Curative Fungicide Activity Against *Calonectria pseudonaviculata*, the Boxwood Blight Pathogen[1]

J. A. LaMondia[2]

─── **Abstract** ───

Azoxystrobin, azoxystrobin plus benzovindiflupyr, kresoxim-methyl, propiconazole, pyraclostrobin, pyraclostrobin plus fluxapyroxad, tebuconazole, tetraconazole, thiophanate-methyl, and triflumizole fungicides were evaluated for curative and anti-sporulant activity against boxwood blight caused by *Calonectria pseudonaviculata* on detached leaves and whole boxwood plants (*Buxus* spp.). Pretreating detached leaves with 30 or 300 ppm a.i. 24 h prior to inoculation reduced disease compared to the untreated control for all fungicides. Fungicides were also applied 24 to 96 h post-inoculation. Only propiconazole reduced diseased leaf incidence to at least half of the control. When leaves were treated post-infection with 300 ppm propiconazole, tetraconazole, tebuconazole, or triflumizole, the pathogen did not sporulate over 2 wks. Propiconazole also reduced the percent of leaf area diseased; lesions were nearly 80% smaller with 300 ppm applied 48 h after inoculation. 'True Dwarf' boxwood plants treated with 450 ppm thiophanate-methyl, 120 ppm pyraclostrobin or 150 ppm propiconazole 48 h after inoculation demonstrated that only propiconazole reduced the number of diseased leaves, blight lesions and the frequency of pathogen re-isolation. Experiments with 'Green Mound' and 'Green Mountain' boxwood cultivars and additional fungicides applied 48 h after inoculation demonstrated that propiconazole at 300 ppm, pyraclostrobin plus fluxapyroxad (150 ppm each) and azoxystrobin (135 ppm) plus benzovindiflupyr (67.5 ppm) reduced disease.

Index words: fungicide management, *Buxus*, chemical disease management.

Chemicals used in this study: azoxystrobin (Heritage 50 WG), azoxystrobin plus benzovindiflupyr (Mural 30, 50 WG), kresoxim-methyl (Cygnus 50 WG), propiconazole (ProCon-Z 14.3 L), pyraclostrobin (Insignia 20 WG), pyraclostrobin plus fluxapyroxad (Orkestra Intrinsic 21.26 SC), tebuconazole (Torque 38.7 SC), tetraconazole (Minerva 11.6 SC), thiophanate-methyl (3336 50% WP), triflumizole (Procure 480 SC).

Species used in this study: boxwood (*Buxus* L.), boxwood blight (*Calonectria pseudonaviculata* (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M. J. Wingf. & Crous.

Significance to the Horticulture Industry

Boxwood blight caused by the fungal pathogen *Calonectria pseudonaviculata* has been a destructive disease affecting boxwood nursery production and valuable landscape plantings. Management of the disease has been heavily dependent on sanitation and protection with a number of fungicides. While pretreatment with fungicides is most efficacious against the boxwood blight pathogen, infectious periods may occur almost continuously over a long wet period and environmental conditions may limit the ability to apply protectant fungicides prior to infection. In those instances, the reduced incidence, lesion size and inhibition of sporulation demonstrated by post-infection treatment with propiconazole, fluxapyroxad and benzovindiflupyr fungicides demonstrated in these experiments should combine to slow the development of disease and significantly reduce epidemic development, aiding boxwood blight management.

Introduction

Boxwood blight in the United States is caused by the fungal pathogen *Calonectria pseudonaviculata* (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M. J. Wingf. & Crous (Crous et al. 2002, 2004) (synonym:*Cylindrocladium pseudonaviculatum* Crous, J.Z. Groenew. & C.F. Hill). The pathogen appears to be clonal with limited genetic diversity within its known range (LeBlanc et al. 2018). The disease was first identified in the USA in 2011 (Ivors et al. 2012) and resulted in very significant losses in the ornamental nursery industry due to direct losses of plants due to disease, the removal of potentially infected plant debris, destruction of exposed plants and the subsequent costs associated with changes in plant production to prevent re-introduction of the pathogen (AmericanHort 2017). The disease has been confirmed from at least 25 states to date (Calabro 2018, LaMondia and Shishkoff 2017). In 2018, boxwood blight was diagnosed and confirmed from more than 400 samples from landscape settings in Connecticut (Y. Li Connecticut Agricultural Experiment Station, personal communication). *C. pseudonaviculata* infection efficiency is strongly influenced by the frequency and length of extended wet periods (Avenot et al. 2017) so it may not be unexpected that disease incidence was greatest in 2011 and 2018, the wettest 161.8 cm precipitation) and second wettest (159.5 cm) years recorded in Connecticut in the last 30 years (http://www.nrcc.cornell.edu/regional/tables/tables.html).

Once *C. pseudonaviculata* is present in a boxwood planting or plants are under threat of exposure to the pathogen, management of the disease is heavily dependent

on the application of fungicides. A number of fungicides have been identified with efficacy against the pathogen *in vitro* (Brand 2006, Henricot et al. 2008, LaMondia 2014) and *in planta* (Henricot and Wedgwood 2013, LaMondia 2015) and combinations of protectant and systemic fungicides with different modes of action may be most efficacious and protect against the development of fungicide resistance (LaMondia 2015, Maurer et al. 2017). Two-week spray intervals are common, but the wide range of temperatures conducive for disease may result in disease risk predictions over a large portion of the year, at least 6 months in Connecticut, making frequent sprays impractical (Coop 2013 and 2014). Therefore, it may not be possible to have continual protectant fungicide coverage on plants in advance of conducive environmental conditions. A number of fungicides have been demonstrated to have curative activity against plant disease in other crops (Dario 2010). This research was conducted to determine if selected fungicides had curative activity, the ability to control the pathogen for periods of time after infection had occurred, against *C. pseudonaviculata* in boxwood. Such curative efficacy might allow more effective disease management if fungicides were applied not only preventatively, but at the end of an infectious period.

Materials and Methods

A mixture of isolates, Cps-CT-L1 and Cps-CT-S1 (LaMondia 2015) and CT-WH1 (Maurer et al. 2017) of the anamorph of *C. pseudonaviculata* were used in these experiments. Isolate virulence was maintained by periodic inoculation of detached boxwood leaves and collecting conidia from lesions. Inoculum was obtained by placing approximately 20 drops of a 100-200 conidia per drop suspension onto half-strength potato dextrose agar (½ PDA) in a petri dish. Drops were allowed to dry by passing sterile air over open dishes in a sterile hood. Petri dishes were then covered and conidia collected after 2-3 days at 22-25 C (72-77 F) by flooding plates with a solution of sterile distilled water and Tween 20 (1 drop Tween 20 per 200 ml). Conidia were dislodged with a sterile bent glass rod and the suspension was filtered through a double layer of sterile cheesecloth. Conidia were counted using a hemacytometer or by directly counting the numbers in single drops of suspension on a glass slide.

Detached leaf assays. Potential fungicide suppression of disease after infection was investigated for azoxystrobin (Heritage 50% WG, Syngenta Crop Protection LLC, Basel, Switzerland), azoxystrobin plus benzovindiflupyr (Mural 30% and 15% WG, Syngenta Crop Protection LLC, Basel, Switzerland), kresoxim-methyl (Cygnus 50% WG, BASF Corp., Ludwigshafen, Germany), pyraclostrobin (Insignia 20% WG, BASF Corp., Ludwigshafen, Germany), pyraclostrobin plus fluxapyroxad (Orkestra Intrinsic 21.26% SC, BASF Corp., Ludwigshafen, Germany), propiconazole (Procon-Z 14.3% L, Loveland Products, Inc., Loveland, CO), tebuconazole (Torque 38.7% SC, Cleary Chemical LLC, Dayton, NJ), tetraconazole (Minerva 11.6% SC, SipcamAdvan, Durham, NC), thiophanate-methyl (3336

50% WP, Cleary Chemical LLC, Dayton, NJ) and triflumizole (Procure 480SC 42.14% WG, Chemtura Corp., Philadelphia, PA).

Fully-expanded mature leaves of greenhouse-grown boxwood plants 'Green Velvet' (*B. sinica* var. *insularis* × *B. sempervirens*) were collected and surface-sterilized in 0.525% sodium hypochlorite for 30 seconds, then rinsed in sterile distilled water and air dried to remove surface water. Leaves were placed with the abaxial surface up on 2.5 × 7.5 cm glass slides. There were ten replicate leaves per slide. Slides were placed on a sheet of paper over a 1.25 cm^2 wire mesh frame in a 13.25 L (45 × 30 × 15 cm) clear plastic bin with a tight sealing cover designed to hold the slides 3 cm (1.02 in) above the bottom of the bin which contained 1 cm (0.4 in) of water to maintain humidity in the bin.

Detached leaves were inoculated with a single drop containing 200 *C. pseudonaviculata* conidia in suspension on the abaxial surface. After 24 hours, the drop was shaken off and leaves were returned to the bins. Leaves were treated with the test fungicide by dipping leaves into water alone or 30 or 300 ppm concentrations of azoxystrobin, kresoxim-methyl, propiconazole, pyraclostrobin, tebuconazole, tetraconazole, thiophanate-methyl and triflumizole for 5 seconds prior to wicking any excess liquid by touching the tip of the leaf to a dry paper towel. Leaves were dipped in fungicide 24 hours prior to inoculation and allowed to dry 0, 24, 48, or 96 hours after inoculation. The leaves were scored as diseased or not and whether the pathogen sporulated or not (binomial data) 14 days after inoculation. The experiment was performed at least twice for each fungicide.

Additional experiments were conducted to evaluate the effects of 3, 30 or 300 ppm propiconazole applied as a pre-inoculation treatment for 24, 48 or 96 hours after inoculation as described above. The percent of the leaf area diseased was rated visually after 14 days. The experiment was conducted twice.

The binomial data consisting of diseased or healthy leaves and sporulation on lesions or not were each analyzed using an online Fisher's Exact Test calculator (http://vassarstats.net/tab2x2.html) for which significant differences from the non-fungicide treated control were determined by the total number of events for each fungicide/concentration/treatment time combination. Data for the percent of diseased leaf area were analyzed by the Kruskal-Wallis nonparametric one-way analysis of variance on ranks and means were separated by the Kruskal-Wallis Multiple-Comparison Z-Value Test (Dunn's Test).

Whole plant assays. *C. pseudonaviculata* conidia (1.1 × 10^5 per plant) were inoculated on to pre-moistened foliage of *Buxus sempervirens* L. 'True Dwarf'© as a directed spray using a hand-held hand-pump spray bottle on a coarse spray setting. Four sprays from each of four directions delivering 3 ml total volume were used to inoculate plants as uniformly as possible. Thiophanate-methyl was applied as Cleary 3336 at 90.0 g per 100 L (450 ppm ai) (0.75 lb per 100 gallons), pyraclostrobin applied as Insignia at 60.0 g per 100 L (120 ppm ai) (0.5 lb per 100 gallons) and propiconazole applied as ProCon-Z at 93.6 ml

per 100 L (146 ppm ai) (12 ounces per 100 gallons) to wet foliage to run-off. Fungicides were applied 48 hours after inoculation, symptoms recorded and re-isolation conducted 7 days after inoculation. The number of leaf and stem lesions per plant were counted. The number of dropped symptomatic leaves were added to the total lesions and categorized as 'diseased'. Re-isolation of the pathogen was attempted for three symptomatic leaves per plant for each of the six replicates of each treatment and the percent successful recovery recorded. The experiment was conducted twice and data were combined for analysis. Data were analyzed by one-way analysis of variance and means separated by Fisher's LSD Multiple Comparison Test (NCSS 12 Statistical Software (2018) NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss).

The effects of additional fungicides applied 48 hours after inoculation were evaluated in *Buxus sinica* var. *insularis* ×·*B. sempervirens* 'Green Mountain' and 'Green Mound'. Azoxystrobin plus benzovindiflupyr was applied as Mural at 45.0 g per 100 L (135 ppm azoxystrobin and 67.5 ppm benzovindiflupyr) (0.375 lb per 100 gallons), pyraclostrobin plus fluxapyroxad applied as Orkestra at 62.4 ml per 100 L (156 ppm each ai) (8 ounces per 100 gallons), propiconazole applied as ProCon-Z at 93.6 ml per 100 L (146 ppm ai) (12 ounces per 100 gallons), triflumizole applied as Procure at 62.4 ml per 100 L (156 ppm ai) (8 ounces per 100 gallons) and tebuconazole applied as Torque at 78.0 ml per 100 L (336 ppm ai) (10 ounces per 100 gallons). Two factorial experiments were conducted with the same treatments but different inoculum levels and analyzed separately. In Experiment 1, four replicate plants per cultivar per treatment were inoculated with 3 sprays of a 1×10^5 suspension of *C. pseudonaviculata* conidia (175,000 per plant) as described above, then treated with the appropriate fungicides 48 hours after inoculation. Disease evaluations consisting of the number of boxwood blight lesions per plant and number of leaves with lesions were conducted 14 days after inoculation. Experiment 2 consisted of four replicate plants per cultivar per treatment that were inoculated with 4 sprays of a 1×10^5 suspension of *C. pseudonaviculata* conidia (650,000 per plant); disease evaluations were conducted 14 days after inoculation.

Results and Discussion

A detached leaf assay with *Buxus* 'Green Velvet' leaves was used to evaluate the effects of fungicide active ingredient, application timing, and concentration on disease incidence and sporulation (Table 1). Pretreating detached leaves 24 hours before inoculation with 30 or 300 ppm of all of the fungicides tested significantly reduced disease compared to the untreated control leaves. These fungicides had previously been demonstrated to have efficacy against *C. pseudonaviculata* (LaMondia 2014, Maurer et al. 2017). Fungicides were also applied from 24 to 96 hours post-inoculation in the present study to assess potential curative post-infection efficacy against *C. pseudonaviculata*. We had previously observed that *C. pseudonaviculata* conidia used for inoculum and placed on water agar to test for viability typically germinate on

Table 1. The effects of selected fungicides, application rate and timing on *Calonectria pseudonaviculata* infection, lesion development and sporulation 14 days post-inoculation on detached leaves of *Buxus* 'Green Velvet'.

Fungicide	Rate (ppm a.i.)	Time (h)	Diseased (%)[z]	Sporulating (%)
Control	na	na	90	86
Propiconazole	30	0	5***	0****
		24	40***	20****
		48	55***	5****
		96	45***	0****
	300	0	5***	0****
		24	0***	0****
		48	20***	0****
		96	60***	0****
Azoxystrobin	30	0	40***	35****
		24	90	70**
		48	93	83
		96	100	100
	300	0	10***	20****
		24	77	60****
		48	80	63***
		96	100	100
Thiophanate-methyl	30	0	10***	5****
		24	55***	25****
		48	55***	25****
	300	0	0***	0****
		24	60***	23****
		48	70**	18****
Pyraclostrobin	30	0	3***	0****
		24	68***	62****
		48	78	58****
		96	70	70
	300	0	0***	0****
		24	54***	26****
		48	54***	26****
		96	80	20****
Tetraconazole	30	0	50***	20****
		24	80	0****
		48	90	0****
		96	100	0****
	300	0	0***	0****
		24	20***	0***
		48	63***	0***
		96	70	0***
Triflumizole	30	0	17***	0***
		24	53***	0***
		48	82	0***
	300	0	0***	0***
		24	53***	0***
		48	70***	0***
Tebuconazole	30	0	15***	10***
		24	75	50***
		48	80	20***
		96	95	45***
	300	0	5***	5***
		24	55***	0***
		48	80	0***
		96	85	0***
Kresoxim-methyl	30	0	5***	5***
		24	75	60*
		48	65**	35***
		96	90	65*
	300	0	5***	0***
		24	65***	0***
		48	75	5***
		96	100	0***

[z]The binomial data consisting of diseased or healthy leaves and leaves with pathogen sporulation or not were each analyzed using an online Fisher's Exact Test calculator (http://vassarstats.net/tab2x2.html) for which significant differences from the non-fungicide treated control were determined by the total number of events for each fungicide/concentration/treatment time combination. Note: Sporulation only occurred on diseased leaves. Significance is indicated as: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Table 2. The effect of propiconazole concentration and timing of application on the development of boxwood blight lesions caused by *Calonectria pseudonaviculata* 14 days post-inoculation on detached leaves of *Buxus* 'Green Velvet'.

Propiconazole rate (ppm) and timing	Diseased leaf area (%) [z]
Untreated Control	90.0 a
3 ppm pretreated	20.0 cd
3 ppm 24 h post-inoculation	70.0 ab
3 ppm 48 h post-inoculation	100.0 a
3 ppm 96 h post-inoculation	90.5 a
30 ppm pretreated	5.0 cd
30 ppm 24 h post-inoculation	58.3 b
30 ppm 48 h post-inoculation	57.2 b
30 ppm 96 h post-inoculation	44.8 bc
300 ppm pretreated	5.0 cd
300 ppm 24 h post-inoculation	1.5 d
300 ppm 48 h post-inoculation	20.2 cd
300 ppm 96 h post-inoculation	59.5 b
	$P < 0.0001$

[z]The percent of diseased leaf area data was analyzed by the Kruskal-Wallis nonparametric one-way analysis of variance on ranks and means were separated by the Kruskal-Wallis Multiple-Comparison Z-Value Test (Dunn's Test) at $P < 0.05$.

water agar and also on leaves within 3 hours of placement and that infection of boxwood leaves through stomates or through direct penetration occurs fairly quickly, within 24 hours.

The only fungicide that did not differ from the untreated control at either 30 or 300 ppm for any time period after inoculation was azoxystrobin, which also had low efficacy against *C. pseudonaviculata* in *in vitro* experiments (LaMondia 2014). At the 300-ppm fungicide rate, all other fungicides were significantly different from the untreated control for at least 24 hours after inoculation. However, from a practical rather than statistical perspective, only propiconazole reduced diseased leaf incidence to less than half that of the untreated control. We also observed that boxwood plants treated post-infection with the demethylation inhibitor (DMI) fungicides propiconazole, tetraconazole, and tebuconazole and 30 or 300 ppm triflumizole at 300 ppm did not exhibit pathogen sporulation over the 2 weeks of the experiment. Leaves treated with 300 ppm propiconazole were held an additional 2 weeks for a total of 4 weeks under humid moist chamber conditions and the pathogen did not sporulate over that time, with leaves eventually rotting from secondary fungal and bacterial causes (data not shown).

Boxwood blight disease severity as measured by percent of leaf area diseased was significantly reduced by pretreatment with as little as 3 ppm of propiconazole in comparison to the untreated control (Table 2). Treatment of leaves with 30 or 300 ppm up to 96 hours after inoculation with the pathogen resulted in significantly smaller boxwood blight lesions. In fact, lesion size was reduced by nearly 80% with 300 ppm as late as 48 hours after inoculation.

Detached leaves do not always respond in a similar manner as leaves on whole plants. Therefore, treatment of whole plants of the very susceptible boxwood *B. sempervirens* 'True Dwarf' with 450 ppm thiophanate-methyl, 120 ppm pyraclostrobin, or 150 ppm propiconazole

Table 3. The effect of fungicide application 48 hours after inoculation on boxwood blight symptoms (number of lesions and number of diseased leaves per plant) and *Calonectria pseudonaviculata* isolation frequency in *Buxus sempervirens* 'True Dwarf' [z].

Treatment and rate[y]	Lesions	Diseased	Percent Isolations[x]
Untreated Control	40.0 a	45.3 a	46.7 a
Thiophanate-methyl 450 ppm ai	47.8 a	51.0 a	66.7 a
Pyraclostrobin 120 ppm ai	37.0 ab	40.0 a	43.3 ab
Propiconazole 150 ppm ai	26.5 b	27.7 b	10.0 b
$P <$	0.015	0.01	0.03

[z]1.1×10^5 *Calonectria pseudonaviculata* conidia per plant applied to *Buxus sempervirens* 'True Dwarf" as a directed spray. Fungicides were applied after 48 hours and symptoms and re-isolation conducted 7 days after inoculation.

[y]Thiophanate-methyl was applied as Cleary's 3336 at 90.0 g per 100 L, pyraclostrobin applied as Insignia at 60.0 g per 100 L, and propiconazole applied as ProCon-Z at 93.6 ml per 100 L.

[x]Percent isolations with successful recovery of *C. pseudonaviculata* from 3 leaves per plant, for each of the 6 replicates.

48 hours after inoculation demonstrated that only propiconazole significantly reduced the number of diseased leaves, number of boxwood blight lesions, and the frequency of re-isolation of the pathogen from symptomatic tissue (Table 3).

Additional experiments with 'Green Mound' and 'Green Mountain' boxwood plants and a different group of fungicides previously shown to have efficacy against boxwood blight (LaMondia and Maurer 2017b, LaMondia and Maurer 2016, LaMondia 2016) applied 48 hours after inoculation demonstrated that propiconazole at 300 ppm, pyraclostrobin plus fluxapyroxad (150 ppm each) and azoxystrobin (135 ppm) plus benzovindiflupyr (67.5 ppm) consistently reduced the number of lesions that developed on plants (Table 4). Fungicide treatment data are reported averaged across cultivars and cultivar data is reported averaged across fungicide treatments for the factorial experiments. These were no significant interactions between treatments and cultivars. As we observed that pyraclostrobin and azoxystrobin did not have post-infection efficacy, fluxapyroxad and benzovindiflupyr were likely responsible for the observed post-infection efficacy. The low application rate of benzovindiflupyr (67.5 ppm) is worth noting. This experiment also confirmed that 'Green Mountain' was significantly more susceptible than 'Green Mound' boxwood (LaMondia and Shishkoff 2017).

Curative fungicide efficacy relies on the ability of an efficacious fungicide to be incorporated into the plant as either a true systemic in the vascular system or as a locally systemic or translaminar fungicide. Post-infection efficacy is also dependent on additional factors such as the host plant susceptibility, disease incidence and severity due to inoculum level and environmental conditions, the fungicide concentration present in plant tissues where the pathogen is present, and even the specific stage of the pathogen that is exposed to the active ingredient. The fungicides that demonstrated curative post-infection efficacy in these experiments are systemic active ingredients in FRAC group 3 (the DMI fungicide propiconazole) (FRAC 2013, Ziogas and Malandrakis 2015) or in group 7 with

J. Environ. Hort. 38(2):44–49. June 2020

47

Table 4. The effect of fungicide application 48 hours after inoculation on boxwood blight symptoms (number of lesions and number of diseased leaves per plant) in *Buxus* 'Green Mountain' and 'Green Mound'.

Treatment and rate[x]	Experiment 1[z]		Experiment 2[y]	
	Lesions	Leaves	Lesions	Leaves
Untreated Control	9.5 a	5.3 a	21.6 ab	12.3 a
Azoxystrobin 135 ppm plus				
Benzovindiflupyr 67.5 ppm	0.7 b	0.6 b	8.8 b	6.2 ab
Pyraclostrobin 150 ppm plus				
Fluxapyroxad 150 ppm	1.6 b	1.3 b	7.8 b	5.4 b
Propiconazole 300 ppm	0.7 b	0.6 b	9.1 b	4.6 b
Triflumizole 300 ppm	1.0 b	0.6 b	24.5 a	12.3 a
Tebuconazole 270 ppm	3.8 b	2.0 b	18.7 ab	7.5 ab
Cultivar				
Green Mound	1.0 b	0.5 b	8.4 b	5.3 b
Green Mountain	4.4 a	2.7 a	20.7 a	10.2 a
Source *P* <				
Treatment	0.04	0.4	0.04	0.04
Cultivar	0.03	0.01	0.002	0.01
Trt×Cv	0.05	0.04	ns	ns

[z]Experiment 1: 4 replicate plants per cultivar per treatment were inoculated with 3 sprays of a 1×10^5 suspension of *C. pseudonaviculata* conidia (175,000 per plant); disease evaluations conducted 14 days after inoculation.

[y]Experiment 2: 4 replicate plants per cultivar per treatment were inoculated with 4 sprays of a 1×10^5 suspension of *C. pseudonaviculata* conidia (650,000 per plant); disease evaluations conducted 14 days after inoculation.

[x]All fungicides were applied 48 hours after inoculation. Azoxystrobin plus benzovindiflupyr applied as Mural at 90.0 g per 100 L, pyraclostrobin plus fluxapyroxad applied as Orkestra at 62.4 ml per 100 L, propiconazole applied as ProCon-Z at 93.6 ml per 100 L, triflumizole applied as Procure at 62.4 ml per 100 L, and tebuconazole applied as Torque at 78.0 ml per 100 L.

translaminar activity (benzovindiflupyr, and fluxapyroxad) (Guicherit et al. 2014).

Our results are consistent with previously observed results from experiments using different pathogens and plant species. Mueller et al. (2004) demonstrated that propiconazole and azoxystrobin were effective against rust diseases preventatively and also had post-infection efficacy up to 7 days after inoculation for certain plants and that post-infection effects on the pathogen decreased as time from inoculation to fungicide application increased. Effective curative efficacy resulted in the cessation of lesion development. Propiconazole was also shown to suppress disease development during the latent period for *Tranzschelia discolor* infecting prune (*Prunus domestica* L.) (Kable et al. 1987). After symptoms developed, lesion size and pathogen sporulation was suppressed. Wilcox et al. (1990) documented that DMI fungicides, including propiconazole, had postinfection efficacy and antisporulant activity against *Monilinia fructicola* in sour cherry (*Prunus cerasus* L.) when applied within 48 hours of inoculation. The efficacy was affected by fungicide dose and the time after inoculation. Post-infection treatment with propiconazole also inhibited sporulation of *Cercospora* on peanut (*Arachis hypogaea* L.) (Dahmen and Staub 1992).

Fungicides may have different efficacy against isolates with different levels of virulence (Maloney et al. 2018) or different stages of pathogenic fungi. This was demonstrat-

ed for *C. pseudonaviculata* by LaMondia and Maurer (2017a) as fungicides at concentrations effective against conidia and hyphae did not necessarily affect the viability of microsclerotia. Direct exposure of microsclerotia to thiophanate-methyl did not affect viability at concentrations and exposure times up to 316 ppm for 96 hours. Kresoxim-methyl did not affect viability but reduced the growth rate of hyphae from microsclerotia by 50%. Propiconazole at 100 ppm for more than 48 hours or 316 ppm for more than 24 hours reduced viability of microsclerotia by 90 to 100%. The melanized hyphae and microsclerotia survived fungicide concentrations and exposure times that were lethal to hyphae and conidia. Coincidentally, we have also observed that melanized hyphae of *C. pseudonaviculata* form in culture and in infected boxwood leaves at about the 48-hour time period when curative efficacy of propiconazole is reduced. In addition, the 48-hour limit of post-infection efficacy may be related to the relatively short incubation/latent period prior to lesion development, similar to the 48-hour curative efficacy results obtained with *M. fructicola* blossom blight, which has a similar incubation/latent period (Wilcox 1990).

While pretreatment with fungicides is most efficacious against the boxwood blight pathogen, infectious periods may occur almost continuously over a long wet period and environmental conditions may limit the ability to apply protectant fungicides prior to infection. In those instances, the reduced incidence, lesion size, and inhibition of pathogen sporulation in infected leaves demonstrated by post-infection treatment with propiconazole, fluxapyroxad and benzovindiflupyr fungicides demonstrated in the current experiments should combine to slow the development of disease and significantly reduce epidemic development, aiding boxwood blight management. As curative fungicide treatments are not only less effective than protectant applications, but also increase the risk of fungicide resistance development, it will be especially important to rotate or mix fungicides to manage this risk (Gehesquière et al. 2015, Gisi et al. 2000, Maurer et al. 2017).

Literature Cited

AmericanHort. 2017. Boxwood Blight Best Management Practices. Boxwood Blight Working Group Horticultural Research Institute National Plant Board. (https://hortknowledgecenter.org/getattachment/7068c31f-fee0-4541-bf4a-ac89350be97b/BoxwoodBlightBMPs2017.pdf?lang=en-US. Accessed February 28, 2019.

Avenot, H. F., C. King, T. Edwards, A. Baudoin, and C. X. Hong. 2017. Effects of inoculum dose, temperature, cultivar, and interrupted leaf wetness period on infection of boxwood by *Calonectria pseudonaviculata*. Plant Dis 101:866–873.

Brand, T. 2006. In vitro-Wirkung fungizider Wirkstoffe auf Konidienkeimung und Myzelwachstum von Cylindrocladium buxicola. Nachrichtenbl. Deut. Pflanzenschutzd. (Berlin) 58:117–121.

Calabro, J. 2018. Reclaiming boxwood from boxwood blight. Nursery Management. http://www.nurserymag.com/article/reclaiming-boxwood-from-blight-calabro/. Accessed 3 January 2019.

Coop, L. 2014. The Best/Worst Time for Pathogens. New, weather-driven risk models indicate when box blight and apple scab are more likely to spread. Growing Knowledge Article in Digger Magazine Pub. by The Ore. Assoc. of Nurseries. Oct. 2014.

Coop, L. 2013. Brief Documentation for Boxwood Blight Infection Risk Model. Online publication. Oregon State University. http://uspest.org/wea/Boxwood_blight_risk_model_summary.pdf. Accessed February 28, 2019.

Crous, P. W., J. Z. Groenewald, and C. F. Hill. 2002. *Cylindrocladium pseudonaviculata* sp. nov. from New Zealand, and new *Cylindrocladium* records from Vietnam. Sydowia. 54:23–34.

Crous, P. W., J. Z. Groenewald, J. M. Risède, P. Simoneau, and N. L. Hywel-Jones. 2004. *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. Stud. Mycol. 50:415–430.

Dahmen, H. and T. Staub. 1992. Protective, curative and eradicant activity of difenconazole against *Venturia inequalis*, *Cercospora arachidicola*, and *Alternaria solani*. Plant Dis. 76:774–777.

Dario, I. 2010. Curative and Eradicative Effects of Fungicides, Fungicides, Odile Carisse (Ed.), ISBN: 978-953-307-266-1, InTech, Available from: http://www.intechopen.com/books/fungicides/curative-and-eradicative-effects-of-fungicides.

Fungicide Resistance Action Committee (FRAC). 2013. List of plant pathogenic organisms resistant to disease control agents. Online publication. http://www.frac.info/. Accessed May 26, 2016.

Gehesquière, B., J. A. Crouch, R. E. Marra, K. Van Poucke, F. Rys, M. Maes, B. Gobin, M. Höfte, and K. Heungens. 2015. Characterization and taxonomic reassessment of the box blight pathogen *Calonectria pseudonaviculata*, introducing *Calonectria henricotiae* sp. nov. Plant Pathol. 65:37–52.

Gisi, U., K. M. Chin, G. Knapova, R. Küng Färber, U. Mohr, S. Parisi, H. Sierotzki, and U. Steinfeld. 2000. Recent developments in elucidating modes of resistance to phenylamide, DMI and strobilurin fungicides. Crop Prot. 19:863–872.

Guicherit, E., D. Bartlett, S. M. Dale, H. U. Haas, G. Scalliet, and H. Walter, 2014. "Solatenol-the second generation benzonorbornene SDHI carboxamide with outstanding performance against key crop diseases," in *Modern Fungicides and Antifungal Compounds VII*, eds H. W. Dehne, H. B. Deising, B. Fraaije, U. Gisi, D. Hermann, A. Mehl, et al. (Braunschweig: Deutsche Phytomedizinische Gesellschaft e.V. Selbstverlag), 67–72.

Henricot, B., C. Gorton, G. Denton, and J. Denton. 2008. Studies on the control of *Cylindrocladium buxicola* using fungicides and host resistance. Plant Dis. 92:1273–1279.

Henricot, B., and E. Wedgwood. 2013. Evaluation of foliar fungicide sprays for the control of boxwood blight, caused by the fungus *Cylindrocladium buxicola*. Plant Health Prog. doi: 10.1094/PHP-2013-1024-01-RS.

Ivors, K. L., L. W. Lacey, D. C. Milks, S. M. Douglas, M. K. Inman, R. E. Marra, and J. A. LaMondia. 2012. First report of boxwood blight caused by *Cylindrocladium pseudonaviculatum* in the United States. Plant Dis. 96:1070.

Kable, P. F., B. J. Keen and R. W. Bambach. 1987. Evaluation of fungicides for curative activity against *Tranzschelia discolor*, cause of the rust disease of French prune (*Prunus domestica* L.). Aust .J. Agric. Res. 38:577–585.

LaMondia, J. A. 2016. Evaluation of fungicides for management of boxwood blight, 2014. Plant Dis. Manag. Rep. 10:O7010. http://www.plantmanagementnetwork.org/pub/trial/pdmr/reports/2016/OT010.pdf. Accessed May 26, 2016.

LaMondia, J. A. 2015. Management of *Calonectria pseudonaviculata* in boxwood with fungicides and less susceptible host species and varieties. Plant Dis. 99:363–369.

LaMondia, J. A. 2014. Fungicide efficacy against *Calonectria pseudonaviculata*, causal agent of boxwood blight. Plant Dis. 98:99–102.

LaMondia J. A. and K. Maurer. 2017a. *Calonectria pseudonaviculata* microsclerotia viability after exposure to fungicides. Phytopathol. 107:S5 104.

LaMondia, J. A. and K. Maurer. 2017b. Evaluation of fungicides for management of boxwood blight, 2016. Plant Dis. Manag. Rep. 11:OT16 http://www.plantmanagementnetwork.org/pub/trial/pdmr/reports/2017/OT016.pdf. Accessed May 26, 2016.

LaMondia, J. A. and K. Maurer. 2016. Evaluation of fungicides for management of boxwood blight, 2015. Plant Dis. Manag. Rep. 10:OT11 http://www.plantmanagementnetwork.org/pub/trial/pdmr/reports/2016/OT011.pdf. Accessed May 26, 2016.

LaMondia J. A. and N. Shishkoff. 2017. Susceptibility of boxwood accessions from the National Boxwood Collection to boxwood blight and potential for differences between *Calonectria pseudonaviculata* and *C. henricotiae*. HortScience 52:873–879.

LeBlanc, N., C. Salgado-Salazar, and J. Crouch. 2018. Boxwood blight: an ongoing threat to ornamental and native boxwood. Appl. Microbiol. Biotechnol. (2018) 102:4371–4380 https://doi.org/10.1007/s00253-018-8936-2. Accessed May 26, 2016.

Maloney, K.; N. D. Havis, D. E. L. Cooke, G. Loake, and R. A. Bain. 2018. Development of a decision aid to support the use of curative late blight fungicides *In:* Proceedings Crop Production in Northern Britain 2018. 27-28 February, Dundee, UK. p. 197–202.

Maurer, K. A., R. S. Cowles and J. A. LaMondia. 2017. Sensitivity of *Calonectria pseudonaviculata*, the pathogen of boxwood blight, to strobilurin and demethylation inhibitor fungicides. J. Environ. Hort. 35:138–145.

Mueller, D. S., S. N. Jeffers, and J. W. Buck. 2004. Effect of timing of fungicide applications on development of rusts on daylily, geranium and sunflower. Plant Dis. 88:657–661.

Wilcox, W. F. 1990. Postinfection and antisporulant activity of selected fungicides in control of blossom blight of sour cherry caused by *Monilinia fructicola*. Plant Dis. 74:808–811.

Ziogas, B. N., and A. A. Malandrakis. 2015. Sterol Biosynthesis Inhibitors: C14 Demethylation. In: Fungicide Resistance in Plant Pathogens. Ishii, H., and Hollomon, D.W. eds. Springer, Japan, p. 199–216.

J. Environ. Hort. 38(2):44–49. June 2020

49