Evaluation of Fungicides, Host-plant Defense Inducer, and Anti-transpirant in Management of Boxwood Blight

Bhawana Ghimire[1], Madhav Parajuli[1], Terri Simmons[1], Prabha Liyanapathiranage[1], and Fulya Baysal-Gurel[1]

Keywords. *Calonectria pseudonaviculata*, chemical control, fungal pathogen, fungicide rotation, sustainable management

Abstract. Boxwood, valued at over $140.9 million annually in the United States, faces a significant threat from boxwood blight disease. This study evaluated 24 treatment combinations involving three fungicides (Daconil Weatherstik, Postiva, and F6123-1), a disinfectant/fungicide (KleenGrow), a host-plant defense inducer (Actigard), and an anti-transpirant (Vapor Gard) for managing boxwood blight. Preventive applications were performed 24 h before pathogen inoculation, while curative treatments were applied at 14-d intervals, starting 14 d after inoculation. The disease severity (0% to 100% plant affected), area under disease progress curve (AUDPC), plant growth, defoliation (0% to 100%), and stem water potential (MPa) were assessed. All the applications significantly reduced disease severity and AUDPC compared with the non-treated, inoculated control. Preventative applications of Actigard alternated (alt.) with Vapor Gard and Actigard alone consistently reduced the disease progression, while Actigard alt. with Vapor Gard reduced the disease severity. Curative application of the low rate of Postiva exhibited the highest disease suppression, comparable to applications of F6123-1 alt. with Vapor Gard and the high rate of Postiva alt. with Vapor Gard. The preventative and curative application of the high rate of Postiva alt. with Vapor Gard was the most effective in reducing disease severity and slowing disease progression. Preventive and curative application of Daconil Weatherstik, KleenGrow, and Daconil Weatherstik alt. with KleenGrow were also effective. None of the preventive treatments significantly reduced defoliation. However, curative applications of F6123-1 alt. with Vapor Gard and preventive and curative treatments of the high rate of Postiva alt. with Vapor Gard consistently reduced the defoliation. Preventive application of Actigard alt. with Vapor Gard, curative application of the low rate of Postiva alt. Vapor Gard, and preventive and curative treatments of the high rate of Postiva alt. Vapor Gard, as well as Vapor Gard alone, resulted in the highest stem water potential among treated plants. A weak positive correlation was observed between stem water potential and disease development. The identified combinations and application strategies provide options for effective boxwood blight management when combined with other management strategies.

Boxwood (*Buxus* spp. L., Buxales, Buxaceae) is an evergreen shrub commonly used in garden design that holds a significant commercial value (Briggs 1980; Donhardt 2007; Hall et al. 2011, 2021). Boxwood has been used in American gardens in the Colonial, Antebellum, and Colonial Revival eras, and these historic gardens still feature the original boxwood plants (Holleran et al. 2005; Malone 1992; Matrana 2009). Boxwood is a popular choice among today's gardening and landscape consumers in the United States, as indicated by the increase in boxwood sales from 2009 ($102.9 million) to 2014 ($126.5 million) and then to 2019 ($140.9 million) (Hall et al. 2021).

Boxwood production is negatively impacted by boxwood blight disease, which is caused by the fungal pathogen *Calonectria pseudonaviculata* (Dhakal et al. 2022; Lombard et al. 2010). Disease symptoms develop in leaves as hazy black spots with indistinct margins, subsequently transforming into a tawny lesion with a graduated margin exhibiting shades of light brown, tan, and yellow. Pathogen signs are visible on the abaxial surface of the lesion as abundant white spore masses during high-moisture conditions (Dart et al. 2011; Ivors et al. 2012). These symptoms can expand rapidly, growing over whole leaves and culminating in defoliation (Baysal-Gurel 2023; Baysal-Gurel and Liyanapathiranage 2017; Bika et al. 2021). In a recent study, commercial boxwood growers have acknowledged boxwood blight as a current and future threat to boxwood production, and boxwood transplants and cutting tools were identified as critical sources impacting the entry and spread of the disease in the production nurseries (Ghimire et al. 2023).

Current boxwood blight management strategies include the use of host resistance, sanitizers (Bika et al. 2021; Dart et al. 2015; Shishkoff 2016), good agronomic practices, biocontrol agents (Kong and Hong 2017), and chemical methods (Dhakal et al. 2022). However, chemical control is the method most preferred by the growers (Ghimire et al. 2023). Some fungicides, such as strobilurins, demethylation inhibitors, chlorothalonil, azoxystrobin + benzovindiflupyr, triflumizole, fluopyram, fludioxonil, metconazole, mancozeb, tebuconazole, trifloxystrobin, captan, thiophanate-methyl, mancozeb, tolyfluanid, difenoconazole + azoxystrobin, metiram + pyraclostrobin, epoxiconazole + metconazole, fludioxonil + cyprodinil, carbendazim + flusilazole, and propiconazole, have been evaluated and demonstrated to be effective for boxwood blight control (Baysal-Gurel et al. 2021, 2022a, 2022b; Brand et al. 2023; Henricot and Wedgwood 2013; LaMondia 2020; Maurer and LaMondia 2016, 2017). Those fungicides belong to single-site (1, 3, 7, 9, 11, 12, and 19) or multisite (M3, M4, M5, and M6) target modes of action in Fungicide Resistance Action Committee (FRAC) groups (FRAC 2021). With the continued use of fungicides for disease management, some fungal populations have been reported to develop resistance and cross-resistance between fungicides in the same FRAC group especially those at medium to high risk (FRAC 2021). Limiting the use of the same chemicals and alternating or combining fungicides with non–cross-resistant or alternative modes of action are recommended strategies for managing pathogen resistance to fungicides. Although boxwood has been treated extensively with fungicides to suppress boxwood blight, no research has evaluated whether strains with lower fungicide sensitivity have emerged. Nonetheless, it has been shown that several fungicides targeted to manage boxwood blight lose some of their efficacy over time (Brand et al. 2023).

For controlling disease of different plants, various preventive (Aiello et al. 2013; Cinquerrui et al. 2017; Miller et al. 2019; Pedersen et al. 2012) and curative applications (Bika et al. 2020; Henricot and Wedgwood 2013; Mizell et al. 2011; Mueller et al. 2004) of fungicides are employed. Preventive applications of fungicides control disease through either inhibition of spore germination, prevention of hyphal penetration, disruption of fungal metabolism, creating a physical barrier, or a combination of these effects, whereas curative applications control disease through inhibition of fungal growth and development, direct antifungal activity, and reducing systemic infections through translocation into the host (El-Baky and Amara 2021; Lukens 2013; Tsuda et al. 2004; Walter 2011; Yang et al. 2011). The preventive treatments are appropriate when a disease is known to occur regularly in a specific area and predictions indicate a high disease risk, environmental conditions are favorable, high value crops are at stake, and these applications can be incorporated as a part of integrated disease-management strategy (Keinath and DuBose 2004; McGrath 2004; Mizell et al. 2011; Mueller et al. 2004). In contrast, curative treatments are applied when disease is detected early and a prompt treatment can prevent its further spread and minimize damage, when infections are localized and can be effectively treated without significant impact on the crop, when there are severe outbreaks where preventive measures have failed, and when an immediate action is required to save the crop (Brown et al. 2019; Buck and Williams-Woodward 2003; Copes et al. 2003; Holb 2009; McGrath 2004; Neupane et al. 2022).

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[1]Department of Agricultural Sciences and Engineering, College of Agriculture, Tennessee State University, Otis L. Floyd Nursery Research Center, 472 Cadillac Lane, McMinnville, TN 37110, USA

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F.B.-G. is the corresponding author. E-mail: fbaysalg@tnstate.edu.

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Also, curative applications may be applied in combination with preventive measures as a part of combined strategy (also known as preventive and curative treatments) in which initial prevention is supplemented with curative applications due to unexpected outbreaks targeted for immediate and long-term protection against fungal diseases (Holb 2009; McGrath 2004). These strategies can be essential in boxwood blight management, but there is a requirement to evaluate these fungicides, their modes of action, and application strategies while minimizing the risk of resistance development. More alternation programs will be available when new combinations and applications approaches are evaluated.

Anti-transpirants are generally being used for their role in reducing plant water demand in various production systems including nurseries (Fitzpatrick 1982; McKenney 1986; Mphande et al. 2023). Generally, anti-transpirants with three different modes of action to reduce the water loss from the plants are reported. These modes are metabolic or stomatal closing (Koyama et al. 2018), reflective (Yee 2012), and film forming (Miller 2023). Numerous film-forming anti-transpirants have been reported to protect against several foliar plant diseases including gray mold, leaf blight, anthracnose, rust, powdery mildew, fruit rots, downy mildew, and black spot (Haggag 2002; Han 1990; Nasraoui et al. 1999; Roark et al. 2000; Ziv 1992; Ziv and Hagiladi 1993). Foliar application of anti-transpirant as a pre-inoculation treatment to prevent pathogen establishment in plant tissues (Haggag 2002) or application in alternation with various fungicide chemistries (Roark et al. 2000) has been identified as a promising strategy in reducing the risk of resistance development. The anti-transpirant interferes with the adhesion of spores to the leaf surface, inhibits spore germination, or inhibits germ tube growth and exerts a direct toxic effect on the fungus, ultimately affecting the colonization of leaf tissues by the pathogen (Sutherland and Walters 2001; Wade et al. 1993; Walters 2006). Measurement of plant water potential is crucial in understanding the amount of water retained by the plants and in understanding the impact of evapotranspiration load on the water content of plants (Prasad et al. 2015). A recent study has documented the effect of applying anti-desiccants or anti-transpirant on microbial communities of boxwood indicating the effect of Vapor Gard on fungal communities and altering their diversity in boxwood phyllosphere (Li et al. 2023). However, these anti-desiccants have not been evaluated in managing boxwood blight disease as a sole application or in combination or alternation with commercially available chemical fungicides.

The objective of this study was to evaluate the efficacy of fungicides, a host-plant defense inducer (Actigard), a disinfectant/fungicide (KleenGrow), and an anti-transpirant (thin film forming Vapor Gard), either singly or in alternation, applied preventatively, curatively, or preventatively and curatively for controlling boxwood blight in *C. pseudonaviculata*–inoculated boxwood plants.

Materials and methods

Plant materials and inoculum preparation. One-year-old boxwood 'Green Velvet' (*Buxus sinica* var. *insularis* × *Buxus sempervirens*) plants were potted in 25.8-cm^2 nursery containers filled with nursery mix [processed pine bark (55% to 65%), Canadian sphagnum peat (17% to 22%), and sand (18% to 23%)]. The plants were placed in a Biosafety Level 2 facility at the Otis L. Floyd Nursery Research Center in McMinnville, TN, USA 1 month after re-potting. The plants were manually irrigated once a week with 100 mL of water. A constant temperature of 23 °C, 14 h of light and 10 h of darkness, and a relative humidity of 80% were maintained in Expts. 1 and 2.

Isolate FBG 2019_393 (GenBank accession no. MH078193) of *C. pseudonaviculata* was isolated from a symptomatic 'Green Velvet' boxwood plant received from a commercial nursery in Tennessee and maintained on potato dextrose agar (PDA) medium (Sigma-Aldrich, St. Louis, MO, USA). Long-term storage of the isolate was done by storing the mycelial plugs in slants filled with PDA at 4 °C; the mycelial plugs were transferred into fresh medium every 6 months. The pathogenicity of the isolate FBG 2019_393 was previously confirmed by Bika et al. (2021). The *C. pseudonaviculata* inoculum was prepared from 3-week-old cultures grown on PDA medium at 25 °C with 12 h of fluorescent light and 12 h of dark. The surface of the mycelia of *C. pseudonaviculata* growing on the petri dish was flooded with 30 mL of sterile distilled deionized (DI) water for 2 h and drained. The water-soaked mycelia were then gently scraped off using a sterile spatula and discarded. The mycelia that remained in the petri dish were washed out using sterile DI water, and the plates were sealed using parafilm tape. The plates were then incubated at 23 °C with continuous fluorescent light (150 μmol·m^{-2}·s^{-1}) for 5 d, and freshly formed conidia were harvested by spraying 30 mL of sterile DI water amended (5%) with Tween 20 (Avenot et al. 2022; Bika et al. 2021) and swirling with sterile silica beads. Then the conidial suspensions were passed through double-layered Miracloth and diluted with sterile DI water to adjust the concentrations as needed.

Experimental design. Treatments were arranged in a completely randomized design with six replications (each consisting of one potted boxwood plant) per treatment. A total of 27 treatments including different combinations [applications (preventive and curative) and doses] of three fungicides, one disinfectant/fungicide (KleenGrow), one host-plant defense inducer (Actigard), and one anti-transpirant (Vapor Gard), including the positive and negative controls, were evaluated in the study (Table 1). The study was conducted from 31 Aug to 20 Oct 2022 (Expt. 1) and from 25 Jan to 23 Mar 2023 (Expt. 2). All the preventive applications of fungicides, Actigard, and Vapor Gard were done on 31 Aug 2022 (24 h before inoculation) for Expt. 1 and 25 Jan 2023 for Expt. 2 by spraying on the foliage until runoff (Tables 2 and 3). The plants were inoculated with conidial suspension of 1 × 10^5 conidia/mL using a handheld sprayer until runoff on 1 Sep 2022 (Expt. 1) and 26 Jan 2023 (Expt. 2). Transparent plastic bags were placed over plants for 48 h for incubation. Non-treated, non-inoculated treatments included a spray of sterile water until runoff. Non-treated, non-inoculated and non-treated, inoculated plants served as the negative and positive controls, whereas non-inoculated Vapor Gard–treated plants were also tested as a sole application treatment to evaluate their effect on stem water potential. However, for the Actigard treatment, 50 mL of chemical solution was applied as a drench application. The volume of 50 mL was determined using a pour-through test (Bilderback 2001; Cavins et al. 2008). All the curative applications were conducted 14 d after inoculation (dai) [Expt. 1 (15 Sep 2022), Expt. 2 (9 Feb 2023)], 28 dai [Expt. 1 (29 Sep 2022), Expt. 2 (23 Feb 2023)], and 42 dai [Expt. 1 (13 Oct 2022), Expt. 2 (9 Mar 2023)].

Assessment of plant growth, disease severity, and stem water potential. Initial plant height and width were measured 24 h before inoculation. Final height and width were measured at 56 dai. The height was measured from the base of the stem at the substrate level to the top of the terminal bud on the main stem. Plant width was measured as the average of the widest width, and a second measurement perpendicular to it [(widest width + perpendicular width) ÷ 2]. Changes in height and width were determined by subtracting the initial measurement from the final measurement. Disease severity was assessed at 7, 14, 21, 28, 35, 42, 49, and 56 dai in both experiments. Disease severity was determined visually using a scale of 0% to 100% of leaves and stems showing symptoms of boxwood blight disease. The defoliation (%) was visually assessed using a scale of 0% to 100% of the defoliated leaves in comparison with the total canopy. The typical symptoms of boxwood blight disease were circular spots and zonate lesions on leaves, distinctive black streaks on stems, and defoliation on stems (Castroagudín et al. 2020). The area under disease progress curve (AUDPC) was calculated using the formula:

$$\sum \left[\frac{(x_i + x_{i-1})}{2} \right] (t_i - t_{i-1}) \qquad [1]$$

where x_i is the rating at each evaluation time and $(t_i - t_{i-1})$ is the number of days between evaluations.

Stem water potential was measured by using a model 600 pressure chamber instrument at 56 dai (PMS Instrument Company, Albany, OR, USA) (Shimada et al. 2012). Gasket

Table 1. Details of fungicides, disinfectant, Actigard, and Vapor Gard used in this study.

Trade name	Type	Active ingredient (% content)	Application rate (mL or g/L)	Mode of action	Primary use (label)
Actigard 50 WG	Defense inducer	Acibenzolar-*S*-methyl (50)	2.02 g	Activation of the natural defenses mimicking role of salicylic acid and methyl jasmonate	Preventive
Daconil Weatherstik	Fungicide	Chlorothalonil (54)	1.71 mL	Multisite mode of action	Preventive and curative
F6123-1	Fungicide	Flutriafol (11.8)	1.09 mL	Sterol demethylation inhibition	Preventive
KleenGrow	Disinfectant	Quaternary ammonium chloride (7.5)	1.95 mL	Hydrophobic interference with plasma membrane, intracellular targets, and binding to DNA	Preventive and curative
Postiva	Fungicide	Pydiflumetofen and difenoconazole (6.9 and 11.5)	1.09 mL and 1.95 mL	Succinate dehydrogenase inhibitor + sterol demethylation inhibition	Curative
Vapor Gard	Anti-transpirant and anti-desiccant	Di-1-*p*-menthene (96)	19.75 mL	Reduction in water loss via evapotranspiration	Preventive

[i] Not applicable.

Table 2. Mean (± *SE*) defoliation of boxwood (*Buxus* spp.) transplants treated with preventive applications of fungicides, Actigard, and Vapor Gard treatments.

Treatment	Application type/dates[i]	Mean defoliation[ii]	
		Expt. 1	Expt. 2
Actigard	1	14.1 ± 1.5 a[iii]	9.1 ± 2.4 a
Actigard alt. Vapor Gard	1, 2	9.16 ± 0.5 b	7.9 ± 2.8 ab
F6123-1	2	3.33 ± 0.5 c	9.1 ± 2.9 a
F6123-1 alt. Vapor Gard	1, 2	14.10 ± 0.8 a	8.3 ± 3.0 ab
Vapor Gard	2	4.16 ± 0.5 c	7.9 ± 1.2 ab
Non-treated, inoculated control[iv]	1	8.33 ± 0.5 b	13.1 ± 5.8 a
Non-treated, non-inoculated control[iv]	1	0.0 ± 0.0 d	0.0 ± 0.0 b
F value		49.56	1.68
P value		<0.0001	0.1543

[i] The application dates were as follows: 1 = 31 Aug 2022 morning (Expt. 1) or 25 Jan 2023 (Expt. 2) morning; 2 = 31 Aug 2022 afternoon (Expt. 1) or 25 Jan 2023 (Expt. 2) afternoon.
[ii] Defoliation (mean ± *SE*) of boxwood plants treated with anti-transpirant, host-plant defense inducer, disinfectant, and fungicides at the end of both experiments. Defoliation on each plant was evaluated using a 0% to 100% scale. The values are the means ± *SE* of six single-plant replications in both trials.
[iii] Treatment means followed by different lowercase letters in the column denote significance at $P \leq 0.05$. The values are the means per plant for six single-plant (one potted boxwood plant) replicates. A one-way analysis of variance was used to evaluate treatment effects. When the effects were significant, Fisher's least significant difference test was used for mean comparison with $\alpha = 0.05$.
[iv] Control treatments included non-treated *C. pseudonaviculata*–inoculated plants and non-inoculated, non-treated plants.
Preventive applications of products were conducted 24 h before inoculation. alt. = alternated with.

size of 1/4 inch was used, and three stems (of length and width to fit into the pressure chamber) were randomly sampled from each boxwood plant to measure the stem water potential.

Statistical analysis. The preventive, curative, and preventive and curative treatments were grouped together and analyzed separately. Treatment effects on height increase, width increase, AUDPC, final disease severity, defoliation, and stem water potential 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 (α = 0.05). The means were separated using the least square means. The disease progress (AUDPC) in Expts. 1 and 2 were compared using unpaired *t* test (SAS Inc.). Pearson's correlation analysis between AUDPC and stem water potential and between final disease severity and stem water potential was conducted in R Statistical Computing Environment (R-4.2.2) (Dalgaard 2010) using cor.test function with a significance level of 0.05.

Table 3. Mean (± *SE*) defoliation of boxwood (*Buxus* spp.) transplants treated with curative applications of fungicides, Actigard, and Vapor Gard treatments.

Treatment	Mean defoliation[i]	
	Expt. 1	Expt. 2
F6123-1	127.7 ± 16.4 b[ii]	159.1 ± 1.9 b
F6123-1 alt. Vapor Gard	85.6 ± 3.4 c	138.7 ± 4.9 de
Postiva low	81.7 ± 3.7 c	137.2 ± 2.0 de
Postiva high	78.1 ± 4.3 c	146.5 ± 1.5 c
Postiva low alt. Vapor Gard	77.6 ± 4.6 c	132.6 ± 3.3 e
Postiva high alt. Vapor Gard	90.6 ± 6.0 c	143.2 ± 2.0 cd
Vapor Gard	81.1 ± 4.0 c	136.6 ± 2.1 de
Non-treated, inoculated control[iii]	257.9 ± 24.4 a	192.1 ± 0.9 a
Non-treated, non-inoculated control	64.5 ± 1.1 c	97.41 ± 0.6 f
F value	32.95	44.84
P value	<0.0001	<0.0001

[i] Defoliation (mean ± *SE*) of boxwood plants treated with anti-transpirant, host-plant defense inducer, disinfectant, and fungicides at the end of both experiments. Defoliation on each plant was evaluated using a 0% to 100% scale. The values are the means ± *SE* of six single-plant replications in both trials.
[ii] Treatment means followed by different lowercase letters in the column denote significance at $P \leq 0.05$. The values are the means per plant for six single-plant (one potted boxwood plant) replicates. A one-way analysis of variance was used to evaluate treatment effects. When the effects were significant, Fisher's least significant difference test was used for mean comparison with $\alpha = 0.05$.
[iii] Control treatments included non-treated *C. pseudonaviculata*–inoculated plants and non-inoculated non-treated plants. The application dates were as follows: 3 = 15 Sep 2022 (Expt. 1) or 9 Feb 2023 (Expt. 2); 4 = 29 Sep 2022 (Expt. 1) or 23 Feb 2023 (Expt. 2); 5 = 13 Oct 2022 (Expt. 1) or 9 Mar 2023 (Expt. 2).
Curative application of the products started 14 d after inoculation with two more consecutive applications at 14-d intervals. alt. = alternated with.

Results

Effectiveness of fungicides, Actigard, and Vapor Gard to control boxwood blight

The disease pressure in Expt. 1 was significantly higher than the disease pressure in Expt. 2 (disease severity and AUDPC). Significant differences among the treatments were observed for disease progress in Expt. 1 and Expt. 2 (Figs. 1–6). Non-inoculated and non-treated plants and non-inoculated and Vapor Gard–treated plants remained asymptomatic (Figs. 1–6).

Preventive treatments. *Disease severity and progress.* All treatments resulted in significantly lower disease severity and progress in comparison with non-treated inoculated control (Figs. 1 and 4). In Expt. 1, Vapor Gard resulted in the lowest disease progress and was comparable to the disease progress of plants treated with F6123-1, Actigard alternated (alt.) with Vapor Gard and F6123-1 alt. with Vapor Gard but was statistically different from the rest of the treatments (Fig. 1). In Expt. 2, applications of Actigard resulted in the lowest disease progress, and the disease progress was comparable to plants treated with Actigard alt. with Vapor Gard but was statistically different from the rest of the treatments (Fig. 1). Additionally, applications of Actigard alt. with Vapor Gard demonstrated the lowest disease severity, comparable to the disease severity of plants treated with Vapor Gard, F6123-1, and Actigard but statistically different from the non-treated inoculated control in Expt. 1 (Fig. 4), whereas in Expt. 2, applications of Actigard alt. with Vapor Gard resulted in the lowest disease severity, comparable to the disease severity of plants treated with Vapor Gard, F6123-1, F6123-1 alt. with Vapor Gard, and Actigard but statistically different from the rest of the treatments (Fig. 4).

Defoliation. Significant differences were observed among the treatments for

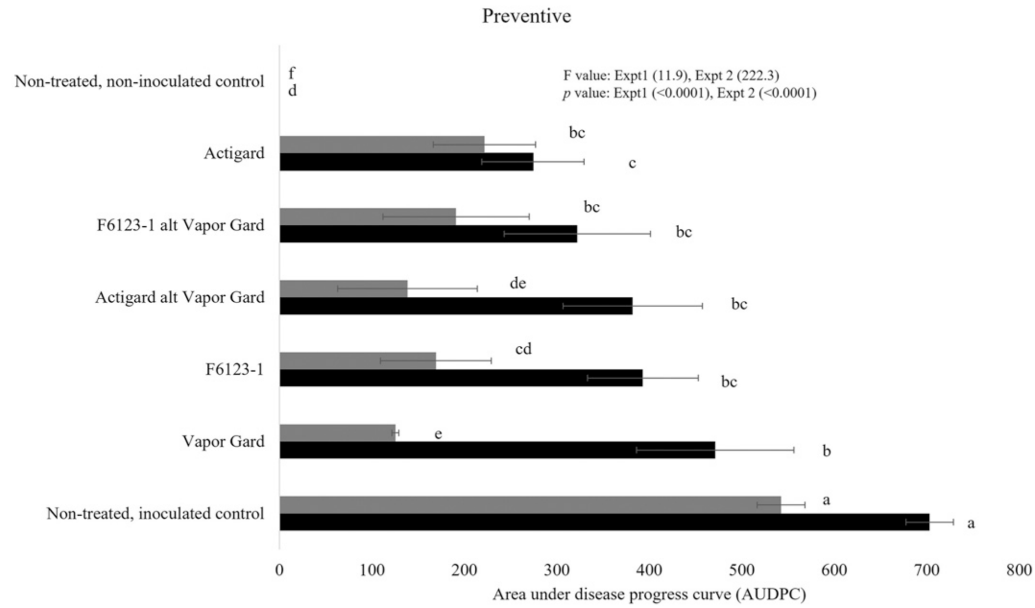

Fig. 1. Mean (± *SE*) area under disease progress curve (AUDPC) of blight disease (*C. pseudonaviculata*) of boxwood (*B. sinica* var. *insularis* × *B. sempervirens* 'Green Velvet') in Expt. 2 evaluated on 2 Feb, 9 Feb, 16 Feb, 23 Feb, 2 Mar, 9 Mar, and 16 Mar 2023. The values are the means per plant for six-single plant (one potted boxwood plant) replicates. The black bars indicate Expt. 1, and the gray bars indicate Expt. 2. Lowercase letters on the bars denote significance at $P \leq 0.05$. The AUDPC values were calculated using the following equation: $\sum[\frac{(x_i+x_{i-1})}{2}](t_i - t_{i-1})$. Control treatments included non-treated *C. pseudonaviculata*–inoculated plants and non-inoculated non-treated plants. Preventive application of products was conducted 24 h prior to inoculation.

defoliation in Expt. 1 but not in Expt. 2 (Table 2). Vapor Gard and F6123-1 resulted in the lowest defoliation in Expt. 1, whereas in Expt. 2, the treatments were not significantly different from the non-treated inoculated control (Table 2). The lowest defoliation (0% to 5%) was observed in plants treated with Actigard alt. with Vapor Gard, Actigard, and F6123-1 alt. with Vapor Gard, and defoliation (%) was significantly lower than in the non-treated inoculated control in Expt. 2. However, defoliation in plants treated with treatments of F6123-1 and Vapor

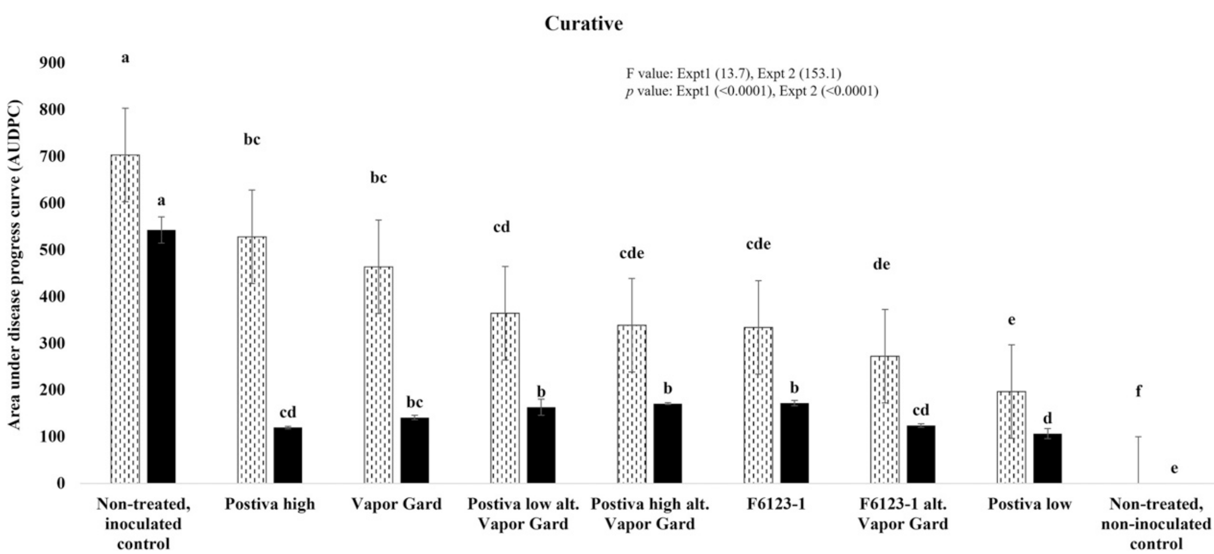

Fig. 2. Mean (± *SE*) area under disease progress curve (AUDPC) of blight disease (*C. pseudonaviculata*) of boxwood (*B. sinica* var. *insularis* × *B. sempervirens* 'Green Velvet') in Expt. 2 evaluated on 2 Feb, 9 Feb, 16 Feb, 23 Feb, 2 Mar, 9 Mar, and 16 Mar 2023. The values are the means per plant for six-single plant (one potted boxwood plant) replicates. The patterned bars indicate Expt. 1, and the solid black bars indicate Expt. 2. Lowercase letters on the bars denote significance at $P \leq 0.05$. The AUDPC values were calculated using the following equation: $\sum[\frac{(x_i+x_{i-1})}{2}](t_i - t_{i-1})$. Control treatments included non-treated *C. pseudonaviculata*–inoculated plants, non-inoculated non-treated plants, and non-inoculated Vapor Gard–treated plants (preventative and curative). Curative application of the products started 14 d after inoculation with two more consecutive applications at 14-d intervals.

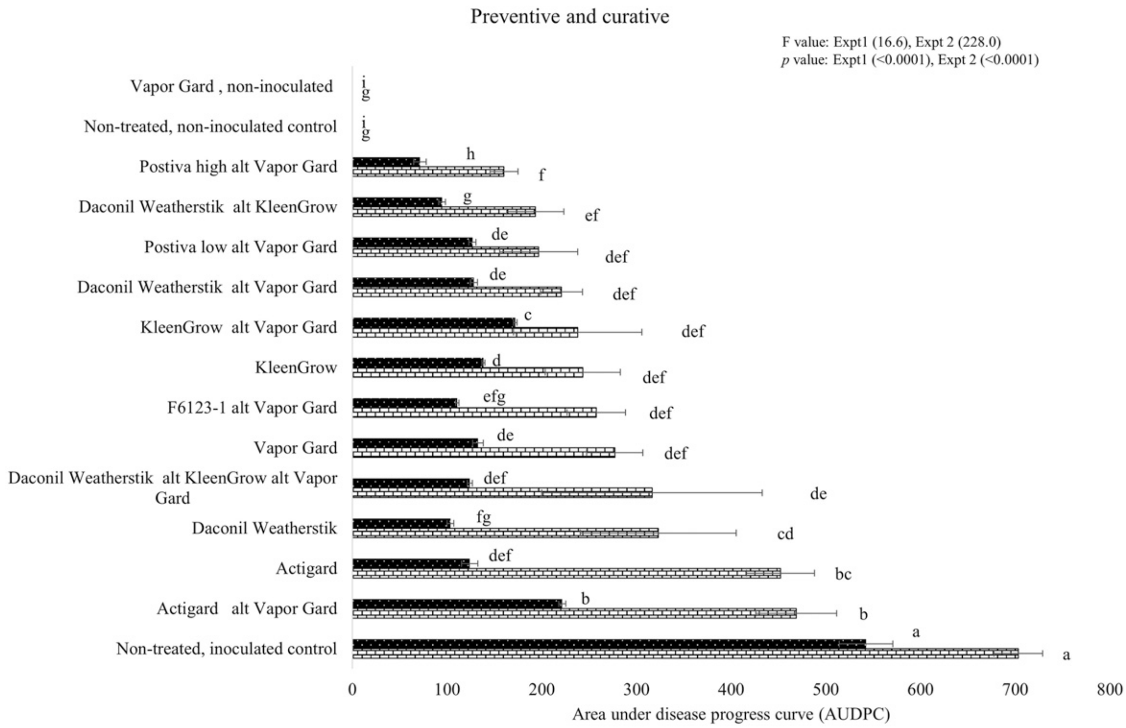

Fig. 3. Mean (± *SE*) area under disease progress curve (AUDPC) of blight disease (*C. pseudonaviculata*) of boxwood (*B. sinica* var. *insularis* × *B. sempervirens* 'Green Velvet') in Expt. 2 evaluated on 2 Feb, 9 Feb, 16 Feb, 23 Feb, 2 Mar, 9 Mar, and 16 Mar 2023. The values are the means per plant for six-single plant (one potted boxwood plant) replicates. The patterned bars indicate Expt. 1, and the solid black bars indicate Expt. 2. Lowercase letters on the bars denote significance at $P \leq 0.05$. The AUDPC values were calculated using the following equation: $\sum[\frac{(x_i+x_{i-1})}{2}](t_i - t_{i-1})$. Control treatments included non-treated *C. pseudonaviculata*–inoculated plants, non-inoculated non-treated plants, and non-inoculated Vapor Gard–treated plants (preventative and curative). There was a preventive application of product 24 h prior to inoculation, followed by inoculation and a second application 14 d after inoculation and two more consecutive applications at 14-d intervals.

Gard were the lowest and were significantly different from the non-treated inoculated controls in Expt. 1.

Curative treatments. *Disease severity and progress.* All the treatments resulted in significantly lower disease severity and progress in comparison with the non-treated inoculated control in both experiments (Figs. 2 and 5). In Expt. 1, applications of the low rate of Postiva resulted in the lowest disease progress and was comparable to the disease progress of plants treated with F6123-1 alt. with Vapor Gard, F6123-1 and the high rate of Postiva alt. with Vapor Gard but was statistically different from the rest of the treatments (Fig. 2), whereas in Expt. 2, applications of the low rate of Postiva resulted in the lowest disease progress, which was comparable to the disease progress of plants treated with the high rate of Postiva, F6123-1 alt. with Vapor Gard, and Vapor Gard alone but was statistically different from the rest of the treatments (Fig. 2).

Additionally, applications of the low rate of Postiva demonstrated the lowest disease severity comparable to the disease severity of plants treated with F6123-1 alt. with Vapor Gard and the high rate of Postiva alt. with Vapor Gard but statistically different from other treatments in Expt. 1 (Fig. 5). However, in Expt. 2, applications of the low rate of Postiva resulted in the lowest disease severity comparable to the disease severity of plants treated with F6123-1 alt. with Vapor Gard, the high rate of Postiva, Vapor Gard, and the high rate of Postiva alt. with Vapor Gard but statistically different from the rest of the treatments (Fig. 5).

Defoliation. The low rate of Postiva, the low rate of Postiva alt. with Vapor Gard, and F6123-1 alt. with Vapor Gard resulted in significantly lower defoliation in Expt. 1 in comparison with the non-treated inoculated control. In Expt. 2, plants treated with F6123-1 alt. with Vapor Gard, the high rate of Postiva alt. with Vapor Gard, the low rate of Postiva, and the low rate of Postiva alt. with Vapor Gard resulted in the least defoliation (Table 3).

Preventive and curative treatments. *Disease severity and progress.* All treatments resulted in significantly lower disease severity and progress in comparison with the non-treated inoculated control in both experiments (Figs. 3 and 6). In Expt. 1, applications of the high rate of Postiva alt. with Vapor Gard resulted in the lowest disease progress, which was comparable to the disease progress of plants treated with Daconil Weatherstik alt. with KleenGrow, the low rate of Postiva alt. with Vapor Gard, Daconil Weatherstik alt. with Vapor Gard, KleenGrow alt. with Vapor Gard, KleenGrow alone, F6123-1 alt. with Vapor Gard, and Vapor Gard alone but statistically different from the rest of the treatments (Fig. 3). However, in Expt. 2, applications of the high rate of Postiva alt. Vapor Gard resulted in the lowest disease progress, which was statistically different from the rest of the treatments (Fig. 3).

Additionally, applications of Daconil Weatherstik alt. with KleenGrow demonstrated the lowest disease severity comparable to the disease severity of plants treated with KleenGrow alone, the low rate of Postiva alt. with Vapor

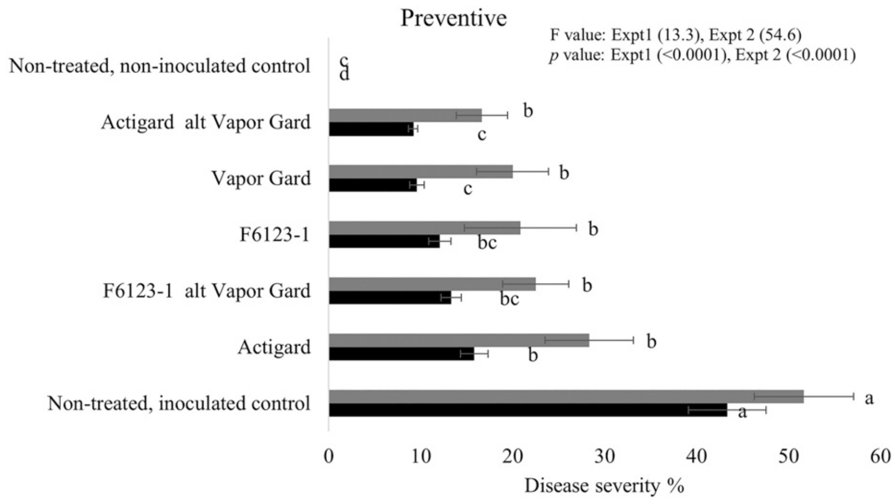

Fig. 4. Mean (± *SE*) final boxwood blight (*C. pseudonaviculata*) disease severity (percentage of whole plant affected) of boxwood (*B. sinica* var. *insularis* × *B. sempervirens* 'Green Velvet') in Expt. 1 evaluated on 20 Oct 2022. The values are the means per plant for six-single plant (one potted boxwood plant) replicates. Disease severity was evaluated using a 0% to 100% disease severity scale. The black bars indicate Expt. 1, and the gray bars indicate Expt. 2. Treatment means followed by different lowercase letters in the column denote significance at $P \leq 0.05$. Control treatments included non-treated *C. pseudonaviculata*–inoculated plants and non-inoculated non-treated plants. Preventive application of products was conducted 24 h prior to inoculation.

Gard, the high rate of Postiva alt. with Vapor Gard, Daconil Weatherstik alt. with KleenGrow alt. with Vapor Gard, and KleenGrow alt. with Vapor Gard but statistically different from other treatments in Expt. 1 (Fig. 6). In contrast, in Expt. 2, applications of Daconil Weatherstik alt. with KleenGrow resulted in the lowest disease severity, comparable to the disease severity of plants treated with the high rate of Postiva alt. with Vapor Gard, Daconil Weatherstik alt. with Vapor Gard, Actigard alone, Daconil Weatherstik alone, the low rate of Postiva alt. with Vapor Gard, F6123-1 alt. with Vapor Gard, and Daconil Weatherstik alt. with KleenGrow alt. Vapor Gard but statistically different from the rest of the treatments (Fig. 6).

Defoliation. Treatments of Daconil Weatherstik alt. with KleenGrow alt. with Vapor Gard, the high rate of Postiva alt. with Vapor Gard, and Actigard alone resulted in the lowest defoliation (%) (Table 4) in Expt. 1. The treatments of the low rate of Postiva alt. with Vapor Gard and Daconil Weatherstik alt. with Vapor Gard resulted in significantly lower defoliation in comparison with non-treated inoculated control in Expt. 1 (Table 4). However, in Expt. 2, treatments of Daconil Weatherstik alt. with KleenGrow, the high rate of Postiva alt. with Vapor Gard, KleenGrow alone, F6123-1 alt. with Vapor Gard, the low rate of Postiva alt. with Vapor Gard, KleenGrow alt. with Vapor Gard, Vapor Gard alone, Actigard alt. with Vapor Gard, and Actigard alone demonstrated the lowest defoliation (Table 4).

Effect of fungicides, Actigard, and Vapor Gard on plant growth and stem water potential

There were no significant differences in height increase and width increase among the treatments in both experiments (data not shown). The lowest stem water potential was observed for the non-treated, inoculated controls in both experiments. Significant ($P < 0.05$) weak positive correlations between disease severity and stem

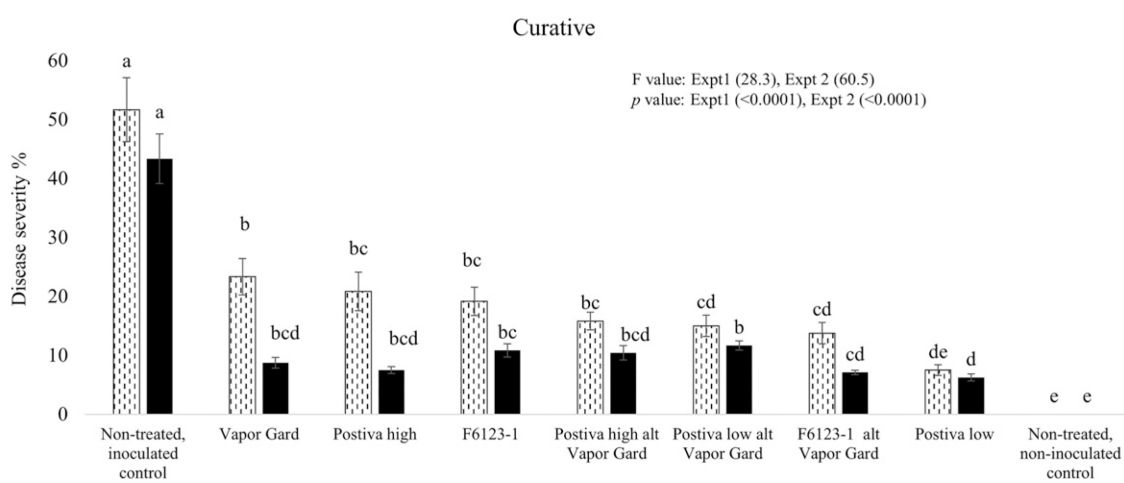

Fig. 5. Mean (± *SE*) final boxwood blight (*C. pseudonaviculata*) disease severity (percentage of whole plant affected) of boxwood (*B. sinica* var. *insularis* × *B. sempervirens* 'Green Velvet') in Expt. 1 evaluated on 20 Oct 2022. The values are the means per plant for six-single plant (one potted boxwood plant) replicates. Disease severity was evaluated using a 0% to 100% disease severity scale. The patterned bars indicate Expt. 1, and the solid black bars indicate Expt. 2. Treatment means followed by different lowercase letters in the column denote significance at $P \leq 0.05$. Control treatments included non-treated *C. pseudonaviculata*–inoculated plants, non-inoculated non-treated plants, and non-inoculated Vapor Gard–treated plants (preventative and curative). Curative application of the products started 14 d after inoculation with two more consecutive applications at 14-d intervals.

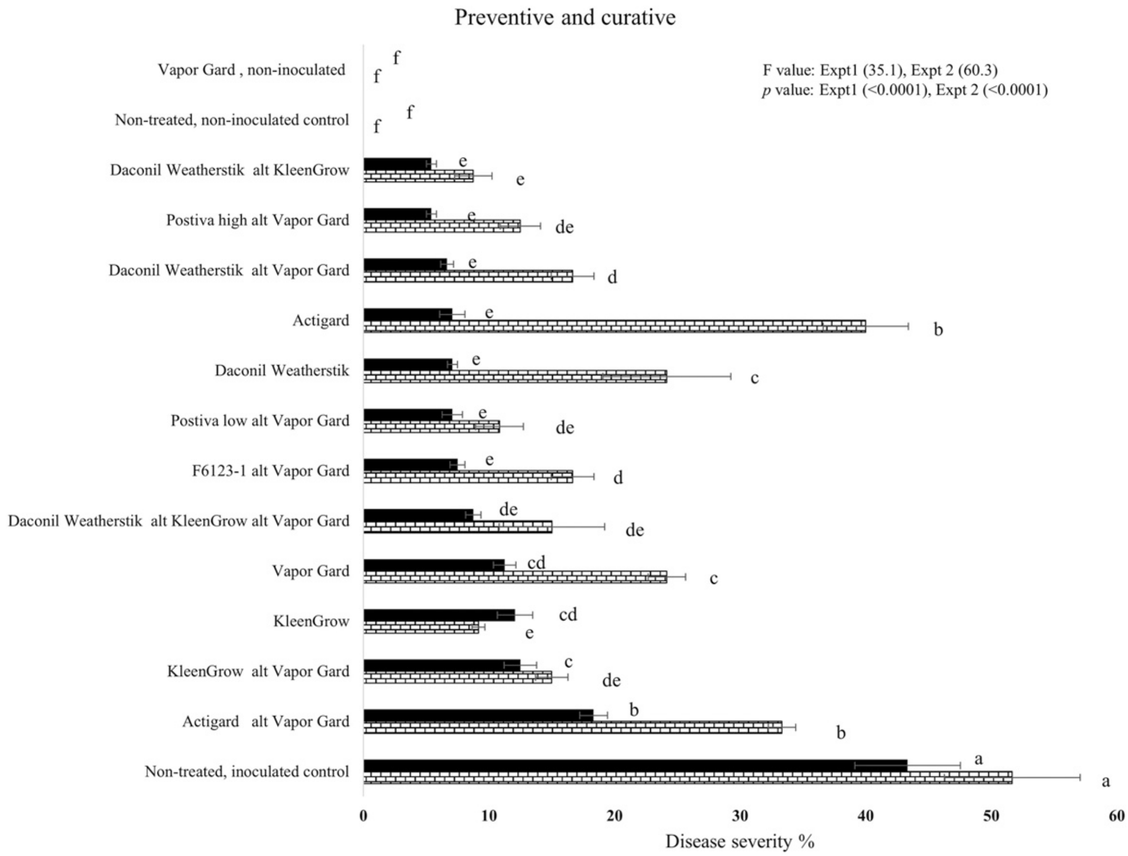

Fig. 6. Mean (± *SE*) final boxwood blight (*C. pseudonaviculata*) disease severity (percentage of whole plant affected) of boxwood (*B. sinica* var. *insularis* × *B. sempervirens* 'Green Velvet') in Expt. 1 evaluated on 20 Oct 2022. The values are the means per plant for six-single plant (one potted boxwood plant) replicates. Disease severity was evaluated using a 0% to 100% disease severity scale. The patterned bars indicate Expt. 1, and the solid black bars indicate Expt. 2. Treatment means followed by different lowercase letters in the column denote significance at $P \leq 0.05$. Control treatments included non-treated *C. pseudonaviculata*–inoculated plants, non-inoculated non-treated plants, and non-inoculated Vapor Gard–treated plants (preventative and curative). There was a preventive application of product 24 h prior to inoculation, followed by inoculation and a second application 14 d after inoculation and two more consecutive applications at 14-d intervals.

water potential (r = 0.25) and between AUDPC and stem water potential (r = 0.13) were also observed (data not shown).

Preventive treatments. Significant differences were observed among the treatments in Expts. 1 and 2 for stem water potential (Table 5). The highest stem water potential (MPa) was observed for Vapor Gard–treated and non-inoculated plants in both experiments. Among the inoculated treatments, plants treated with Actigard alt. with Vapor Gard resulted in the highest stem water potential in both Expt. 1 and Expt. 2 (Table 5).

Curative treatments. Significant differences were observed among the treatments in Expts. 1 and 2 for stem water potential (Table 6). In Expt. 1, among the inoculated and treated plants, plants treated with the low rate of Postiva alt. with Vapor Gard resulted in the highest stem water potential (Mpa), and the water potential was comparable to the stem water potential of plants treated with the high rate of Postiva alone, Vapor Gard alone, the low rate of Postiva, F6123-1 alt. with Vapor Gard and the high rate of Postiva alt. with Vapor Gard but was statistically different from the rest of the treatments, whereas in Expt. 2, plants treated with the low rate of Postiva alt. with Vapor Gard resulted in the highest stem water potential (Mpa), and the stem water potential was comparable to the stem water potential of plants treated with Vapor Gard, the high rate of Postiva, the low rate of Postiva, and F6123-1 alt. with Vapor Gard but was statistically different from the rest of the treatments.

Preventive and curative treatments. Significant differences were observed among the treatments in Expt. 1 and Expt. 2 for stem water potential (Table 7). In Expt. 1, among the inoculated and treated plants, plants treated with the high rate of Postiva alt. with Vapor Gard resulted in the highest stem water potential (Mpa), and the water potential was comparable to the stem water potential of plants treated with Vapor Gard, the low rate of Postiva alt. with Vapor Gard, Daconil Weatherstik alt. with Vapor Gard, KleenGrow alt. with Vapor Gard, and F6123-1 alt. with Vapor Gard but was statistically different from the rest of the treatments. However, in Expt. 2, plants treated with Vapor Gard resulted in the highest stem water potential (Mpa), which was statistically different from the rest of the treatments. The treatments of the low rate of Postiva alt. with Vapor Gard, the high rate of Postiva alt. with Vapor Gard, KleenGrow alt. with Vapor Gard, and Daconil Weatherstik alt. with Vapor

Table 4. Mean (± *SE*) defoliation of boxwood (*Buxus* spp.) transplants treated with preventive and curative applications of fungicides, Actigard, and Vapor Gard treatments.

Treatment	Application type/dates[i]	Mean defoliation[ii]	
		Expt. 1	Expt. 2
Actigard	1, 3, 4, 5	5.4 ± 0.4 c–e[iii]	4.5 ± 1.0 cd
Actigard alt. Vapor Gard	1, 3, 4, 5	9.6 ± 2.2 ab	5.8 ± 1.4 b–d
Daconil Weatherstik alt. KleenGrow alt. Vapor Gard	1, 3, 4, 5	3.7 ± 0.9 d–f	10.8 ± 2.9 ab
Daconil Weatherstik alt. KleenGrow	1, 3, 4, 5	7.5 ± 0.9 a–d	3.0 ± 1.5 cd
KleenGrow	1, 3, 4, 5	7.9 ± 1.4 a–c	5.0 ± 1.1 b–d
Daconil Weatherstik	1, 3, 4, 5	8.3 ± 1.2 a–c	7.0 ± 2.0 bc
Daconil Weatherstik alt. Vapor Gard	1, 3, 4, 5	8.7 ± 0.9 a–c	7.0 ± 1.7 a–c
F6123-1 alt. Vapor Gard	1, 3, 4, 5	10.8 ± 1.4 a	5.4 ± 1.5 b–d
KleenGrow alt. Vapor Gard	1, 3, 4, 5	10.8 ± 1.4 a	5.4 ± 0.8 b–d
Postiva low alt. Vapor Gard	1, 3, 4, 5	6.6 ± 3.9 b–d	5.8 ± 2.3 b–d
Postiva high alt. Vapor Gard	1, 3, 4, 5	0.0 ± 0.0 ef	3.0 ± 1.1 cd
Vapor Gard	2, 3, 4, 5	2.08 ± 0.4 ef	5.8 ± 1.4 b–d
Vapor Gard, non-inoculated[iv]	2, 3, 4, 5	0.0 ± 0.0 f	0.0 ± 0.0 d
Non-treated, inoculated control[iv]		8.3 ± 0.5 a–c	13.3 ± 5.8 a
Non-treated, non-inoculated control[iv]		0.0 ± 0.0 f	0.0 ± 0.0 d
F value		8.72	2.61
P value		<0.0001	0.0039

[i] The application dates were as follows: 1 = 31 Aug 2022 morning (Expt. 1) or 25 Jan 2023 (Expt. 2) morning; 2 = 31 Aug 2022 afternoon (Expt. 1) or 25 Jan 2023 (Expt. 2) afternoon; 3 = 15 Sep 2022 (Expt. 1) or 9 Feb 2023 (Expt. 2); 4 = 29 Sep 2022 (Expt. 1) or 23 Feb 2023 (Expt. 2); 5 = 13 Oct 2022 (Expt. 1) or 9 Mar 2023 (Expt. 2).

[ii] Defoliation (mean ± *SE*) of boxwood plants treated with anti-transpirant, host-plant defense inducer, disinfectant, and fungicides at the end of both experiments. Defoliation on each plant was evaluated using a 0% to 100% scale. The values are the means ± *SE* of six single-plant replications in both trials.

[iii] Treatment means followed by different lowercase letters in the column denote significance at $P \leq 0.05$. The values are the means per plant for six single-plant (one potted boxwood plant) replicates. A one-way analysis of variance was used to evaluate treatment effects. When the effects were significant, Fisher's least significant difference test was used for mean comparison with $\alpha = 0.05$.

[iv] Control treatments included non-treated *C. pseudonaviculata*–inoculated plants, non-inoculated non-treated plants, and non-inoculated anti-transpirant–treated plants (preventative and curative).

Preventive application of product 24 h before inoculation followed by inoculation and second application 14 d after inoculation with two more consecutive applications at 14-d intervals. alt. = alternated with.

Gard demonstrated moderately high stem water potential in Expt. 2 (Table 7).

Discussion

This study evaluated the efficacy of 24 treatment combinations, including three fungicides (Postiva, Daconil Weatherstik, and F6123-1), one disinfectant/fungicide (KleenGrow), one host-plant defense inducer (Actigard), and one anti-transpirant (Vapor Gard), applied with different timings (preventive, curative, and preventive and curative) for reducing the boxwood blight disease over time. The tested treatments, whether applied alone, in combination, or in alternation, significantly reduced disease severity and progression compared with the non-treated, inoculated control plants. It is important to note that in both Expt. 1 and Expt. 2, the disease severity gradually developed (<5%) over the first 4 weeks and then rapidly increased, suggesting that the initial disease pressure was low, but it intensified toward the end of the experiment (data not shown).

Preventive applications of Actigard alt. with Vapor Gard significantly reduced disease severity and progress. Defoliation was also significantly reduced by preventive applications of Actigard alt. with Vapor Gard. Preventive applications of Actigard alt. with Vapor Gard resulted in the highest stem water potential in both experiments. Acibenzolar-*S*-methyl, the active ingredient in Actigard (US Environmental Protection Agency 2016), is a previously reported protective fungicide. It suppresses disease by inducing synthetic acquired host defenses and adversely affecting the pathogen's growth and vigor (LaMondia 2009; Latunde-Dada and Lucas 2001; Rohilla et al. 2002). It is recommended to be integrated with other fungicides as an integrated plant disease management strategy of various plant diseases (Ji et al. 2011; LaMondia 2009). Actigard has been demonstrated to effectively control boxwood blight when applied preventively (Melo et al. 2019), whereas the alternated application of a fungicide (chlorothalonil) with Vapor Gard has been previously reported (Roark et al. 2000) to effectively suppress the plant disease (black spot disease severity of naturally infected rose), which was (statistically) comparable to suppression of disease severity by a sole application of fungicide (chlorothalonil). During foliar disease development, the leaves lose the integrity of their cell membranes, hence leading to the loss of a large amount of apoplastic water and an increase in leaf temperature eventually causing higher loss of water by transpiration (Lindenthal et al. 2005). Anti-transpirants are often applied as foliar sprays or dips that reduce the transpiration rate of plants by forming a protective waxy or thin film coating on foliage or inducing stomatal closure to help conserve water and prevent excessive water loss through stomatal pores in leaves (Javan et al. 2013; Mphande et al. 2023; Pandey et al. 2017). Based on the outcomes of this study, preventive application of Actigard alt. with Vapor Gard has the potential to have significant disease control effects in comparison with non-treated inoculated control when preventive applications are required. Treatments of fungicides (Actigard – preventive; the low rate and high rate of Postiva,

Table 5. Mean (± *SE*) stem water potential of boxwood (*Buxus* spp.) transplants treated with preventive applications of fungicides, Actigard, and Vapor Gard treatments.

Treatment	Application type/dates[i]	Mean stem water potential (MPa ± *SE*)[ii]	
		Expt. 1	Expt. 2
Actigard	1	109.6 ± 1.7 c[iii]	147.4 ± 2.4 de
Actigard alt. Vapor Gard	1, 2	96.8 ± 6.6 cd	143.3 ± 1.4 e
F6123-1	2	208.8 ± 20.7 b	159.1 ± 4.4 b
F6123-1 alt. Vapor Gard	1, 2	123.3 ± 7.3 c	153.7 ± 1.8 bc
Vapor Gard	2	121.9 ± 3.3 c	150.6 ± 0.9 cd
Non-treated, inoculated control[iv]	1	257.9 ± 24.4 a	192.1 ± 0.9 a
Non-treated, non-inoculated[iv] control	1	64.5 ± 1.1 d	97.4 ± 0.6 f
F value		28.56	171.68
P value	1	<0.001	<0.0001

[i] The application dates were as follows: 1 = 31 Aug 2022 morning (Expt. 1) or 25 Jan 2023 (Expt. 2) morning; 2 = 31 Aug 2022 afternoon (Expt. 1) or 25 Jan 2023 (Expt. 2) afternoon.
[ii] Stem water potential was measured on the day of experiment termination by using a model 600 pressure chamber instrument. The values of mean (± *SE*) stem water potential are negative (−).
[iii] Treatment means followed by different lowercase letters in the column denote significance at $P \leq 0.05$. The values are the means per plant for six single-plant (one potted boxwood plant) replicates. A one-way analysis of variance was used to evaluate treatment effects. When the effects were significant, Fisher's least significant difference test was used for mean comparison with $\alpha = 0.05$.
[iv] Control treatments included non-treated *C. pseudonaviculata*–inoculated plants and non-inoculated non-treated plants. Preventive applications of products were conducted 24 h before inoculation. alt. = alternated with.

F6123-1-1 – curative; the low rate and high rate of Postiva, Daconil Weatherstik, and KleenGrow – preventive and curative) alternated with Vapor Gard demonstrated highest stem water potentials and were effective in boxwood blight disease suppression. Although the exact mechanism of the role of Vapor Gard in disease suppression is unknown, there might be several factors contributing to the observed disease suppression. Vapor Gard creates a thin, nearly invisible layer on the leaves and surfaces of plants and helps to decrease water vapor loss (Iriti et al. 2009; Miller 2023). In addition to this, Vapor Gard is reported to be fungitoxic in vitro against conidial germination, appressoria formation, and hyphal elongation of various fungi such as *Botrytis cinerea*, *Sphaerotheca fuliginea*, and *Blumeria graminis* f. sp. *hordei* (Ela et al. 1989; Elad et al. 1990; Nasraoui et al. 1999; Sutherland and Walters 2001), which could have contributed to the disease suppression. However, the effects of Vapor Gard on germination of *C. pseudonaviculata* spores, hyphal elongation, and on the sporulation on the infected tissues have yet to be explored for understanding the exact mechanism of action in disease suppression. Also, the role of reduced water potential in disease suppression is not clearly understood.

Among the curative treatments, the low rate of Postiva, F6123-1 alt. with Vapor Gard and the high rate of Postiva alt. with Vapor Gard consistently resulted in significant reduction of disease progress and final disease severity in both experiments. Similarly, defoliation was consistently lowest for the treatments of the low rate of Postiva, F6123-1 alt. with Vapor Gard in both experiments. Postiva is a fungicide that consists of two active ingredients: pydiflumetofen at a concentration of 6.9% and difenoconazole at a concentration of 11.5%. These active ingredients belong to different groups of the FRAC mode of action, specifically groups 7 and 3, respectively (FRAC 2021). Pydiflumetofen, categorized as a succinate-dehydrogenase inhibitor, acts by inhibiting an enzyme involved in fungal cell respiration, thus impeding fungal growth (Hägerhäll 1997; Ruprecht et al. 2009). Difenoconazole, on the other hand, is a demethylation inhibitor fungicide that hampers fungal development by interfering with the synthesis of ergosterol, a crucial component of the plasma membrane in certain fungi (Bowyer and Denning 2014). The fungicides that contain pydiflumetofen and difenoconazole as active ingredients are recommended for controlling fungal diseases in ornamental crops. Postiva has a combined succinate dehydrogenase inhibitor + sterol demethylation inhibition mode of action to suppress plant disease development. The compound pydiflumetofen has a medium to high risk of resistance development, whereas difenoconazole has a medium risk of resistance development (FRAC 2021). In this study, curative applications of the low rate of Postiva ($1.09\ mL \cdot L^{-1}$) and the high rate of Postiva ($1.6\ mL \cdot L^{-1}$) alt. with Vapor Gard were demonstrated

Table 6. Mean (± *SE*) stem water potential of boxwood (*Buxus* spp.) transplants treated with curative applications of fungicides, Actigard, and Vapor Gard treatments.

Treatment	Mean stem water potential (MPa ± *SE*)[i]	
	Expt. 1	Expt. 2
F6123-1	127.7 ± 16.4 b[ii]	159.1 ± 1.9 b
F6123-1 alt. Vapor Gard	85.6 ± 3.4 c	138.7 ± 4.9 de
Postiva low	81.7 ± 3.7 c	137.2 ± 2.0 de
Postiva high	78.1 ± 4.3 c	146.5 ± 1.5 c
Postiva low alt. Vapor Gard[iii]	77.6 ± 4.6 c	132.6 ± 3.3 e
Postiva high alt. Vapor Gard[iii]	90.6 ± 6 c	143.2 ± 2 cd
Vapor Gard	81.1 ± 4 c	136.6 ± 2.1 de
Non-treated, inoculated control	257.9 ± 24.4 a	192.1 ± 0.9 a
Non-treated, non-inoculated control	64.5 ± 1.1 c	97.41 ± 0.6 f
F value	32.95	44.84
P value	<0.0001	<0.0001

[i] Stem water potential was measured on the day of experiment termination by using a model 600 pressure chamber instrument. The values of mean (± *SE*) stem water potential are negative (−).
[ii] Treatment means followed by different lowercase letters in the column denote significance at $P \leq 0.05$. The values are the means per plant for six single-plant (one potted boxwood plant) replicates. A one-way analysis of variance was used to evaluate treatment effects. When the effects were significant, Fisher's least significant difference test was used for mean comparison with $\alpha = 0.05$.
[iii] Control treatments included non-treated *C. pseudonaviculata*–inoculated plants and non-inoculated non-treated plants. The application dates were as follows: 3 = 15 Sep 2022 (Expt. 1) or 9 Feb 2023 (Expt. 2); 4 = 29 Sep 2022 (Expt. 1) or 23 Feb 2023 (Expt. 2); 5 = 13 Oct 2022 (Expt. 1) or 9 Mar 2023 (Expt. 2).
Curative application of the products started 14 d after inoculation with two more consecutive applications at 14-d intervals. alt. = alternated with.

Table 7. Mean (± *SE*) stem water potential of boxwood (*Buxus* spp.) transplants treated with preventive and curative applications of fungicides, Actigard, and Vapor Gard treatments.

Treatment	Application type/dates[i]	Mean stem water potential (MPa ± *SE*)[ii]	
		Expt. 1	Expt. 2
Actigard	1, 3, 4, 5	101.9 ± 2.1 bc[iii]	145.8 ± 11.6 bc
Actigard alt. Vapor Gard	1, 3, 4, 5	88.8 ± 3.6 cd	137.5 ± 4.4 cd
Daconil Weatherstik alt. KleenGrow alt. Vapor Gard	1, 3, 4, 5	85.5 ± 4.1 cd	138.5 ± 0.5 c
Vapor Gard	2, 3, 4, 5	119.0 ± 8 b	149.0 ± 0.9 b
Daconil Weatherstik alt. KleenGrow	1, 3, 4, 5	106.1 ± 1.4 bc	136.1 ± 2.6 c–e
Daconil Weatherstik alt. Vapor Gard	1, 3, 4, 5	73.0 ± 2.6 de	128.0 ± 2.2 d–f
F6123-1 alt. Vapor Gard	1, 3, 4, 5	79.9 ± 2 de	136.3 ± 1 c–e
KleenGrow alt. Vapor Gard	1, 3, 4, 5	75.8 ± 8.1 de	126.6 ± 2.1 ef
KleenGrow	1, 3, 4, 5	101.4 ± 4.4 bc	144.5 ± 2.3 bc
Postiva low alt. Vapor Gard	1, 3, 4, 5	71.7 ± 1.9 de	123.7 ± 2.4 f
Postiva high alt. Vapor Gard	1, 3, 4, 5	68.0 ± 2.3 de	121.2 ± 0.9 f
Vapor Gard	1, 3, 4, 5	71.6 ± 4.8 de	102.1 ± 2.3 g
Vapor Gard, non-inoculated[iv]	2, 3, 4, 5	64.0 ± 3.2 e	94.2 ± 1.4 g
Non-treated, inoculated control[iv]	1, 3, 4, 5	257.9 ± 24.4 a	192.1 ± 0.9 a
Non-treated, non-inoculated control[iv]	1, 3, 4, 5	64.5 ± 1.1 e	97.4 ± 0.6 g
F value		48.12	102.21
P value		<0.0001	<0.0001

[i] The application dates were as follows: 1 = 31 Aug 2022 morning (Expt. 1) or 25 Jan 2023 (Expt. 2) morning; 2 = 31 Aug 2022 afternoon (Expt. 1) or 25 Jan 2023 (Expt. 2) afternoon; 3 = 15 Sep 2022 (Expt. 1) or 9 Feb 2023 (Expt. 2); 4 = 29 Sep 2022 (Expt. 1) or 23 Feb 2023 (Expt. 2); 5 = 13 Oct 2022 (Expt. 1) or 9 Mar 2023 (Expt. 2).

[ii] Stem water potential was measured on the day of experiment termination by using a model 600 pressure chamber instrument. The values of mean (± *SE*) stem water potential are negative (−).

[iii] Treatment means followed by different lowercase letters in the column denote significance at $P \leq 0.05$. The values are the means per plant for six single-plant (one potted boxwood plant) replicates. A one-way analysis of variance was used to evaluate treatment effects. When the effects were significant, Fisher's least significant difference test was used for mean comparison with $\alpha = 0.05$.

[iv] Control treatments included non-treated *C. pseudonaviculata*–inoculated plants, non-inoculated non-treated plants, and non-inoculated anti-transpirant–treated plants (preventative and curative).

Preventive application of product 24 h before inoculation followed by inoculation and second application 14 d after inoculation with two more consecutive applications at 14-d intervals. alt. = alternated with.

to be efficient treatments in controlling the disease. Postiva has been previously established as an effective curative fungicide (1.1 and 1.6 mL·L^{-1}) in controlling naturally occurring powdery mildew in hydrangea and black spot in roses (Jennings et al. 2024a, 2024b), whereas flutriafol (the active ingredient of F6123-1) is an established disease management fungicide and is demonstrated to be effective to control various foliar diseases (Bhuiyan et al. 2015; Claassen et al. 2022; Silva et al. 2021). Flutriafol is a systemic fungicide used both preventively and curatively to suppress various foliar and soil-borne pathogens (Grichar 2023; Li et al. 2021; Pijls and Shaw 1997). Because the low rate of Postiva is a cost-effective option between the high rate of Postiva and the low rate of Postiva and F6123-1 alternated with Vapor Gard being a sustainable approach, these curative treatments could potentially be used as an immediate application approach when the disease is suspected to be established or has already been established. These treatments consistently reduced the disease in both experiments.

In this study, preventive and curative treatments of the high rate of Postiva (1.6 mL·L^{-1}) alt. with Vapor Gard was the most effective in reducing disease severity and progress and defoliation in both experiments. In a recent study by Jennings et al. (2024a, 2024b), applications of 1.1 mL·L^{-1} of Postiva were able to reduce the naturally infected powdery mildew disease of big leaf hydrangea and black spot disease of roses, respectively, in greenhouse and shade house trials, and the treatments were equally effective (statistically) when applied at the rate of 1.6 and 2.2 mL·L^{-1}. Although not consistent throughout the experiments, the treatments such as the low rate of Postiva (1.09 mL·L^{-1}) alt. with Vapor Gard, Daconil Weatherstik alt. with Vapor Gard, and KleenGrow alt. with Vapor Gard also demonstrated some level of efficacy in reducing the disease. Daconil Weatherstik (chlorothalonil) and KleenGrow (quaternary ammonium compounds) have previously been demonstrated to be effective in controlling boxwood blight disease, which is similar to the results of this study (Bika et al. 2021; Henricot and Wedgwood 2013). In previous studies, the preventive and curative treatments of chlorothalonil, epoxiconazole + kresoxim-methyl + pyraclostrobin, propiconazole, myclobutanil, thiophanate-methyl, fludioxonil, pyraclostrobin, and kresozim-methyl have been demonstrated to be effective in management of boxwood blight (Henricot and Wedgwood 2013; LaMondia 2015). For consistency in disease control, it is recommended that the high rate of Postiva (1.6 mL·L^{-1}) alt. with Vapor Gard may be recommended as a reliable and reproducible preventive and curative disease control treatment. It is likely that the consistent curative applications of the low rate of Postiva (1.09 mL·L^{-1}) are effective in reducing disease, whereas a higher rate (1.6 mL·L^{-1}) is required if the treatment is applied as a preventive and curative application when used in alternation with Vapor Gard. Because preventive and curative applications of the high rate of Postiva alt. with Vapor Gard were consistent in controlling the disease pressure, combining (alternating) the high rate of Postiva (1.6 mL·L^{-1}) with

Vapor Gard could be a sustainable rotation option to minimize the risk associated with the reduction in efficacy.

To our knowledge, there is no previous report in the literature of an experiment that used Postiva in alternation with an anti-transpirant to control fungal disease. It is important to note that preventive fungicides act by creating a protective barrier on the plant surface that inhibits fungal spores from germinating and infecting the plant; preventive fungicides are applied before the onset of disease symptoms. The preventative activity occurs when a fungicide is present on or in the plant before the pathogen arrives or beings to develop (Manoharachary and Kunwar 2014; Martinez 2012; Oliver and Hewitt 2014), whereas the effectiveness of a curative fungicide depends on its ability to penetrate the plant as a systemic or locally systemic fungicide. Factors influencing post-infection efficacy include host-plant susceptibility, disease incidence and severity, fungicide concentration in plant tissues, and the pathogen's exposure stage to the active ingredient (LaMondia 2020). It is possible that the initial higher dose of the preventive fungicide complemented with the benefits of the anti-transpirant after inoculation, followed by the fungicide and the anti-transpirant was mostly likely able to reduce the overall disease progress, final disease severity, and defoliation in both experiments. Also, it is important to note that curative applications of the low rate of Postiva and the high rate of Postiva were equally effective in controlling boxwood blight disease severity in both experiments. Because the curative application of the low rate of Postiva (1.09) has previously been demonstrated to be an effective application rate and application type for management of ornamental plant diseases (Jennings et al. 2024a, 2024b), the low rate of Postiva (1.1 $mL \cdot L^{-1}$) may be one of the effective curative blight disease control measures. Chlorothalonil (Daconil Weatherstik) is a multisite contact activity compound, falling in the low-risk group (FRAC code of M 05) and is reported to be very effective in controlling boxwood blight in several studies (Baysal-Gurel et al. 2021; Ivors et al. 2013). Quaternary ammonium compounds (QACs) (KleenGrow) are non-oxidizing disinfectants generally acting by causing cell membrane disruption and leading to cytoplasm leakage and interaction with phospholipids leading to coagulation of the cytoplasm (Jakovljevic 2022) and have been reported to reduce boxwood blight disease severity in previous studies (Baysal-Gurel et al. 2021, 2022b; Brand et al. 2023). Preventive and curative applications of chlorothalonil alt. with QACs also provided disease control throughout the experiments in comparison with the non-inoculated control, which provides an option for fungicide rotation and helps in reducing the risk of resistance development in the pathogen population, which are consistent with previous research findings (Baysal-Gurel et al. 2021). The curative and preventive and curative applications of Vapor Gard with fungicides were more efficient in terms of disease suppression than solitary applications of Vapor Gard, but none of the preventive applications of Vapor Gard when applied as a solitary treatment were able to consistently reduce the disease progress throughout the experiments. It is not clearly understood but highly likely that a higher frequency of applications of these compounds would increase the disease control.

No significant differences were observed in height and width increase ($P > 0.05$) between treatments, which could be attributed to the slow growth behavior of boxwood (3 to 6 inches/year) (Palmer and Shishkoff 2014) and the experiment being conducted for a short period (7 weeks). The plants demonstrating the lowest disease severity and progress demonstrated higher stem water potential, and the observed weak positive correlation demonstrated that the application of anti-transpirant in alternation could potentially be associated with disease suppression, although the mechanism of control is still unknown. The effectiveness of curative applications of Vapor Gard, when alternated with chemicals, was comparatively less than the combined impact of preventive and curative treatments. This could indicate that employing preventive and curative measures could be more effective than starting chemical applications after the infection has been initiated. It is noteworthy that these experiments were conducted in a controlled environment that involved single inoculations and the disease developed after inoculation potentially be the further development of infections which were established. However, the disease pressure could be different in field conditions, where new infections eventually develop; the efficacy of these treatments under such conditions could be a topic of further exploration.

In integrated plant health management of ornamental crops, preventive, curative, and preventive and curative treatments of fungicides, bio-fungicides, and biorational products remain essential strategies in addition to other strategies such as sanitation, use of clean stock, treatment of cuttings, host resistance, early detection, and cultural control (Daughtrey and Benson 2005; Gullino and Garibaldi 2007; Mizell et al. 2011). To reduce the reliance on traditional chemical methods, integration of various approaches such as exploring novel modes of action, novel target sites for fungicides, genetic advancements, biological controls, and low-resistance-risk chemicals (Leadbeater 2015) can be further explored. This study has shown different options for preventive, curative, and preventive and curative applications of chemicals alternated with anti-transpirant that provide equally effective or superior disease control of boxwood blight disease. Preventive applications of Actigard alt. with Vapor Gard, curative applications of the low rate of Postiva and F6123-1 alt. with Vapor Gard, and preventive and curative applications of the high rate of Postiva alt. with Vapor Gard demonstrated significant disease control. These treatments also consistently exhibited higher stem water potential. The results of this study could serve as viable options in boxwood blight disease management. Adopting such integrated approaches demonstrates responsible fungicide usage, optimizing fungicide use efficiency and safeguarding the efficacy of these crucial disease control tools for the future.

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