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Comparative Performance of Fungicides in Management of Phytophthora Root Rot on Boxwood

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Abstract. **Nursery growers rely on fungicides to manage root rot disease of boxwood caused by *Phytophthora nicotianae* and *P. cinnamomi.* Repeated use of the same fungicide may lead to the fungicide resistance. In this study, fungicides pyraclostrobin + cyazofamid (Empress + Segway), ametoctradin + dimethomorph (Orvego), ametoctradin + dimethomorph alternated with pyraclostrobin (Orvego *alt* Empress), ametoctradin + dimethomorph alternated with fluxapyroxad + pyraclostrobin (Orvego *alt* Orkestra), and oxathiapiprolin (Segovis) were evaluated for their efficacy in managing Phytophthora root rot under greenhouse conditions in Tennessee. One-year-old container-grown boxwood 'Green Velvet' plants were inoculated with *P. nicotianae* or *P. cinnamomi*. The first applications of fungicide treatments were made preventatively as a drench 48 h before pathogen inoculation. Following inoculation, four applications of fungicide treatments were performed on a 14-day intervals. Initial and final plant height and width were measured. Total plant fresh weight and root fresh weight were measured at the end of the trials, and plants were evaluated for root rot severity (0% to 100% roots affected) and pathogen recovery. All fungicides significantly reduced root rot severity and pathogen recovery of *P. nicotianae* and *P. cinnamomi*. Ametoctradin + dimethomorph alternated with pyraclostrobin (Orvego *alt* Empress) provided similar protection against *P. cinnamomi* to that of a single application of ametoctradin + dimethomorph (Orvego) or oxathiapiprolin (Segovis). For *P. nicotianae*, ametoctradin + dimethomorph alternated with pyraclostrobin + fluzapyroxad (Orvego *alt* Orkestra) was found to be as effective as a single application of either ametoctradin + dimethomorph (Orvego) or oxathiapiprolin (Segovis) in one of the two trials. Effects of fungicides on plant growth such as height, width, total, and root fresh weight were not significant. These findings will be useful to nursery growers in selecting the right fungicide program for the management of root rot disease of boxwood caused by *P. nicotianae* and *P. cinnamomi*.**

Boxwood (Buxus spp.) is a woody ornamental shrub commonly cultivated for its vibrant green color and evergreen growth. The evergreen growth makes boxwood a popular landscaping shrub, particularly for hedges or topiary. In 2019, boxwood was reported as the highest-grossing broadleaf evergreen in the United States, with an annual wholesale value of ∼$140 million (National Agriculture Statistics Service 2020). However, the boxwood industry in the United States has been threatened by a growing number of serious plant pest insects and diseases (Dhakal et al. 2022). Phytophthora root rot caused by *Phytophthora* spp., is a common and destructive disease of boxwood (Vettraino et al. 2010; Weiland 2021). Boxwood species susceptible to root rot are American/common, English, and Japanese boxwood (Baysal-Gurel et al. 2017). Several species of *Phytophthora* are responsible for causing root rot in boxwood, such as *P. cactorum* (Lebert & Cohn) J. Schröter; *P. cinnamomi Rand*; *P. citricola Sawada*; *P. citrophthora* [R.E. Sm. & E.H. Sm.] *Leonian*, *P. elongate* A. Rea, M. Stukely & T. Jung; P. *gonapodyides* (Petersen) Buisman, *P. multivora* P.M. Scott & T. Jung; *P. nicotianae* Breda de Hann; *P. occultans* Man in't Veld & Rosendahl, and *P. pini Leonian* (Andrus 1933; Bienapfl and Balci 2014; Daughtrey et al. 2016; Gitto et al. 2018; Haasis 1961; Reeser et al. 2015; Vettraino et al. 2010).

In Tennessee, *P. nicotianae* and *P. cinnamomi* are the most common causal agents of Phytophthora root rot in boxwood (Baysal-Gurel et al. 2017; Neupane and Baysal-Gurel 2022). Root rot symptoms first appear as dark brown to black discoloration in the roots (Parajuli et al. 2022). As the disease progresses, aboveground symptoms appear as a slow decline in growth, branch dieback, leaf chlorosis/leaves turning a straw color, and potential plant death (Baysal-Gurel et al. 2017). Zoospores serve as the primary infecting propagules of both *P. nicotianae* and *P. cinnamomi.* They are chemotactically attracted to plant roots, which is favored by surface water (Erwin and Ribeiro 1996; Van West et al. 2002). Moreover, resting structures such as oospores and chlamydospores enables them to survive in dead plant materials such as fallen leaves, branches, or organic matter for multiple years (Agrios 1997; Gallup et al. 2018). When favorable soil moisture and temperature returns, resting structures become active and infect plants. *P. nicotianae* and *P. cinnamomi* are active throughout the year in greenhouse production systems and during the warm season in the field.

Strategies recommended to manage Phytophthora root rot in boxwood include the integration of sanitation, cultural practices, water management, and the repeated application of fungicides and biofungicides (Baysal-Gurel et al. 2019; Dhakal et al. 2022; Neupane and Baysal-Gurel 2022). There are no boxwood cultivars documented to be resistant to Phytophthora. Fungicides belonging to Fungicide Resistance Action Committee (FRAC) groups 33, 43, 28, 1 + 14, 11, 4, 12 + 4, 40, 7 + 11, 45 + 40, U15, 21, and 14 are widely used for Phytophthora root rot management in boxwood (Baysal-Gurel et al. 2017). Although some of these fungicides have been successful in suppressing Phytophthora root rot (Neupane and Baysal-Gurel 2022), the application of the same fungicide continuously may result in fungicide resistance (Shew 1985). For instance, *P. nicotianae* has already been reported as a high-risk pathogen for fungicide resistance (Hu et al. 2008). Moreover, because most of the newly developed fungicides are site-specific that act on certain metabolic pathways of the pathogen (FRAC 2021), the effectiveness of fungicides can be reduced over time due to resistance development. As such, alternative fungicide application strategies that can effectively manage Phytophthora are essential to reduce resistance.

In recent years, several new fungicides with different modes of action have been developed and have provided excellent control of Phytophthora in woody ornamental nursery plants (Neupane et al. 2022; Parajuli and Baysal-Gurel 2022). These fungicides can be used in a rotation program or by mixing fungicides of different modes of action to manage resistance (Damicone and Smith 2009; van den Bosch et al. 2014). This study aims to evaluate the efficacy of fungicides alone, in combination, or in rotation against Phytophthora root rot under

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greenhouse conditions using boxwood plants inoculated with *P. nicotianae* or *P. cinnamomi*.

Materials and Methods

Inoculum preparation. Isolates FBG4871 of *P. cinnamomi* and FBG2017_132 of *P. nicotianae* (GenBank accession no. OQ836532 and ON208989, respectively) were obtained from cultures maintained at Baysal-Gurel laboratory at Tennessee State University. Isolates FBG4871 and FBG2017_132 were isolated from a symptomatic boxwood plant 'Green Velvet'. Symptomatic plants showed branch dieback, leaf chlorosis/leaves turning a straw color, and roots were dark brown to black. Cultures of *P. nicotianae* and *P. cinnamomi* were maintained on V8 agar medium at room temperature (25 °C). The V8 agar medium was prepared according to the procedure followed by Jeffers and Martin (1986). Inoculum was prepared by growing *P. nicotianae* or *P. cinnamomi* in rice grains (Holmes and Benson 1994). Rice grains (∼25 g) were mixed with 18 mL of deionized water in a conical flask and were consecutively autoclaved twice (each for 30 min). The second autoclave was done 1 h after the first to allow complete cooling of the flask. Six V8 agar plugs measuring 7 mm in diameter were taken from 14-day-old cultures of *P. cinnamomi* or *P. nicotianae* and placed into a flask and allowed to colonize rice grains for 10 d. The flask was lightly shaken every day until rice grains were used.

Treatments. Fungicides treatments included pyraclostrobin (Empress) + cyazofamid (Segway), ametoctradin + dimethomorph (Orvego), ametoctradin + dimethomorph alternated with (*alt*) pyraclostrobin (Orvego *alt* Empress), ametoctradin + dimethomorph *alt* fluzapyroxad + pyraclostrobin (Orvego *alt* Orkestra) and oxathiapiprolin (Segovis). Fungicide treatment details are included in Table 1.

Experimental design and condition. The experiments were conducted in a greenhouse at the Otis L. Floyd Nursery Research Center in McMinnville, TN, USA. On 22 Apr, one-year-old boxwood 'Green Velvet' rooted cuttings were potted in 1-gal containers filled with potting mix [Morton's Nursery Mix: Canadian sphagnum peat (55% to 65%)] (Morton's Horticultural Products, McMinnville, TN, USA). On 8 June, plants were fertilized with 18N–6P–8K Nutricode controlled-release granular fertilizer (Florikan E.S.A. LLC, Sarasota, FL) at a rate of 10 g/plant. Five single-plant replicates per treatment were arranged in a completely randomized design. The experiment was conducted twice, the first from 16 Aug to 27 Oct 2022 (Trial 1) and the second from 23 Aug to 3 Nov 2022 (Trial 2) for both *P. nicotianae* and *P. cinnamomi*. All treatments were applied as a drench application on 16 Aug 2022 (Trial 1) and 23 Aug 2022 (Trial 2). After 48 h, boxwood plants were inoculated with *P. nicotianae* or *P. cinnamomi* colonized rice grains by placing four grains 5 cm below the surface of the potting mix on four opposite sides of the plant. Nontreated, noninoculated and nontreated, and inoculated boxwood plants served as controls. Treatments were continuously applied as drench applications on a 14-d interval until 13 Oct 2022 (Trial 1) and 20 Oct 2022 (Trial 2). Plants were irrigated using overhead mist/water nozzle spinnet brown (Hummert International, Earth City, MO, USA) emitters for 2 min twice daily (250 mL/d) in Aug, Sept, Oct, and Nov. During the first trial, the maximum greenhouse temperatures were 28.8, 28.2, and 27.7 °C; minimum temperatures were 19.7, 17.9, and 17. °C; and average relative humidity was 97.3%, 98.7%, and 99.0% for Aug, Sept, and 1–13 Oct, respectively. During the second trial, the maximum greenhouse temperatures were 29.5, 28.2, 27.7, and 27.6 °C; minimum temperatures were 19.5, 17.9, 17.5, and 14.4 °C; and average relative humidity were 97.3, 98.7, 99.5, and 99.0% for 23–31 Aug, Sept, Oct, and 1–3 Nov, respectively.

Recording plant growth and root rot disease. On 16 Aug 2022 (Trial 1) and 23 Aug 2022 (Trial 2), initial plant height and width were measured. Final height and width, total plant fresh 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 the experiment on 27 Oct 2022 (Trial 1) and 3 Nov 2022 (Trial 2). The height was measured from the base of the stem at the soil level to the top of the terminal bud on the main stem. Plant width was measured as the average of the widest part from leaf tip to leaf tip and the width perpendicular to the widest part. The increase in plant height or width was calculated by subtracting the initial height/width from the final height/width. Root rot severity caused by *P. nicotianae* or *P. cinnamomi* was assessed using a scale from 0% to 100% roots affected. Ten randomly selected root pieces (∼1 cm long) were plated on a V8 medium to observe pathogen recovery from treated and nontreated roots. Pathogen recovery was determined as a percentage value by counting the number of roots with pathogen growth to the total number of roots plated.

Statistical analysis. The effects of fungicide treatments on height increase, width increase, total plant fresh weight, and root fresh weight were analyzed using one-way analysis of variance with PROC GLM procedure (SAS Inc., Cary, NC, USA). Means were compared using Fisher's least significant difference test ($\alpha = 0.05$). Phytophthora root rot severity and pathogen recovery from roots were analyzed using general linear mixed model with a logit link and beta distribution (PROC GLIMMIX). Root rot severity and Phytophthora recovery data were converted from 0 to 1 to meet the PROC GLIMMIX assumption before analysis.

Results

Effects of fungicides on growth and health of boxwood inoculated with P. cinnamomi*.* Phytophthora root rot disease pressure caused by *P. cinnamomi* was moderate in both trials (Table 2). Phytophthora root rot severity was the highest for nontreated, inoculated boxwood plants with 52% (Trial 1) and 49% (Trial 2). In both trials, all tested fungicide treatments significantly reduced Phytophthora root rot severity compared with the nontreated, inoculated boxwood plants. In Trial 1, boxwood plants treated with oxathiapiprolin (Segovis) showed significantly lower root rot severity compared with plants treated with pyraclostrobin + cyazofamid (Empress + Segway) and ametoctradin + dimethomorph alternated with pyraclostrobin + fluzapyroxad (Orvego *alt* Orkestra). Root rot severity was statistically similar in ametoctradin + dimethomorph (Orvego), ametoctradin + dimethomorph alternated with pyraclostrobin (Orvego *alt* Empress) and oxathiapiprolin (Segovis) treated boxwood roots. In Trial 2, ametoctradin + dimethomorph alternated with pyraclostrobin (Orvego *alt* Empress) treated boxwood plants exhibited significantly lower root rot severity compared with ametoctradin + dimethomorph alternated with pyraclostrobin +

Table 1. List of fungicides used in this study.

Treatment[i]	Active ingredient (%)	Trade name	Appl. rate (mL/L)	FRAC code	Appl. interval	Appl. method	No. of appl
Ametoctradin + dimethomorph	26.9 + 20.2	Orvego	1.09	45 + 40	14 d	Drench	5
Ametoctradin + dimethomorph *alt* pyraclostrobin	26.9 + 23.3 *alt* 23.3	Orvego *alt* Empress	1.09 *alt* 0.46	45 + 40 *alt* 11	14 d	Drench	5
Ametoctradin + dimethomorph *alt* pyraclostrobin + fluzapyroxad	26.9 + 20.2 *alt* 21.6 + 21.26	Orvego *alt* Orkestra	1.09 *alt* 0.77	45 + 40 *alt* 11 + 7	14 d	Drench	5
Pyraclostrobin + cyazofamid	23.3 + 34.5	Empress + Segway	0.23 + 0.116	11 + 21	14 d	Drench	5
Oxathiapiprolin	18.7	Segovis	0.154	49	14 d	Drench	5

[i] Fungicide manufacturer: ametoctradin + dimethomorph (BASF Corporation, Florham Park, NJ, USA); pyraclostrobin (BASF Corporation); pyraclostrobin + fluzapyroxad (BASF Corporation); cyazofamid (OHP Inc., Bluffton, SC, USA); oxathiapiprolin (Syngenta International AG, Basel, Switzerland).
alt = alternated with; Appl = application; FRAC = Fungicide Resistance Action Committee.

Table 2. Mean (± *SE*) root rot severity (% root affected) and pathogen recovery from the roots of boxwood (*Buxus* spp.) inoculated with *Phytophthora cinnamomi* in greenhouse studies.

		Root rot severity (%)		Pathogen recovery (%)	
Treatment	Trade name	Trial 1	Trial 2	Trial 1	Trial 2
Ametoctradin + dimethomorph	Orvego	21 ± 1.9 bc[i]	18 ± 4.4 cd	24 ± 2.5 b	24 ± 2.4 bc
Ametoctradin + dimethomorph *alt* pyraclostrobin	Orvego *alt* Empress	18 ± 2.0 bc	16 ± 3.3 d	26 ± 2.5 b	22 ± 2.0 bc
Ametoctradin + dimethomorph *alt* pyraclostrobin + fluzapyroxad	Orvego *alt* Orkestra	26 ± 3.7 b	27 ± 2.6 b	22 ± 2.0 b	26 ± 2.4 b
Pyraclostrobin + cyazofamid	Empress + Segway	26 ± 4.9 b	26 ± 1.9 bc	24 ± 5.1 b	28 ± 3.7 b
Oxathiapiprolin	Segovis	15 ± 1.6 c	18 ± 2.6 cd	18 ± 2.0 b	18 ± 2.0 c
Nontreated, inoculated control		52 ± 3.4 a	49 ± 3.7 a	50 ± 3.16 a	52 ± 2.0 a
Nontreated, noninoculated control		0 ± 0 d	0 ± 0 e	0 ± 0 c	0 ± 0 d
P value		<0.0001	<0.0001	<0.0001	<0.0001

[i] Means followed by a different lowercase letter within a column are significantly different ($P \leq 0.05$). Data were analyzed according to general linear mixed model with a logit link and beta distribution (PROC GLIMMIX).
alt = alternated with.

fluzapyroxad (Orvego *alt* Orkestra) and pyraclostrobin + cyazofamid (Empress + Segway) treated plants. Ametoctradin + dimethomorph (Orvego) and oxathiapiprolin (Segovis) treated plants had root rot severity comparable to ametoctradin + dimethomorph alternated with pyraclostrobin (Orvego *alt* Empress) treated plants. Significantly higher pathogen recovery was recorded from nontreated, inoculated boxwood roots. In Trial 1, pathogen recovery was lower in fungicide-treated boxwood plants, but there were no significant differences among the fungicide treatments (Table 2). Oxathiapiprolin (Segovis) treated boxwood plants had significantly lower pathogen recovery compared with plants treated with pyraclostrobin + cyazofamid (Empress + Segway) and ametoctradin + dimethomorph alternated with pyraclostrobin + fluzapyroxad (Orvego *alt* Orkestra) in Trial 2 (Table 2). Nontreated, noninoculated boxwood plants did not exhibit root rot severity symptoms, and *P. cinnamomi* was not recovered in both trials. There were no significant differences among the treatments for height increase, width increase, total plant fresh weight, and root fresh weight in both trials (Table 3).

Effects of fungicides on growth and health of boxwood inoculated with P. nicotianae. Phytophthora root rot disease pressure caused by *P. nicotianae* was moderate in both trials with nontreated, inoculated boxwood plants having the highest level of root rot severity of 56% and 51% in Trials 1 and 2, respectively (Table 4). In both trials, all fungicide treatments significantly reduced root rot severity compared with the nontreated, inoculated boxwood. In Trial 1, Phytophthora root rot severity in oxathiapiprolin (Segovis), ametoctradin + dimethomorph (Orvego) and ametoctradin + dimethomorph alternated with pyraclostrobin + fluzapyroxad (Orvego *alt* Orkestra) treated boxwood plants were lower than pyraclostrobin + cyazofamid (Empress + Segway) and ametoctradin + dimethomorph alternated with pyraclostrobin (Orvego *alt* Empress) treated boxwood plants. In Trial 2, boxwood plants treated with oxathiapiprolin (Segovis) and ametoctradin + dimethomorph (Orvego) had the lowest root rot severity. In both trials, all fungicide treatments significantly reduced pathogen recovery (Table 4) and was significantly higher in nontreated, inoculated boxwood plants (~50%). In Trial 1, oxathiapiprolin (Segovis) and ametoctradin + dimethomorph (Orvego) treated boxwood plants had significantly lower *P. nicotianae* recovery than pyraclostrobin + cyazofamid (Empress + Segway) treated plants. In Trial 2, the lowest pathogen recovery was from oxathiapiprolin (Segovis), ametoctradin + dimethomorph (Orvego) and ametoctradin + dimethomorph alternated with pyraclostrobin + fluzapyroxad (Orvego *alt* Orkestra) treated boxwood plants. In both trials, no root rot severity was observed, and *P. nicotianae* was not recovered from nontreated, noninoculated boxwood plants. Furthermore, there were no significant differences among the treatments in height increase, width increase, total plant fresh weight, and total root fresh weight (Table 5).

Discussion

Phytophthora root rot, caused by *P. nicotianae* and *P. cinnamomi*, is one of the greater challenges to boxwood nursery production and is commonly managed by the repeated applications of the fungicides that belong to the same FRAC code groups. Therefore, the evaluation of fungicide rotation programs with different modes of action is critical in preventing fungicide resistance development in pathogen populations.

In the current study, when the fungicides were evaluated alone, combined, or in rotation programs, all tested fungicide treatments significantly reduced Phytophthora root rot severity of boxwood compared with the nontreated, inoculated control plants. Individual applications of oxathiapiprolin (Segovis) were effective in reducing root rot severity on boxwood plants and recovery of *P. nicotianae* and *P. cinnamomi* consistently throughout the trials. Oxathiapiprolin (Segovis) is a single-site mode of-action fungicide that suppresses oomycetes pathogens by inhibiting oxysterol-binding

Table 3. Effect of fungicides on growth (mean ± *SE*) of boxwood (*Buxus* spp.) inoculated with *Phytophthora cinnamomi* in greenhouse studies.

		Ht increase (cm)[ii]		Width increase (cm)[iii]		Total fresh plant wt (g)		Total fresh root wt (g)	
Treatment	Trade name	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Ametoctradin + dimethomorph	Orvego	3.7 ± 0.4 a[i]	3.4 ± 0.3 a	2.9 ± 0.6 a	3.2 ± 0.4 a	31.4 ± 0.5 a	25.2 ± 1.0 a	22.8 ± 1.3 a	18.3 ± 0.4 a
Ametoctradin + dimethomorph *alt* pyraclostrobin	Orvego *alt* Empress	3.3 ± 0.2 a	3.7 ± 0.3 a	2.2 ± 0.3 a	4.1 ± 0.5 a	32.8 ± 2.6 a	24.7 ± 0.9 a	22.0 ± 2.3 a	16.8 ± 0.9 a
Ametoctradin + dimethomorph *alt* pyraclostrobin + fluzapyroxad	Orvego *alt* Orkestra	3.6 ± 0.2 a	3.6 ± 0.2 a	3.2 ± 0.7 a	3.2 ± 0.7 a	29.8 ± 1.8 a	27.0 ± 2.1 a	20.1 ± 1.7 a	18.8 ± 1.0 a
Pyraclostrobin + cyazofamid	Empress + Segway	3.8 ± 0.3 a	3.8 ± 0.4 a	3.3 ± 0.3 a	3.8 ± 0.6 a	30.5 ± 2.3 a	27.3 ± 0.9 a	21.2 ± 1.6 a	18.2 ± 0.6 a
Oxathiapiprolin	Segovis	3.6 ± 0.2 a	3.2 ± 0.4 a	3.1 ± 0.7 a	3.3 ± 0.8 a	32.8 ± 1.5 a	26.7 ± 1.2 a	23.6 ± 1.7 a	18.4 ± 1.3 a
Nontreated, inoculated control		3.4 ± 0.4 a	3.8 ± 0.5 a	3.1 ± 0.3 a	3.4 ± 0.3 a	30.2 ± 2.5 a	28.2 ± 1.6 a	20.4 ± 1.8 a	19.3 ± 0.5 a
Nontreated, noninoculated control		3.7 ± 0.41 a	3.5 ± 0.3 a	3.2 ± 0.6 a	2.9 ± 1.1 a	25.3 ± 0.5 a	27.8 ± 1.9 a	18.8 ± 0.7 a	20.2 ± 1.0 a
P value		0.907	0.922	0.894	0.901	0.116	0.563	0.433	0.217

[i] Means followed by a different lowercase letter within a column are significantly different ($P \leq 0.05$). One-way analysis of variance was used to evaluate treatment effects. Means were compared using Fisher's least significant difference test with an $\alpha = 0.05$.
[ii] Height increase = final height – initial height.
[iii] Width increase = [(final widest width – initial widest width) + (final perpendicular width – initial perpendicular width)] ÷ 2.
alt = alternated with.

Table 4. Mean (± *SE*) root rot severity (% root affected) and pathogen recovery from the roots of boxwood (*Buxus* spp.) inoculated with *Phytophthora nicotianae* in greenhouse studies.

		Root rot severity (%)		Pathogen recovery (%)	
Treatment	Trade name	Trial 1	Trial 2	Trial 1	Trial 2
Ametoctradin + dimethomorph	Orvego	24 ± 2.9 cd[i]	22 ± 1.2 d	22 ± 3.7 c	28 ± 2.0 cd
Ametoctradin + dimethomorph *alt* pyraclostrobin	Orvego *alt* Empress	38 ± 1.2 b	34 ± 1.0 b	30 ± 3.2 bc	34 ± 2.5 bc
Ametoctradin + dimethomorph *alt* pyraclostrobin + fluzapyroxad	Orvego *alt* Orkestra	25 ± 5.7 cd	27 ± 2.0 c	26 ± 2.5 bc	30 ± 5.4 cd
Pyraclostrobin + cyazofamid	Empress + Segway	33 ± 3.4 bc	35 ± 2.2 b	32 ± 2.0 b	40 ± 3.1 b
Oxathiapiprolin	Segovis	17 ± 3.8 d	19 ± 1.0 d	22 ± 4.9 c	22 ± 2.4 d
Nontreated, inoculated control		56 ± 1.9 a	51 ± 3.3 a	54 ± 4.1 a	50 ± 1.9 a
Nontreated, noninoculated control		0 ± 0 e	0 ± 0 e	0 ± 0 d	0 ± 0 e
P value		<0.0001	<0.0001	<0.0001	<0.0001

[i] Means followed by a different lowercase letter within a column are significantly different ($P \leq 0.05$). Data were analyzed according to General Linear Mixed Model with a logit link and beta distribution (PROC GLIMMIX).
alt = alternated with.

protein homolog, which affects lipid biosynthesis and transport (FRAC 2021). Regardless of oxathiapiprolin (Segovis) being the most effective fungicide identified in this current study, it is also considered a high-risk fungicide for resistance (FRAC 2021). Since mixing high-risk resistance fungicides with low-risk is an effective strategy to manage resistance (Hobbelen et al. 2011), future experiments need to test the effectiveness of oxathiapiprolin (Segovis) in rotation with other fungicides. Segovis was highly effective in reducing root rot disease caused by *P. nicotianae* in red maple (*Acer rubrum*) (Parajuli and Baysal-Gurel 2022) and caused by *P. cinnamomi* in dogwoods (*Cornus florida*) (Neupane et al. 2022).

Individual applications of ametoctradin + dimethomorph (Orvego) and rotational applications of ametoctradin + dimethomorph (Orvego) and pyraclostrobin (Empress) were similarly effective in reducing root rot severity caused by *P. cinnamomi* in both trials. Ametoctradin is a strong inhibitor of the mitochondrial respiratory complex III (cytochrome bc1) of oomycetes (Dreinert et al. 2018; FRAC 2021) and dimethomorph inhibits the life cycle of a pathogen by inhibiting cell wall biosynthesis (Kuhn et al. 1991). This fungicide possesses systemic, curative, and antisporulant attributes that inhibit zoospore encystment and mycelial growth (Cohen et al. 1995). The dual function of ametoctradin + dimethomorph in targeting two sites in the oomycete life cycle, potentially explains the effectiveness of Orvego fungicide in reducing root rot caused by *P. nicotianae* and *P. cinnamomi* and the reduction of their recovery from the infected roots. Ametoctradin + dimethomorph (Orvego) is effective for control of root rot caused by *P. cinnamomi* and *P. nicotianae* on rhododendron (*Rhododendron prostratum*), English ivy (*Hedera helix*) and pansy (*Viola tricolor*) (Norman et al. 2010). It was also effective against root rot caused by *P. cryptogea* in gerbera daisy (*Gerbera jamesonii*) in a greenhouse study (Benson and Parker 2011). Pyraclostrobin, the active ingredient of Empress, was used in the rotation program with Orvego. Orvego is a quinone outside inhibitor that disrupts fungal mitochondria activity, thus preventing spore germination and reducing mycelial growth (Bartlett et al. 2002). Previously, pyraclostrobin was reported to be effective in reducing root rot severity caused by *P. cinnamomi* in flowering dogwoods under flooding (Brown et al. 2019) and drought conditions (Neupane et al. 2022). Pyraclostrobin reduced root rot severity caused by *P. nicotianae* in red maple under greenhouse settings (Parajuli and Baysal-Gurel 2022) and in hydrangea (*Hydrangea* spp.) under both greenhouse and field conditions (Baysal-Gurel and Kabir 2019). Pyraclostrobin also promotes plant growth, delays senescence, and plays a role in hormonal balance (Venancio et al. 2003). However, pyraclostrobin is also listed as a high risk of fungicide resistance (FRAC 2021). Therefore, the rotational application of ametoctradin + dimethomorph (Orvego) with pyraclostrobin (Empress) can maximize efficacy and minimize the risk of developing resistance.

For the management of root rot caused by *P. nicotianae*, individual applications of ametoctradin + dimethomorph (Orvego) and rotational applications of ametoctradin + dimethomorph (Orvego) and pyraclostrobin + fluzapyroxad (Orkestra) were identified as effective approaches in both trials. Fluxapyroxad, one of the active ingredients in Orkestra, is a succinate dehydrogenase inhibitor of oomycetes pathogens (FRAC 2021). Neupane et al. (2022) and Brown et al. (2019) found Orkestra effective in suppressing root rot severity caused by *P. cinnamomi* in flowering dogwoods and Parajuli and Baysal-Gurel (2022) found it effective in controlling root rot severity caused by *P. nicotianae* in red maple. Individual applications of ametoctradin + dimethomorph (Orvego), rotational applications of ametoctradin + dimethomorph (Orvego) and pyraclostrobin (Empress), or rotational application of ametoctradin + dimethomorph (Orvego) and pyraclostrobin + fluzapyroxad (Orkestra) resulted in similar and consistent effects in reducing the *P. nicotianae* and *P. cinnamomi* recovery from infected roots.

Pyraclostrobin + cyazofamid (Empress + Segway) treated boxwood plants consistently

Table 5. Effect of fungicides on growth (mean ± *SE*) of boxwood (*Buxus* spp.) inoculated with *Phytophthora nicotianae* in greenhouse studies.

		Ht increase (cm)[ii]		Width increase (cm)[iii]		Total fresh plant wt (g)		Total fresh root wt (g)	
Treatment	Trade name	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Ametoctradin + dimethomorph	Orvego	3.2 ± 0.4 a[i]	3.5 ± 0.2 a	3.1 ± 0.8 a	3.2 ± 0.6 a	51.8 ± 4.2 a	55.4 ± 1.3 a	40.6 ± 3.9 a	43.8 ± 1.5 a
Ametoctradin + dimethomorph *alt* pyraclostrobin	Orvego *alt* Empress	3.7 ± 0.6 a	3.4 ± 0.2 a	3.4 ± 0.2 a	3.2 ± 0.3 a	45.8 ± 2.0 a	57 ± 2.1 a	33.2 ± 1.6 a	40.6 ± 0.9 a
Ametoctradin + dimethomorph *alt* pyraclostrobin + fluzapyroxad	Orvego *alt* Orkestra	3.3 ± 0.7 a	3.3 ± 0.2 a	3.3 ± 0.3 a	3.8 ± 0.4 a	46 ± 4.2 a	56 ± 1.8 a	34.3 ± 3.7 a	41.1 ± 1.3 a
Pyraclostrobin + cyazofamid	Empress + Segway	3.6 ± 0.8 a	3.6 ± 0.4 a	3.8 ± 0.8 a	3.7 ± 0.4 a	50.2 ± 2.0 a	54.4 ± 1.8 a	36.5 ± 2.0 a	40.2 ± 0.8 a
Oxathiapiprolin	Segovis	3.1 ± 0.2 a	3.3 ± 0.3 a	3.5 ± 0.5 a	4.1 ± 0.4 a	45.5 ± 1.9 a	57.8 ± 4.2 a	33.4 ± 1.3 a	41.4 ± 2.7 a
Nontreated, inoculated control		3.2 ± 0.4 a	3.5 ± 0.1 a	3.3 ± 0.3 a	3.7 ± 0.5 a	40.3 ± 1.4 a	49.4 ± 2.2 a	27.5 ± 0.5 a	38 ± 1.3 a
Nontreated, noninoculated control		3.9 ± 0.6 a	3.8 ± 0.5 a	3.7 ± 0.7 a	3.6 ± 0.6 a	45.1 ± 2.8 a	50.8 ± 3.0 a	34.0 ± 3.2 a	36.2 ± 2.3 a
P value		0.915	0.937	0.983	0.882	0.153	0.199	0.062	0.090

[i] Means followed by a different lowercase letter within a column are significantly different ($P \leq 0.05$). One-way analysis of variance was used to evaluate treatment effects. Means were compared using Fisher's least significant difference test with an $\alpha = 0.05$.
[ii] Height increase = final height – initial height.
[iii] Width increase = [(final widest width – initial widest width) + (final perpendicular width – initial perpendicular width)] ÷ 2.
alt = alternated with.

exhibited lower root rot severity and pathogen recovery compared with the nontreated, inoculated control plants and were identified as moderately effective in managing Phytophthora root rot in boxwood plants compared with other individual and rotational applications evaluated in this study. Cyazofamid provides inhibition of mitochondrial complex III (cytochrome bc1) of oomycetes (FRAC 2021). There are no documented reports on evaluating Segway against *P. cinnamomi* or *P. nicotianae*, but Segway tested against root rot caused by *P. cryptogea* in gerbera daisy was found effective in a greenhouse study (Benson and Parker 2011). An earlier study reported strong inhibition of mycelial growth of Phytophthora spp. in vitro by cyazofamid present in Segovis (Mitani et al. 2001). Therefore pyraclostrobin (Empress) and cyazofamid (Segway) can be included in rotational programs with other identified treatments to manage Phytophthora root rot disease in boxwood.

Fungicide treatments had minimal effects on plant growth, such as height, width, total plant fresh weight, and root fresh weight. Despite root rot disease usually having a detrimental impact on plant growth, this was not seen in our experiment due to the short experimental period of around four months. Because boxwood plants are kept in a nursery for a longer period, root rot disease could reduce their growth and aesthetic value.

References Cited

Agrios GN. 1997. Plant pathology fourth edition. Academic Press, San Diego, CA, USA.

Andrus C. 1933. Fungous flora accompanying decline of boxwood. Plant Dis Rep. 17:169–170.

Bartlett DW, Clough JM, Godwin JR, Hall AA, Hamer M, Parr-Dobrzanski B. 2002. The strobilurin fungicides. Pest Manag Sci. 58:649–662. https://doi.org/10.1002/ps.520.

Baysal-Gurel F, Kabir MN. 2019. Evaluation of fungicides and biocontrol products for the control of Phytophthora root rot of hydrangeas. Arch Phytopathol Pflanzenschutz. 52:481–496. https://doi.org/10.1080/03235408.2019.1648023.

Baysal-Gurel F, Kabir MN, Randaci A. 2017. Boxwood *Phytophthora*. https://digitalscholarship.tnstate.edu/cgi/viewcontent.cgi?article=1108&context=extension. [accessed 17 Mar 2023].

Baysal-Gurel F, Panth M, Simmons T. 2019. Evaluation of fungicide alone and in rotation programs for the control of Phytophthora root rot of boxwood. Plant Dis. Mgt. Rpt. 13:OT017. American Phytopathol. Soc., St. Paul, MN, USA.

Benson D, Parker K. 2011. Efficacy of fungicides and biopesticides for management of Phytophthora crown and root rot of gerber daisy. Plant Health Prog. 12:13. https://doi.org/10.1094/PHP-2011-0512-01-RS.

Bienapfl J, Balci Y. 2014. Movement of *Phytophthora* spp. in Maryland's nursery trade. Plant Dis. 98:134–144. https://doi.org/10.1094/PDIS-06-13-0662-RE.

Brown MS, Baysal-Gurel F, Oliver JB, Addesso KM. 2019. Evaluation of fungicides and biofungicide to control Phytophthora root rot (*Phytophthora cinnamomi* Rands) and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) on flowering dogwoods exposed to simulated flood events. Crop Prot. 124:104834. https://doi.org/10.1016/j.cropro.2019.05.028.

Cohen Y, Baider A, Cohen BH. 1995. Dimethomorph activity against oomycete fungal plant pathogens. Phytopathology. 85:1500–1506.

Damicone J, Smith D. 2009. Fungicide resistance management. Oklahoma Coop. Ext. Serv. https://shareok.org/bitstream/handle/11244/334679/oksa_EPP-7663_2009-04.pdf?sequence=1. [15 Mar 2003].

Daughtrey M, Gilrein D, Vescera M. 2016. Photographic guide of boxwood pests & diseases on Long Island. Cornell Coop. Ext. of Suffolk County, Cornell University, Riverhead, NY. https://ccesuffolk.org/resources/photographic-guide-of-boxwood-pests-diseases-on-long-island. [accessed 15 Mar 2023].

Dhakal K, Bika R, Ghimire B, Parajuli M, Neupane S, Neupane K, Addesso KM, Baysal-Gurel F. 2022. Arthropod and disease management in boxwood production. J Integr Pest Manag. 13:18. https://doi.org/10.1093/jipm/pmac013.

Dreinert A, Wolf A, Mentzel T, Meunier B, Fehr M. 2018. The cytochrome bc1 complex inhibitor ametoctradin has an unusual binding mode. Biochim Biophys Acta Bioenergetics. 1859: 567–576. https://doi.org/10.1016/j.bbabio.2018.04.008.

Erwin DC, Ribeiro OK. 1996. Phytophthora diseases worldwide. Amer. Phytopathol. Soc. (APS Press).

Fungicide Resistance Action Committee. 2021. Fungicide Resistance Action Committee 2021 FRAC code list 2020: Fungicides sorted by mode of action (including FRAC code numbering). https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2021–final.pdf?sfvrsn=f7ec499a_2. [accessed 21 Mar 2023].

Gallup CA, McCorkle KL, Ivors KL, Shew D. 2018. Characterization of the black shank pathogen, *Phytophthora nicotianae*, across North Carolina tobacco production areas. Plant Dis. 102:1108–1114. https://doi.org/10.1094/PDIS-02-17-0295-RE.

Gitto A, Jeffers S, Graney L, Loyd A, Bechtel C. 2018. First report of *Phytophthora occultans* causing root rot on American boxwood planted in residential landscapes in the Eastern United States. Plant Dis. 102:2384. https://doi.org/10.1094/PDIS-04-18-0684-PDN.

Haasis F. 1961. *Phytophthora parasitica*, cause of root rot, canker, and blight of boxwood. Phytopathology. 51:734.

Hobbelen P, Paveley N, Van den Bosch F. 2011. Delaying selection for fungicide insensitivity by mixing fungicides at a low and high risk of resistance development: A modeling analysis. Phytopathology. 101:1224–1233. https://doi.org/10.1094/PHYTO-10-10-0290.

Holmes K, Benson D. 1994. Evaluation of *Phytophthora parasitica* var. *nicotianae* for biocontrol of *Phytophthora parasitica* on *Catharanthus roseus*. Plant Dis. 78:193–199.

Hu J, Hong C, Stromberg E, Moorman G. 2008. Mefenoxam sensitivity and fitness analysis of *Phytophthora nicotianae* isolates from nurseries in Virginia, USA. Plant Pathol. 57:728–736. https://doi.org/10.1111/j.1365-3059.2008.01831.x.

Jeffers S, Martin S. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70:1038–1043.

Kuhn PJ, Pitt D, Lee SA, Wakley G, Sheppard AN. 1991. Effects of dimethomorph on the morphology and ultrastructure of *Phytophthora*. Mycol Res. 95:333–340. https://doi.org/10.1016/S0953-7562(09)81244-6.

Mitani S, Araki S, Yamaguchi T, Takii Y, Ohshima T, Matsuo N. 2001. Antifungal activity of the novel fungicide cyazofamid against *Phytophthora infestans* and other plant pathogenic fungi in vitro. Pestic Biochem Physiol. 70:92–99. https://doi.org/10.1006/pest.2001.2541.

National Agriculture Statistics Service. 2020. 2019 census of horticultural specialties. 2017 census of agriculture. https://www.nass.usda.gov/Publications/AgCensus/2017/Online_Resources/Census_of_Horticulture_Specialties/HORTIC.pdf. [accessed 12 Feb 2023].

Neupane K, Alexander L, Baysal-Gurel F. 2022. Management of *Phytophthora cinnamomi* using fungicides and host plant defense inducers under drought conditions: A case study of flowering dogwood. Plant Dis. 106:475–485. https://doi.org/10.1094/PDIS-04-21-0789-RE.

Neupane S, Baysal-Gurel F. 2022. Comparative performance of fungicides, biofungicides, host-plant defense inducers, and fertilizer in management of Phytophthora root rot on boxwood. HortScience. 57:864–871. https://doi.org/10.21273/HORTSCI16546-22.

Norman D, Benson M, Daughtrey M. 2010. Efficacy of ametoctradin plus dimethomorph for control of *Phytophthora* species infecting ornamental plants in the Eastern United States. Phytopathology. 100:S90–S91.

Parajuli M, Baysal-Gurel F. 2022. Control of Phytophthora and Rhizoctonia root rot on red maple using fungicides and biofungicides. HortScience. 57:1306–1312. https://doi.org/10.21273/HORTSCI16673-22.

Parajuli M, Panth M, Gonzalez A, Addesso KM, Witcher W, Simmons T, Baysal-Gurel F. 2022. Cover crop usage for the sustainable management of soilborne diseases in woody ornamental nursery production system. Can J Plant Pathol. 44:432–452. https://doi.org/10.1080/07060661.2021.2020336.

Reeser P, Sutton W, Hansen E, Goheen E, Fieland V, Grünwald N. 2015. First report of *Phytophthora occultans* causing root and collar rot on ceanothus, boxwood, rhododendron, and other hosts in horticultural nurseries in Oregon, USA. Plant Dis. 99(9). https://doi.org/10.1094/PDIS-02-15-0156-PDN.

Shew H. 1985. Response of *Phytophthora parasitica* var. *nicotianae* to metalaxyl exposure. Plant Dis. 69:559–562.

van den Bosch F, Paveley N, van den Berg F, Hobbelen P, Oliver R. 2014. Mixtures as a fungicide resistance management tactic. Phytopathology. 104:1264–1273. https://doi.org/10.1094/PHYTO-04-14-0121-RVW.

Van West PV, Morris B, Reid B, Appiah AA, Osborne M, Campbell T, Shepherd S, Gow NAR. 2002. Oomycete plant pathogens use electric fields to target roots. Mol Plant Microbe Interact. 15:790–798. https://doi.org/10.1094/MPMI.2002.15.8.790.

Venancio WS, Rodrigues MAT, Begliomini E, de Souza NL. 2003. Physiological effects of strobilurin fungicides on plants. Publicatio UEPG: Ciências Exatas e da Terra. Agrárias e Engenharias. 9. https://doi.org/10.5212/publicatio.v9i03.814.

Vettraino A, Franceschini S, Vannini A. 2010. First report of *Buxus rotundifolia* root and collar rot caused by *Phytophthora citrophthora* in Italy. Plant Dis. 94:272. https://doi.org/10.1094/PDIS-94-2-0272A.

Weiland JE. 2021. The challenges of managing Phytophthora root rot in the nursery industry. Plant Health Prog. 22:332–341. https://doi.org/10.1094/PHP-02-21-0036-FI.