicate the significant difference among the treatments ($F$ = 1.87, $P$ = 0.040).

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(Kong et al., 2020) and *T. harzianum* (84.19%) on *Crossandra* (Sonavane and Sriram, 2021). Fertilizers can reduce disease severity by nourishing the host to keep it healthy or by creating a physical barrier and inhibiting pathogens from reaching the host plants (Chase and Poole, 1984).

Chemical and biocontrol products represent an important option for the management of soilborne diseases. In this experiment, the efficacy of chemical fungicides such as ametoctradin + dimethomorph, fluzapyroxad, mefenoxam, oxathiapiprolin, pyraclostrobin, pyraclostrobin + boscalid, biofungicides such as *B. amyloliquefaciens* and *T. harzianum* + *T. virens,* plant host defense inducers such as aluminum tris-foliar, aluminum tris-drench, potassium salts of phosphoric acid, fertilizer water-soluble nitrogen, potash, and the combination of fertilizer + *Trichoderma* spp. are tested in greenhouse and field conditions. Efficacy of these treatments (all or some) against different oomycetes in different plants was carried out previously in the Baysal-Gurel Laboratory such as against Phytophthora *cinnamomi* Rands in container-grown flowering dogwood (*Cornus florida* L.) (Neupane et al., 2021), *Phytopythium vexans* (de Bary) Abad, de Cock, Bala, Robideau, A.M. Lodhi, and Lévesque in container-grown *Ginkgo biloba* L. and *Acer rubrum* L. (Panth et al., 2021), *P. nicotianae* in container, and field-grown *Hydrangea* spp. (Baysal-Gurel and Kabir, 2019). There are no efficacy reports regarding these commercially available chemical fungicides, biofungicides, plant host defense inducers, and fertilizer against *P. nicotianae* in container- and field-grown boxwood that can be used easily by nursery growers. This experiment was conducted to evaluate the efficacy of these treatments and develop recommendations for Phytophthora root rot management in container- and field-grown boxwood.

## Materials and Methods

*Oomycete culture. P. nicotianae* culture (Isolate FBG2017_132 GenBank accession no. ON208989) isolated from a boxwood plant in 2017 from Green Velvet cultivar in a nursery from McMinnville, TN, was obtained from Baysal-Gurel Laboratory. *P. nicotianae* cultures were maintained on V8 and PARPH-V8 media at room temperature (25 °C). For preparation of V8 medium, V8 juice (Campbell Soup Company, Camden, NJ) and 1% $CaCO_3$ (98% Acros Organics, Geel, Belgium) were mixed and centrifuged for 10 min at 13,440 rcf; 50 mL of the clarified juice was added to 450 mL of deionized water mixed with 9 g of agar (Sigma-Aldrich, St. Louis, MO). The mixture was autoclaved at 121 °C at 15 psi for 15 min and then transferred to a water bath maintaining a temperature of 55 °C. For V8-PARPH selective medium, 500 μL of each antibiotic [ampicillin (Sigma-Aldrich) (25 mg·mL$^{-1}$ in ethanol), pimaricin (2.5%) (MP Biomedicals, Santa Ana, CA), rifampicin (Sigma-Aldrich) (1 mg·mL$^{-1}$ in ethanol)] and 500 μL of each fungicide

Table 2. Height increase, width increase, total plant fresh weight, and root fresh weight (mean ± SE) of boxwood plants treated with fungicides, biofungicides, host-plant defense inducers, and fertilizer in field experiments.

| | Trial 1 | | | | Trial 2 | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Height increase[z] | Width increase[y] | Total fresh weight | Root weight | Height increase | Width increase | Total fresh weight | Root weight |
| Nontreated, noninoculated | 2.25 ± 0.22 a[x] | 1.20 ± 0.11 a | 11.53 ± 3.49 a | 6.25 ± 2.39 a | 1.82 ± 0.04x a | 1.67 ± 0.03 a | 9.20 ± 0.64 a | 4.25 ± 0.35 a |
| Nontreated, inoculated | 2.12 ± 0.17 a | 0.97 ± 0.05 a | 11.65 ± 2.26 a | 6.13 ± 1.47 a | 1.47 ± 0.08 a | 1.87 ± 0.08 a | 7.45 ± 0.84 a | 2.95 ± 0.40 a |
| Potassium salts of phosphorous acid | 2.15 ± 0.41 a | 0.98 ± 0.03 a | 11.58 ± 1.64 a | 5.45 ± 1.08 a | 1.70 ± 0.16 a | 2.30 ± 0.51 a | 8.97 ± 1.02 a | 3.72 ± 0.46 a |
| Aluminum tris-drench | 2.45 ± 0.55 a | 1.20 ± 0.04 a | 10.10 ± 2.50 a | 4.93 ± 1.41 a | 1.52 ± 0.08 a | 1.80 ± 0.38 a | 8.75 ± 0.61 a | 3.57 ± 0.28 a |
| Aluminum tris-foliar | 2.10 ± 0.05 a | 1.16 ± 0.06 a | 8.88 ± 1.43 a | 4.10 ± 0.81 a | 1.77 ± 0.10 a | 1.31 ± 0.05 a | 8.82 ± 0.74 a | 3.85 ± 0.20 a |
| Pyraclostrobin | 2.22 ± 0.31 a | 1.32 ± 0.16 a | 9.78 ± 1.22 a | 4.73 ± 0.77 a | 1.40 ± 0.09 a | 1.45 ± 0.28 a | 9.40 ± 0.77 a | 4.17 ± 0.43 a |
| Water-soluble nitrogen, soluble potash | 2.07 ± 0.28 a | 1.06 ± 0.07 a | 11.65 ± 1.25 a | 6.13 ± 1.33 a | 1.60 ± 0.17 a | 1.61 ± 0.23 a | 7.02 ± 0.81 a | 2.87 ± 0.46 a |
| *Bacillus amyloliquefaciens* | 2.27 ± 0.21 a | 1.23 ± 0.07 a | 11.65 ± 3.63 a | 6.18 ± 1.98 a | 1.55 ± 0.22 a | 1.67 ± 0.18 a | 7.67 ± 0.96 a | 3.60 ± 0.71 a |
| Water-soluble nitrogen, soluble potash + *Trichoderma* spp. | 1.95 ± 0.29 a | 1.18 ± 0.04 a | 11.78 ± 1.70 a | 5.17 ± 0.84 a | 1.40 ± 0.10 a | 1.96 ± 0.26 a | 7.05 ± 0.42 a | 2.72 ± 0.26 a |
| Fluzapyroxad | 1.62 ± 0.21 a | 1.05 ± 0.17 a | 11.15 ± 1.13 a | 5.78 ± 0.54 a | 1.52 ± 0.10 a | 1.55 ± 0.20 a | 10.10 ± 0.80 a | 4.65 ± 0.61 a |
| Ametoctradin + dimethomorph | 1.70 ± 0.36 a | 1.22 ± 0.08 a | 12.40 ± 2.38 a | 6.63 ± 1.38 a | 1.72 ± 0.18 a | 1.07 ± 0.20 a | 7.30 ± 0.85 a | 3.05 ± 0.45 a |
| Pyraclostrobin + boscalid | 1.75 ± 0.22 a | 1.32 ± 0.31 a | 10.43 ± 2.06 a | 5.35 ± 1.30 a | 1.67 ± 0.06 a | 1.76 ± 0.28 a | 9.00 ± 1.17 a | 3.95 ± 0.57 a |
| *Trichoderma harzianum* + *Trichoderma virens* | 2.42 ± 0.06 a | 1.33 ± 0.06 a | 11.35 ± 1.38 a | 5.58 ± 0.98 a | 1.82 ± 0.10 a | 2.02 ± 0.15 a | 7.40 ± 0.30 a | 3.00 ± 0.24 a |
| Oxathiapiprolin | 2.22 ± 0.35 a | 1.15 ± 0.07 a | 11.99 ± 1.18 a | 6.85 ± 0.95 a | 1.72 ± 0.12 a | 1.13 ± 0.08 a | 12.80 ± 4.71 a | 3.57 ± 0.41 a |
| Mefenoxam | 2.20 ± 0.29 a | 1.27 ± 0.09 a | 12.58 ± 2.17 a | 6.1 ± 1.22 a | 1.47 ± 0.06 a | 1.92 ± 0.23 a | 9.82 ± 1.19 a | 4.15 ± 0.67 a |
| *F* | 0.69 | 0.93 | 0.23 | 0.34 | 1.36 | 1.83 | 1.1 | 1.57 |
| *P* | 0.767 | 0.531 | 0.996 | 0.984 | 0.212 | 0.062 | 0.386 | 0.125 |

[z]Height increment was measured by subtracting height of the plant at the beginning of the experiment from the height of the plant at the end of the experiment.
[y]Width increment was measured by subtracting height of the plant at the beginning of the experiment from the height of the plant at the end of the experiment.
[x]Treatment means that do not share the same letter are significantly different (α = 0.05).

Table 3. Recovery of *Phytophthora nicotianae* (mean ± SE) of boxwood plants treated with fungicides, biofungicides, host-plant defense inducers, and fertilizer in field and greenhouse experiments.

| | Pathogen recovery (%)[z] | | | |
|---|---|---|---|---|
| | Field Expt. | | Greenhouse Expt. | |
| Treatment | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| Nontreated, noninoculated | 23.5 ± 3.86 d | 21.5 ± 2.21 e | 0 | 6 ± 2.44 e |
| Pyraclostrobin | 34.50 ± 10.1 4 cd[y] | 27.5 ± 3.5 de | 16 ± 5.09 fg | 26 ± 4.0 cd |
| Oxathiapiprolin | 37.50 ± 6.13 cd | 28 ± 3.46 bcde | 28 ± 3.74 def | 20 ± 3.16 d |
| Water-soluble nitrogen, soluble potash + *Trichoderma* spp. | 47 ± 10.40 abc | 30 ± 3.74 cde | 42 ± 8.00 bc | 38 ± 3.74 b |
| Fluzapyroxad | 50.5 ± 9.39 abc | 32.5 ± 3.59 bcde | 26 ± 6.00 ef | 34 ± 5.09 bc |
| Potassium salts of phosphorous acid | 53.5 ± 11.11 abc | 33 ± 2.38 bcde | 44 ± 7.48 bc | 26 ± 2.44 cd |
| Ametoctradin + dimethomorph | 43.00 ± 10.63 bcd | 33 ± 7.0 bcde | 32 ± 5.83 cde | 24 ± 2.44 cd |
| Mefenoxam | 34.50 ± 10.7 8 cd | 33 ± 4.12 bcde | 28 ± 3.74 def | 32 ± 4.89 bc |
| Water-soluble nitrogen, soluble potash | 49.5 ± 7.27 abc | 33.5 ± 3.59 bcd | 40 ± 4.47 cd | 32 ± 3.74 bc |
| *Bacillus amyloliquefaciens* | 47.5 ± 7.41 abc | 33.5 ± 3.40 bcd | 54 ± 6.00 b | 38 ± 3.74 b |
| Aluminum tris-drench | 55.5 ± 8.34 abc | 36 ± 6.68 bcd | 16 ± 6.78 fg | 34 ± 5.09 bc |
| *Trichoderma harzianum* + *Trichoderma virens* | 42.00 ± 9.89 bcd | 37 ± 4.50 bcd | 36 ± 6.78 cde | 38 ± 5.83 b |
| Aluminum tris-foliar | 62 ± 7.07 ab | 40.5 ± 3.30 abc | 24 ± 7.48 efg | 34 ± 5.09 bc |
| Pyraclostrobin + boscalid | 46.5 ± 0.50 bcd | 44 ± 2.94 ab | 26 ± 2.44 ef | 32 ± 3.74 bc |
| Nontreated, inoculated | 70.5 ± 4.99 a | 50 ± 2.94 a | 88 ± 4.89 a | 58 ± 3.74 a |
| *F* | 1.93 | 2.91 | 13.59 | 7.49 |
| *P* | 0.048 | 0.003 | <0.001 | <0.001 |

[z]For each plant, 10 randomly selected roots were plated on V8-PARPH oomycete-selective medium to determine recovery of *P. nicotianae* from root samples.

[y]Treatment means that do not share the same letter are significantly different ($\alpha = 0.05$).

[hymexazol (Sigma-Aldrich) (5 $mg \cdot mL^{-1}$ in sterilized water) and pentachloronitrobenzene (PCNB) (99% [GC] Sigma-Aldrich) (12.6 $mg \cdot mL^{-1}$ in ethanol)] were added to the V8 agar medium after autoclaving (Jeffers and Martin, 1986) and mixed well before pouring into petri plates.

*Inoculum preparation.* Inoculum was prepared by infesting rice grains with *P. nicotianae* (Holmes and Benson, 1994). First, 25 g long-rice grains were autoclaved twice consecutively with 18 mL of deionized water in a 250-mL conical flask. After the autoclaving process, three 7-mm-diameter V8 agar plugs from 14-d-old *P. nicotianae* cultures were placed into the flask. The infected rice grains were shaken daily for 2 weeks, and then used to inoculate boxwood plants.

*Field experimental design and condition.* The field trial was conducted at the TSUNRC in a field plot with Waynesboro loam soil. The field was cultivated and labeled, and plots were measured and marked in a completely randomized design with four replications. Six chemical fungicides, two biofungicides, three host-plant defense inducers, one fertilizer, and one combination of fertilizer and biofungicide were evaluated for their ability to control *P. nicotianae* on boxwood (*Buxus sempervirens* 'Green Velvet') (Table 1). Field was divided into four parts considered as replications. In each replication, there were 15 small plots of 2.4 m × 0.5 m dimension and each plot received different treatments. The experiment was repeated twice using the same plots. Plots were inoculated with *P. nicotianae*–infested rice grains on 10 May 2019 (Trial 1) and 3 June 2020 (Trial 2). Four rice grains were placed 5.0 cm below the soil surface 30.0 cm apart. Nontreated, noninoculated, and nontreated, inoculated plots served as controls. One-year-old rooted boxwood cuttings (*B. sempervirens* 'Green Velvet') of ≈15 to 20 cm height were planted on 24 May 2019 (Trial 1) and 23 June 2020 (Trial 2). Each plot consisted of five plants spaced 60 cm apart with 2 m between plots. Plants were watered as needed using a drip irrigation system. All treatments except aluminum tris (foliar application) were drench applied according to label requirements for rate and application intervals with a volume of 600 $mL \cdot plot^{-1}$ starting 2 weeks after transplantation of boxwood rooted cuttings on 7 June and ending on 16 Aug. 2019 (Trial 1) and starting from 7 July and ending on 15 Sept. 2020 (Trial 2). The initial and final height and average width of boxwood plants were recorded on 31 May and 29 Aug. 2019 (Trial 1) and 30 June and 28 Sept. 2020 (Trial 2), respectively. Total plant fresh weight, root fresh weight, and Phytophthora root rot disease severity of boxwood plants were recorded on 30 Aug. 2019 (Trial 1) and 29 Sept. 2020 (Trial 2). In 2019, average maximum temperatures for 24–31 May, 1–31 June, 1–30 July, 1–30 Aug., and 1–16 Sept. were 36.5 °C, 31.8 °C, 32.1 °C, and 28.4 °C, respectively; average minimum temperatures were 19.3 °C, 19.4 °C, 19.8 °C, and 16.5 °C, respectively; and total rainfall amounts were 63.0, 97.0, 221.7, 75.9, and 38.0 mm, respectively. In 2020, average maximum temperatures for 3–30 June, and 1–31 July, 1–30 Aug., and 1–29 Sept. were 29.82 °C, 32.91 °C, 32.07 °C, and 27.35 °C, respectively; average minimum temperatures were 17.63 °C, 20.90 °C, 19.36 °C, and 15.23 °C, respectively. Total rainfall amounts were 65.0, 76.9, 146.3, and 72.6 mm, respectively.

*Greenhouse experimental design and condition.* Six chemical fungicides, two biofungicides, three host-plant defense inducers, one fertilizer, and combination of fertilizer and biofungicide were evaluated for their ability to control *P. nicotianae* on boxwood (*B. sempervirens* 'Green Velvet') (Table 1). One-year-old rooted cuttings, ≈15–20 cm height, were grown in containers under greenhouse condition in a completely randomized design with five single plant replications. The experiment was conducted twice from 4 Jan. to 22 Mar. 2019 (Trial 1) and from 8 Sept. 2020 to 11 Jan. 2021 (Trial 2) at the TSUNRC. Plants were watered twice a day for 2 min using an overhead irrigation system with 200 mL water per event. Plants grown in 16.0 × 20.0-cm diameter containers (Nursery Supplies Inc., Chambersburg, PA) using potting mix [Morton's Nursery Mix: Canadian sphagnum peat (55% to 65%)] (Morton's Horticultural Products, McMinnville, TN) were inoculated with pathogen-infested rice grains by placing 4 grains 5 cm below the surface of potting mix in each container except for the noninoculated, nontreated control. Nontreated, noninoculated and nontreated, inoculated containers served as controls. All treatments except aluminum tris (foliar application) were drench applied according to label requirements for rate and application intervals with a volume of 120 mL treatment solution per container. The initial and final height and average width were recorded on 3 Jan. and 21 Mar. 2019 (Trial 1) and 8 Sep. 2020 and 10 Jan. 2021 (Trial 2), respectively. Total fresh weight, root weight, and Phytophthora root rot disease severity were recorded on 22 Mar. 2019 (Trial 1) and 11 Jan. 2021 (Trial 2). Average greenhouse maximum temperatures for 4 to 31 Jan., 1 to 28 Feb., and 1 to 22 Mar. 2019 were 31.0 °C, 27.1 °C, and 34.6 °C; and average minimum temperatures were 16.5 °C, 18.5 °C, and 16.2 °C for the first trial, respectively. Average greenhouse maximum temperatures for 8 to 30 Sept., 1 to 31 Oct., 1 to 30 Nov., 1 to 31 Dec., and 1 to 11 Jan. 2021 were 28.5 °C, 27.3 °C, 29.5 °C, 28.2 °C, and 29.0 °C and average minimum temperatures were 15.1 °C, 16.1 °C, 15.5 °C, 14.8 °C, and 14.5 °C for the second trial, respectively.

*Data collection.* Data such as plant height and width were measured at the beginning and end of each experiment; total plant fresh

Table 4. Height increase, width increase, total plant fresh weight and root fresh weight (mean ± SE) of boxwood plants treated with fungicides, biofungicides, host-plant defense inducers, and fertilizer in the greenhouse experiment.

| Treatment | Ht increase (cm)[z] | | Width increase (cm)[y] | | Total fresh wt (g) | | Total root wt (g) | |
|---|---|---|---|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| Nontreated, noninoculated | 2.8 ± 0.73 a[x] | 3.1 ± 1.88 a | 1.6 ± 0.43 a | 1.6 ± 0.52 a | 27.4 ± 2.21 a | 20.96 ± 1.57 a | 18.1 ± 1.52 a | 14.92 ± 1.14 a |
| Nontreated, inoculated | 2.6 ± 0.40 a | 1.3 ± 0.43 a | 1.3 ± 0.33 a | 0.75 ± 0.17 a | 26.6 ± 2.85 a | 18.63 ± 2.63 a | 15.1 ± 3.73 a | 14.82 ± 3.00 a |
| Aluminum tris-drench | 2.8 ± 0.37 a | 1.1 ± 0.29 a | 1.8 ± 0.46 a | 0.65 ± 0.12 a | 28.7 ± 8.12 a | 18.69 ± 1.37 a | 19.8 ± 5.89 a | 12.47 ± 1.20 a |
| Aluminum tris-foliar | 3.4 ± 0.60 a | 1.9 ± 0.55 a | 2.1 ± 0.29 a | 0.35 ± 0.16 a | 26 ± 5.49 a | 19.474 ± 1.07 a | 18.3 ± 3.90 a | 13.13 ± 1.9 a |
| Ametoctradin + dimethomorph | 2.6 ± 0.50 a | 1.5 ± 0.44 a | 1.7 ± 0.30 a | 0.55 ± 0.18 a | 24.3 ± 2.93 a | 18.72 ± 2.34 a | 16 ± 2.97 a | 14.71 ± 2.05 a |
| *Bacillus amyloliquefaciens* | 3.6 ± 0.50 a | 1.8 ± 0.37 a | 2 ± 0.41 a | 1.05 ± 0.30 a | 22 ± 3.29 a | 17.56 ± 1.23 a | 14.2 ± 2.4 a | 11.43 ± 1.22 a |
| Fluzapyroxad | 3.8 ± 1.11 a | 1.1 ± 0.18 a | 1.6 ± 0.24 a | 0.65 ± 0.21 a | 22 ± 5.8 a | 21.83 ± 1.16 a | 15.1 ± 4.16 a | 14.65 ± 1.36 a |
| Mefenoxam | 2.8 ± 0.48 a | 1.2 ± 0.20 a | 2.5 ± 0.52 a | 0.65 ± 0.12 a | 21.4 ± 4.74 a | 18.66 ± 2.73 a | 13.1 ± 3.90 a | 14.30 ± 1.60 a |
| Potassium salts of phosphorous acid | 2.4 ± 0.92 a | 1.8 ± 0.64 a | 1.8 ± 0.60 a | 1.05 ± 0.20 a | 31.8 ± 7.00 a | 18.92 ± 1.42 a | 22.2 ± 4.89 a | 15.22 ± 2.17 a |
| Oxathiapiprolin | 2.8 ± 0.66 a | 0.9 ± 0.24 a | 1.2 ± 0.48 a | 0.65 ± 0.15 a | 23 ± 1.31 a | 19.05 ± 1.93 a | 15.6 ± 1.57 a | 13.96 ± 1.48 a |
| Pyraclostrobin | 3.2 ± 1.11 a | 1.4 ± 0.29 a | 1 ± 0.27 a | 0.9 ± 0.12 a | 32.4 ± 3.37 a | 20.93 ± 2.96 a | 23.5 ± 2.99 a | 15.02 ± 2.01 a |
| Pyraclostrobin + boscalid | 3.6 ± 0.74 a | 2.1 ± 0.50 a | 1.7 ± 0.46 a | 0.85 ± 0.50 a | 23.4 ± 5.04 a | 18.05 ± 3.56 a | 16.6 ± 3.35 a | 12.77 ± 2.56 a |
| *Trichoderma harzianum* + *Trichoderma virens* | 2.6 ± 1.02 a | 1.2 ± 0.37 a | 1.9 ± 0.55 a | 0.9 ± 0.33 a | 22.6 ± 5.35 a | 20.09 ± 2.96 a | 15.6 ± 3.97 a | 15.10 ± 2.30 a |
| Water-soluble nitrogen, soluble potash | 2.2 ± 0.48 a | 1.2 ± 0.40 a | 1 ± 0.27 a | 0.55 ± 0.20 a | 22.9 ± 1.60 a | 20.15 ± 2.67 a | 16.1 ± 1.4 a | 14.45 ± 2.82 a |
| Water-soluble nitrogen, soluble potash plus *T. harzianum* + *T. virens* | 2.8 ± 0.37 a | 2.2 ± 0.33 a | 0.9 ± 0.36 a | 1.05 ± 0.42 a | 35 ± 5.06 a | 18.28 ± 1.89 a | 25.2 ± 3.74 a | 13.49 ± 1.41 a |
| *F* | 0.44 | 0.86 | 1.18 | 1.12 | 0.84 | 0.29 | 0.99 | 0.33 |
| *P* | 0.954 | 0.603 | 0.317 | 0.357 | 0.625 | 0.992 | 0.475 | 0.987 |

[z]Height increment was measured by subtracting height of the plant at the beginning of the experiment from the height of the plant at the end of the experiment.
[y]Width increment was measured by subtracting height of the plant at the beginning of the experiment from the height of the plant at the end of the experiment.
[x]Treatment means that do not share the same letter are significantly different ($\alpha = 0.05$).

weight (root and shoot), and total root fresh weight (roots were cut from the plant at the base of the root collar) were recorded at the end of each experiment for analyses. Total height and width increment was measured by subtracting the height and width of the plant recorded at the beginning from the height and width of the plant recorded at the end of the experiment. Roots were washed with water to remove debris. Phytophthora root rot disease severity was assessed visually using a scale of 0% to 100% of total root system affected at the end of each trial where 0% is no disease severity and 100% is the death of plants. Furthermore, 10 randomly selected root samples (1 cm long) from the root tips of each boxwood plant were plated on V8-PARPH oomycete-selective medium to determine the percent recovery of *P. nicotianae* from root samples. To confirm pathogen identity, total DNA was extracted directly from root tissue with the UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) following the manufacturer's instructions after each experiment. The internal transcribed spacer (ITS) region of the ribosomal DNA was amplified by polymerase chain reaction using primer pair ITS1 and ITS4 (White et al., 1990).

*Statistical analysis.* Two-way analysis of variance (ANOVA) test was performed to see the main and interactive effects of treatments (treatment effect and trial effect) on Phytophthora root rot disease severity, plant height and width increase, total plant fresh weight and root fresh weights, and percent recovery of *P. nicotianae* with SAS statistical software 2016 (SAS Institute Inc., Cary, NC) in field experiment data. Multiple comparisons were done using post hoc test [Fisher's least significant difference (LSD)] at $P < 0.05$ when the interactions were significant. For the greenhouse trial, ANOVA of all data sets (Phytophthora root rot disease severity, plant height and width increase, total plant fresh weight and root fresh weights, and percent recovery of *P. nicotianae*) was performed using the general linear model procedure with SAS statistical software, and means were separated using the Fisher's LSD test.

## Results

*Efficacy of fungicides, biofungicides, host-plant defense inducer, and fertilizer on Phytophthora root rot under field conditions.* There was significant interaction ($F = 7.89$, $P < 0.05$) between treatment (chemical fungicides, biofungicides, host-plant defense inducers, fertilizer) and trial (Trial 1 and Trial 2) effect on root rot disease severity (Fig. 1). Phytophthora root rot disease pressure was moderate, with nontreated, inoculated boxwood plants showing 55.5% (Trial 1) and 57.8% (Trial 2). The lowest root rot severity was from nontreated, noninoculated controls for both field trials. All the treatments significantly reduced root rot severity in Trial 1 and in Trial 2, all treatments except aluminum tris-foliar in Trial 1. Treatments such as pyraclostrobin, aluminum tris-drench, aluminum

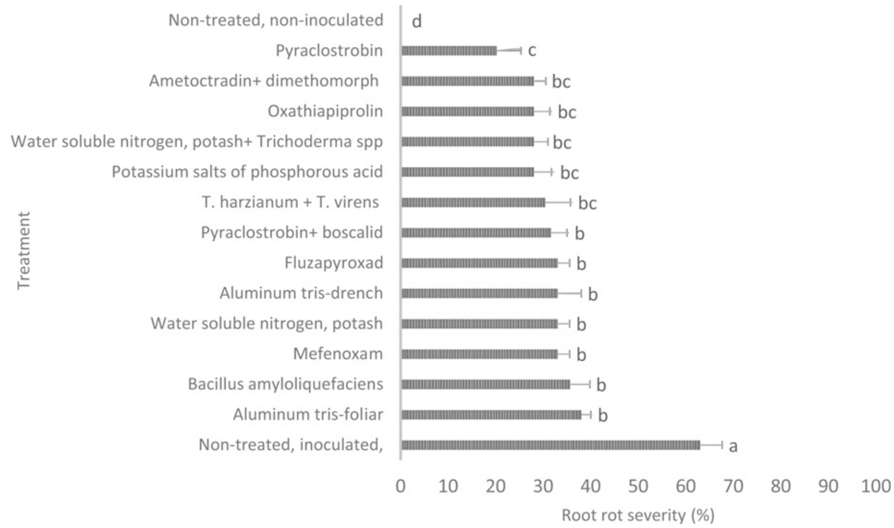


Fig. 2. Phytophthora root rot severity (mean ± SE) of boxwood plants after being treated with different chemical fungicides, biofungicides, host-plant defense inducers, and fertilizer in the greenhouse experiment (Trial 1). Plants were evaluated at 0% to 100% scale based on roots affected. Differences in letters beside the bars indicate the significant difference among the treatments ($F = 12.26$, $P < 0.001$).

tris-foliar, fluzapyroxad, and mefenoxam significantly reduced disease severity in Trial 2 compared with Trial 1. However, fungicides such as oxathiapiprolin, pyraclostrobin, fluzapyroxad, mefenoxam, biofungicides *B. amyloliquefaciens,* and a combined application of water-soluble nitrogen, soluble potash, and *Trichoderma* spp. effectively reduced Phytophthora root rot in field-grown boxwood.

There was no significant interactive effect of treatment and trial on plant height increase, width increase, total plant fresh weight, total root weight, and pathogen recovery (Tables 2 and 3). Pathogen was recovered from roots with all treatments including nontreated, noninoculated control plants. The highest pathogen recovery was from nontreated, inoculated control plants in both trials. Among the treatments used, pyraclostrobin and oxathiapiprolin had the lowest pathogen recovery (Table 3). The sequences of amplicon recovered from pathogen recovery in both trials had 100% coverage and 100% identity to that of *P. nicotianae*.

*Efficacy of fungicides, biofungicides, host-plant defense inducer, and fertilizer on Phytophthora root rot under greenhouse conditions.* Phytophthora root rot disease pressure was moderate, with nontreated, inoculated boxwood plants showing 63% (Trial 1) and 54% (Trial 2). All treatments (fungicides, biofungicides, and fertilizer) significantly reduced Phytophthora root rot severity in both greenhouse trials compared with the nontreated, inoculated control (Figs. 2 and 3). In Trial 1, the treatment that most effectively reduced root rot disease was pyraclostrobin ($F = 12.26$, $P < 0.0001$, Fig. 2). In Trial 2, fungicides such as pyraclostrobin, oxathiapiprolin, and ametoctradin + dimethomorph most effectively reduced disease compared with other treatments applied ($F = 12.05$, $P < 0.0001$, Fig. 3). No disease severity was recorded from the nontreated, noninoculated control plants in Trial 1 but was recorded in Trial 2.

Pathogens were recovered from roots treated with all treatments including nontreated, noninoculated control plants, and the highest recovery was from nontreated, inoculated control plants in both trials. Pathogen recovered from all other treatments was significantly lower compared with the nontreated, inoculated plants. Among the treatments used, pyraclostrobin and oxathiapiprolin had the lowest pathogen recovery (Table 3). The sequences of amplicon recovered from pathogen recovery in both trials had 100% coverage and 100% identity to that of *P. nicotianae*. There were no significant differences in plant height increase, plant width increase, total fresh weight, and root weight between treated and nontreated boxwood plants in both trials (Table 4).

## Discussion

*P. nicotianae* is one of the important soilborne pathogens in nursery crop production with significant yield loss affecting both field and container productions in woody ornamentals. For developing recommendations for management of Phytophthora root rot in boxwood, we evaluated the performance of fungicides, biofungicides, host-plant defense inducers, and fertilizers with artificial pathogen pressure in greenhouse- and field-grown boxwood. In our experiments, there were no significant differences between treatments based on plant height and width increases, total fresh weight, and total root weight. Boxwood, being very slow-growing plants and experiments being carried out for only 4 months could be the reasons for these insignificant results. So, efficacy of the treatments were based on the efficiency to lower Phytophthora root rot severity of experimental plants. Among the treatments used, fungicides such as oxathiapiprolin, pyraclostrobin, and ametoctradin + dimethomorph in container-grown boxwood, and fungicides such as pyraclostrobin, oxathiapiprolin, fluzapyroxad, and mefenoxam; biofungicide such as *B. amyloliquefaciens;* and a combination of water-soluble nitrogen, soluble potash, and *Trichoderma* spp. in field-grown boxwood were highly effective to reduce Phytophthora root rot in our experiments. Oxathiapiprolin and pyraclostrobin are the chemical fungicides that effectively reduced Phytophthora root rot severity in both field- and container-grown production systems and biofungicides were effective only in field conditions.

Oxathiapiprolin (FRAC code 49) is a piperidinyl-thiazole isooxazoline, which acts on pathogens by inhibiting oxysterol-binding proteins (FRAC, 2020; Pasteris et al., 2016). Effective results from our experiments were similar to some previous experiments reported for different *Phytophthora* species: *P. nicotianae* on *Citrus* L. (Hao et al., 2019; Gray et al., 2018), *Nicotiana tabacum* L. (Bittner and Mila, 2016), *Catharanthus roseus* (L.) Don. (Baysal-Gurel et al., 2022), *P. cinnamomi* on *Cornus florida* L. (Brown et al., 2019) and *Persea americana* Mill. (Belisle et al., 2019), *Phytophthora capsici* Leonian on *Capsicum annuum* L. (Ji and Csinos, 2015; Miao et al., 2016), *Phytophthora infestans* (Mont.) De Barry on *S. lycopersicum* (Cohen et al., 2018), *Phytophthora agathidicida* on *Agathis australis* (D.Don) Loudon (Cohen et al., 2018), and *Phytophthora sojae* Kaufm & Gerd. on *Glycine max* (L.) Merr. (Vargas et al., 2022). Pyraclostrobin (FRAC Code 11), developed from derivates of natural fungicides (fungi *Oudemansiella mucida* and *Strobilurus tenacellus* and bacterium *Myxococcus fulvus*), is a Quinone-inhibiting fungicide (Ammermann et al., 2000), and was effective in both conditions of our experiments. This efficacy was similar as suggested for managing *Phytophthora* in ornamental cultivation (Daughtrey and Benson, 2005). Some experiments done previously by different researchers also support the efficacy of pyraclostrobin in managing several species of *Phytophthora* such as *P. cinnamomi* on flowering dogwood, where pyraclostrobin was found to be highly effective (Neupane et al., 2021). A study on leather root rot of *Fragaria ananassa* Duchesna caused by *Phytophthora cactorum* (Libert and Cohn) J. Schröt showed that pyraclostrobin provided excelling control (Rebollar-Alviter and Ellis, 2005). Another experiment on managing *P. sojae* on soybean using pyraclostrobin as seed treatment showed efficacy for increased chlorophyll level, shoot and root length, root activity, and continuous positive effect on enhancing the plant defense (Li et al., 2020). In contrast, some studies showed decreased sensitivity of *P. capsici* causing Phytophthora blight in pepper against quinone-outside inhibitor (QoI) fungicides including pyraclostrobin (Ma et al., 2018), suggesting the necessity of using fungicides in rotation to save crops from increased resistance.

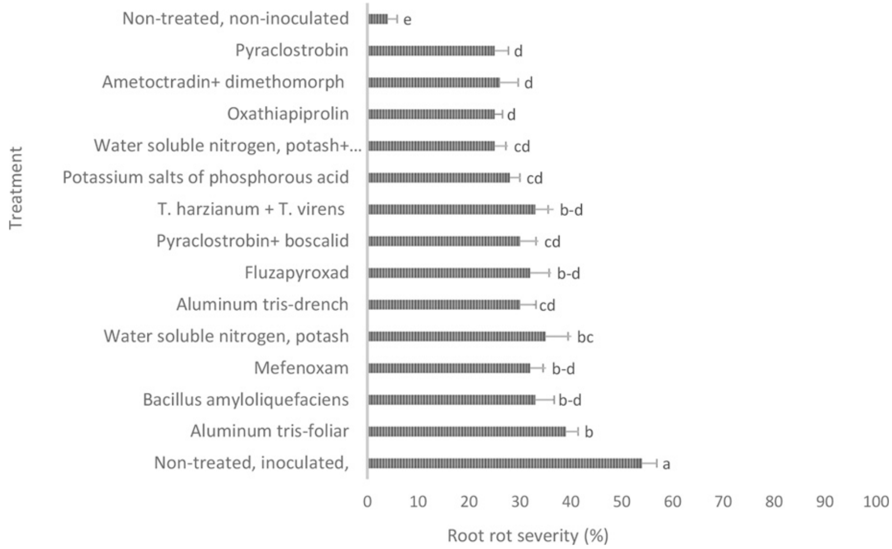


Fig. 3. Phytophthora root rot severity (mean ± SE) of boxwood plants after being treated with different chemical fungicides, biofungicides, host-plant defense inducers, and fertilizer in the greenhouse experiment (Trial 2). Plants were evaluated at 0% to 100% scale based on roots affected. Differences in letters beside the bars indicate the significant difference among the treatments ($F = 12.05$, $P < 0.0001$).

Biofungicides are alternatives to chemical fungicides in plant production conditions with immense opportunity. In this study, the efficacy of biofungicide *B. amyloliquefaciens* was reported in field conditions, which was similar to results previously documented against *P. sojae* (Liu et al., 2019) and *P. cactorum* (Lebert & Cohn) J. Schröt (Lee et al., 2015) in different experiments. In vivo and in vitro experiments of different species of *Trichoderma,* including *T. harzianum* Rifai, *T. virens, Trichoderma viride* Pers., *Trichoderma gamsii, Trichoderma longibrachiatum* Rifai, *Trichoderma asperellum* Samuels, Lieckf. & Nirenberg, and *Trichoderma atroviride,* were effective against soilborne pathogens (La Spada et al., 2020), but in our study the combined application of water-soluble nitrogen, soluble potash, and *T. harzianum* Rifai strain T-22 + *T. virens* strain G-41 was effective, which might be due to the synergistic effect of both fertilizer and biofungicide.

Host-plant defense inducers significantly reduced disease severity compared with the nontreated, inoculated control but were less effective when compared with fungicides and biofungicides. Pathogens that were recovered from nontreated, noninoculated control plant roots could be through cross contamination in the nursery or natural condition of the field, as infested potting mix has been a major route for dissemination of several species of *Phytophthora,* including *P. nicotianae* in nurseries (Herrero et al., 2003).

In conclusion, from our experiment, we were able to find effective fungicides and biofungicides that can be used to manage Phytophthora root rot in field-grown and greenhouse container-grown boxwood. Those treatments can be used as part of an integrated approach along with other measures, and are recommended to nursery producers dealing with Phytophthora root rot problems.

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