Management of *Calonectria pseudonaviculata* in Boxwood with Fungicides and Less Susceptible Host Species and Varieties

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Abstract

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Calonectria pseudonaviculata causes leaf and stem lesions resulting in defoliation and dieback of boxwood. Trials were conducted to evaluate fungicide management of boxwood blight under greenhouse and container nursery conditions in Connecticut using fungicides previously determined to have in vitro activity against conidial germination or mycelial growth. Plants of different boxwood cultivars were inoculated 48 h after fungicide application. Disease progression was monitored over 6 weeks and progressed from leaf and stem lesions to defoliation. The level of disease control achieved by fungicides was generally good, with the most efficacious treatments averaging from 95 to nearly 100% control. Products containing propiconazole, myclobutanil, thiophanate-methyl, fludioxonil, pyraclostrobin, kresoxim-methyl, and chlorothalonil had significant efficacy. The combination of systemic plus protectant fungicides in a single application resulted in superior

disease control compared to the use of a systemic fungicide. There were no differences between the fungicide treatments that included thiophanate-methyl and those that included propiconazole as the systemic fungicide. Korean and 'Winter Gem' (*Buxus sinica* var. *insularis*) were the least susceptible of the cultivars evaluated, common boxwood (*B. sempervirens*) and True Dwarf (*B. sempervirens* 'Suffruticosa') were the most susceptible, and 'Green Mountain' (*B. sinica* var. *insularis* × *B. sempervirens* Suffruticosa) and 'Green Velvet' (*B. sinica* var. *insularis* × *B. sempervirens* Suffruticosa) were intermediate. These results suggest that *B. sinica* var. *insularis* may have some level of resistance to boxwood blight. Management of boxwood blight will rely on integrated best management practices that include inspection of incoming plant material, sanitation, cultural controls including use of cultivars tolerant to infection, and fungicide application.

Boxwood blight, caused by *Calonectria pseudonaviculata* (Crous & al.) L. Lombard & al. (syn ≡ *Cylindrocladium pseudonaviculatum* Crous, J.Z. Groenew. & C.F. Hill, = *Cylindrocladium buxicola* Henricot) is a recent introduction to North America, first recognized in the fall of 2011 in Connecticut and North Carolina (14). Since that time it has been reported from Virginia (3), Alabama (7), Massachusetts, Ohio, Oregon, Rhode Island (12), Delaware, Maryland, New Jersey, and New York (18), and from British Columbia (5), Ontario, and Quebec (12) in Canada. The pathogen causes characteristic leaf and stem lesions and can quickly defoliate plants under conducive environmental conditions (4,9). It has resulted in serious losses in field-grown and container nurseries, more than \$3 million in the first year after detection in Connecticut alone (15), and has also affected valuable landscape plantings. *C. pseudonaviculata* also infects *Sarcococca* spp. (8) as well as *Pachysandra terminalis* (17) and *P. procumbens* (16) in the *Buxaceae*.

Management of boxwood blight will require integrated management practices including effective fungicide management tactics. However, fungicide efficacy data have been limited to date. Published in vitro studies from the United Kingdom concluded that azoxystrobin, chlorothalonil, kresoxim-methyl, mancozeb, boscalid + pyraclostrobin, epoxiconazole + pyraclostrobin, and epoxiconazole + pyraclostrobin + kresoxim-methyl had activity against conidial germination, and that prochloraz, kresoxim-methyl, carbendazim, and epoxiconazole + pyraclostrobin + kresoxim-methyl limited mycelial growth (11). Brand (2) reported that in vitro conidial germination was inhibited by tolylfluanid, mancozeb, chlorothalonil, and fludioxonil + cyprodinil, and that prochloraz, pro-

piconazole, thiophanate-methyl, and carbendazim + flusilazole reduced mycelial growth. LaMondia (15) examined 20 different fungicide active ingredients from 13 different Fungicide Resistance Action Committee (FRAC) groups in in vitro studies and concluded that the strobilurins (FRAC group 11) pyraclostrobin, trifloxystrobin, and kresoxim-methyl as well as fludioxonil, mancozeb, chlorothalonil, and boscalid inhibited *C. pseudonaviculata* conidial germination. The demethylation inhibitor (DMI) fungicides (FRAC group 3) had EC₈₅ values (µg a.i./ml concentration that suppressed fungal growth to 15% of that on nonamended media) of 1.2 µg a.i./ml or less. Propiconazole and triflumizole were the most effective, with EC₈₅ values in the range of 0.28 to 0.65 µg a.i./ml. Thiophanate-methyl (FRAC group 1), fludioxonil (FRAC group 12), the strobilurins pyraclostrobin, trifloxystrobin, and kresoxim-methyl, and the broad-spectrum fungicides mancozeb (FRAC group M-1) and chlorothalonil (M-5) also had efficacy against mycelial growth.

The data from in vitro studies (2,10,15) were important for selection of fungicides for evaluation under field conditions. Henricot and Wedgwood (11) recently reported the first data describing management of boxwood blight under field conditions in the UK. Epoxiconazole plus kresoxim-methyl plus pyraclostrobin (Opponent) was the most effective fungicide product evaluated. Combination treatments of prochloraz, epoxiconazole, chlorothalonil, boscalid, pyraclostrobin, and kresoxim-methyl also resulted in significant control of the disease. Not all of the fungicide active ingredients tested in the in vitro studies were registered for use on boxwood or on ornamentals, and a number of effective fungicides in the UK and Germany, including prochloraz, carbendazim, epoxiconazole, and tolylfluanid, are not currently registered for use in the United States. Ivors et al. (13) evaluated several commercially available fungicides and premixes and determined that fungicides in FRAC groups M5, M5 + 1, M5 + 3, M5 + 11, 1, 2, 3, 7 + 11, 9 + 11, 11, 12, and 19 all significantly reduced boxwood blight, although not all fungicides in the same FRAC group were equally effective.

The objective of this study was to determine the efficacy of fungicides, alone or in combination, against *C. pseudonaviculata* in-

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fecting *Buxus* species under field and greenhouse conditions. These fungicides had been demonstrated to have activity against conidial germination and mycelial growth of the pathogen in in vitro assays in Connecticut and elsewhere. Some of these fungicides are not currently registered for use on boxwood in the United States. Also, limited information on cultivar susceptibility was developed in experiments utilizing multiple species or cultivars in the same experiments.

Materials and Methods

In all experiments, plants were moved to separate locations for fungicide applications to avoid fungicide drift. After 2 days, plants were arbitrarily arranged on greenhouse benches or in the nursery area and spaced so that plants were about 1 cm apart for inoculation with the pathogen. The anamorph of *C. pseudonaviculata* was isolated from leaf and stem lesions of *Buxus sempervirens* L. 'Suffruticosa' grown in Connecticut, and single-spored isolates Cps-CT-L1 (ATCC MYA-4891) (isolated from leaf tissue) and Cps-CT-S1 (ATCC MYA-4890) (from a stem lesion) were cultured on half-strength potato dextrose agar (½PDA). To prepare conidial suspensions for inoculum, approximately 2-week-old cultures were scraped under sterile conditions to remove aerial mycelium above the agar surface and placed under near-UV light for an additional 1 to 2 weeks to encourage sporulation. Cultures were flooded with sterile distilled water to which Tween 20 had been added (1 drop per 200 ml), and conidia were dislodged with a sterile bent glass rod. The suspension was filtered through a double layer of sterile cheesecloth, and numbers of conidia were counted using a hemacytometer. Conidia were inoculated to premoistened plant foliage 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. White polyethylene was suspended over inoculated plants for 24 h

to provide humid conditions suitable for infection. Plants were not watered after fungicide application until at least 24 h after inoculation. After the polyethylene was removed, plants were overhead watered by hand twice per day in the greenhouse or irrigated with overhead sprinklers in the outdoor container nursery production area two times per day, morning and afternoon, delivering approximately 1.7 cm of water daily. Plants grown in no. 3 containers had been fertilized with 5.3 kg per m³ slow release 15-9-12 fertilizer (8 to 9 month longevity). Two-year-old rooted boxwood cuttings (liners) grown in Sunshine no. 3 plant growth media (SunGro Horticulture, Bellevue WA) in 10-cm-diameter, 450-ml containers were fertilized weekly to saturation with 150 ppm nitrogen water soluble fertilizer (8% ammonia nitrogen, 12% nitrate nitrogen, 10% available phosphate P₂O₅, and 20% soluble potash K₂O). Liners are rooted cuttings used to produce larger plants for sale, grown either in pots or in the field.

Experiment 1: Spring 2012. Five- to six-year-old Japanese boxwood 'Green Beauty' *Buxus microphylla* Sieb. & Zucc. var. *japonica* (Mull.-Arg.) Rehder & E.H. Wilson plants grown in no. 3 containers (25-cm top diameter, 10-liter capacity) in a greenhouse were treated with selected fungicides. Fungicides included kresoxim-methyl (Cygnus 50 WG, BASF Corp., Research Triangle Park, NC), boscalid plus pyraclostrobin (Pageant WG, BASF Corp.), famoxadone plus cymoxanil (Palladium WG, Syngenta Crop Protection, LLC, Greensboro, NC), propiconazole (Procon Z 14.3L, Loveland Products, Inc., Greeley, CO), or water alone as a control. Treatments were applied to foliage until run-off using a backpack sprayer (Solo, Newport News, VA). Treatments were applied to the same plants three times at 14-day intervals. There were four replicate plants for each treatment. Two days after each fungicide application, all plants were inoculated with 1.25×10^6 , 5.0×10^5 , and 3.0×10^5 conidia of *C. pseudonaviculata* in 3-ml-volume per plant, respectively. Numbers of characteristic box-

Table 1. Efficacy of fungicides applied 3 times at 2-week intervals against boxwood blight caused by *Calonectria pseudonaviculata* in 5- to 6-year-old *Buxus sinica* var. *insularis* 'Green Beauty' grown in no. 3 containers in a greenhouse, Spring 2012

Fungicide (rate)	Active ingredient(s)	Lesions per plant ^y		
		Evaluation 1	Evaluation 2	Evaluation 3
Untreated control	None	16.8 a ^z	80.8 a	79.3 a
Cygnus 50 WG (25 g/100 liters)	Kresoxim-methyl	0.8 b	1.0 b	1.3 b
Pageant WG (90 g/100 liters)	Boscalid plus pyraclostrobin	0.3 b	2.3 b	4.3 b
Palladium WG (30 g/100 liters)	Fludioxonil plus cyprodinil	0.0 b	0.3 b	1.0 b
Procon-Z 14.3 L (60 ml/100 liters)	Propiconazole	0.8 b	2.5 b	4.0 b
	(P=)	0.01	0.02	0.02

^y Evaluations 1 to 3 were performed 14 days after the first, second, and third fungicide applications, respectively.

^z Data were analyzed by the nonparametric Kruskal-Wallis one-way ANOVA on ranks, and means were separated by the Kruskal-Wallis multiple comparison Z-value test. Means within columns followed by the same letter are not significantly different ($P = 0.05$).

Table 2. Efficacy of fungicides applied 3 times at 2-week intervals against boxwood blight caused by *Calonectria pseudonaviculata* in 2-year-old rooted cuttings of *Buxus sempervirens*

Fungicide (rate)	Active ingredient(s)	Lesions per plant ^y		
		Evaluation 1	Evaluation 2	Evaluation 3
Untreated control	None	98.3 a ^z	72.5 a	29.8 a
Pageant WG (90 g/100 liters)	Boscalid plus pyraclostrobin	5.3 ab	5.0 ab	5.8 ab
Palladium WG (30 g/100 liters)	Fludioxonil plus cyprodinil	1.8 b	2.3 b	3.8 ab
Cygnus 50 WG (25 g/100 liters)	Kresoxim-methyl	0.8 b	1.0 b	0.3 b
Procon-Z 14.3 L (60 ml/100 liters)	Propiconazole	0.8 b	3.0 ab	3.0 ab
Procon Z (60 ml/100 liters) plus Insignia 20 WG (60 g/100 liters)	Propiconazole plus pyraclostrobin	0.2 b	0.8 b	0.5 b
Rally 40 WP (50 g/100 liters)	Myclobutanil	1.4 b	2.4 b	1.2 b
Rally (50 g/100 liters) plus Insignia (60 g/100 liters)	Myclobutanil plus pyraclostrobin	1.2 b	2.8 b	1.0 b
Truban 25 EC (30 ml/100 liters)	Etridiazole	1.2 b	1.6 b	1.4 b
Truban 25 EC (30 ml/100 liters) plus Insignia (60 g/100 liters)	Etridiazole plus pyraclostrobin	5.8 ab	3.4 ab	2.0 b
Daconil (170 ml/100 liters)	Chlorothalonil	2.8 ab	1.2 b	0.4 b
	(P=)	0.002	0.04	0.01

^y Evaluations 1 to 3 were performed 14 days after the first, second, and third fungicide applications, respectively.

^z Data were analyzed by the nonparametric Kruskal-Wallis one-way ANOVA on ranks, and means were separated by the Kruskal-Wallis multiple comparison Z-value test. Means within columns followed by the same letter are not significantly different ($P = 0.05$).

wood blight leaf and stem lesions per plant were counted the day before the second and third fungicide applications and 2 weeks after the third fungicide application. Data were analyzed by the nonparametric Kruskal-Wallis one-way ANOVA on ranks. Means were separated by the Kruskal-Wallis multiple comparison Z-value test.

Experiment 2: Spring 2012. Two-year-old rooted cuttings of the hybrid boxwood cultivar ‘Green Velvet’ *Buxus sinica* (Rehder & E.H. Wilson) M. Cheng var. *insularis* × *B. sempervirens* grown in 10-cm-diameter, 450-ml containers were treated three times with fungicides or water alone at 14-day intervals. Fungicide treatments were: kresoxim-methyl; boscalid plus pyraclostrobin; famoxadone plus cymoxanil; propiconazole; propiconazole plus pyraclostrobin (Insignia 20% WG, BASF Corp.); myclobutanil (Rally 40WSP, Dow AgroSciences LLC, Indianapolis, IN); myclobutanil plus pyraclostrobin; etridiazole (Truban 25% EC, Scotts-Sierra Crop Protection Co., Marysville, OH); etridiazole plus pyraclostrobin; and chlorothalonil (Daconil 54% F Weatherstik, Syngenta Crop Protection, LLC); or water alone as a control. Treatments were applied to foliage until run-off using a backpack sprayer. There were five replicate plants for each treatment. Two days after each of the fungicide applications, all plants were inoculated with 2.5×10^5 , 5.0×10^5 , and 3.0×10^5 conidia of *C. pseudonaviculata* in 3-ml-volume per plant, respectively. Numbers of boxwood blight leaf and stem lesions per plant were counted 13 to 14 days after each of the fungicide applications. Data were analyzed by the nonparametric Kruskal-Wallis one-way ANOVA on ranks. Means were separated by the Kruskal-Wallis multiple comparison Z-value test.

Experiment 3: Fall 2012. Five- to six-year-old hybrid ‘Green Velvet’ *B. sinica* var. *insularis* × *B. sempervirens* in no. 3 containers grown in the container nursery area at the CAES Valley Laboratory Research Farm were treated three times with fungicides or water alone at 14-day intervals. Based on previous experiments, this research was conducted to investigate whether systemic fungicides (defined as xylem-mobile) in combination with protectant or locally systemic translaminar fungicides were more effective than systemic fungicides alone. Ten treatments included water only or three applications of the systemic fungicides thiophanate-methyl (3336 50% WP, Cleary Chemicals LLC, Dayton, NJ) or propiconazole alone or each in combination with the locally systemic/translaminar fungicide pyraclostrobin and protectant fungicides, fludioxonil (Medallion 50% WG, Syngenta Crop Protection, LLC), or chlorothalonil. An additional treatment regime consisted of thiophanate-methyl plus chlorothalonil followed by propiconazole plus pyraclostrobin and finally the locally systemic/translaminar fungicide kresoxim-methyl plus fludioxonil. Two days after the first fungicide spray, foliage was inoculated with 1.0×10^6 conidia per plant in 3 ml per plant. Plants were only inoculated once. Numbers of boxwood blight leaf and stem lesions per plant were counted 2 weeks after the third fungicide application. Data were tested for normality and analyzed by Analysis of Variance, and means were separated by single degree-of-freedom linear contrasts.

Experiment 4: Fall 2012. Two-year-old rooted cuttings of common boxwood (*B. sempervirens*), English boxwood (*B. sempervirens* ‘Suffruticosa’ True Dwarf), Buxus ‘Green Mountain’ (*B. sempervirens* ‘Suffruticosa’ × *B. sinica* var. *insularis*), Korean boxwood (*B. sinica* var. *insularis*), Buxus ‘Winter Gem’ (*B. sinica* var. *insularis*), and Buxus ‘Green Velvet’ (*B. sinica* var. *insularis* × *B. sempervirens*) grown in 10-cm-diameter, 450-ml containers were treated three times with fungicides using the same fungicide treatments as in experiment 3 at 14-day intervals. Two days after the first fungicide spray, foliage was inoculated with 2.0×10^7 conidia per plant in 3 ml per plant. Plants were grown in the CAES container nursery area and irrigated as described above. There were five replicate plants of each cultivar for each of the 10 fungicide treatments. Two weeks after the third fungicide application, the numbers of typical boxwood blight leaf and stem lesions and numbers of dropped leaves per plant were counted. Data were tested for

normality and analyzed by Analysis of Variance, and means were separated by linear contrasts.

Experiment 5: Winter 2012–2013. Two-year-old rooted cuttings of the same *Buxus* cultivars as described in experiment 4 were treated with the same fungicides at the same intervals. Five replicate plants of each cultivar for each of the 10 fungicide treatments were grown in a greenhouse throughout the experiment and evaluated 2 weeks after the third fungicide application for numbers of boxwood blight leaf and stem lesions and numbers of dropped leaves per plant. Two days after the first fungicide spray, foliage was inoculated with 1.3×10^5 conidia per plant in 3 ml per plant. Data were tested for normality and analyzed by Analysis of Variance, and means were separated by linear contrasts.

Results

Experiment 1: Spring 2012. Each of the fungicide treatments evaluated resulted in significantly fewer boxwood blight lesions than the untreated control for each of the three evaluation times (2, 4, and 6 weeks after the first fungicide application) (Table 1). There were no significant differences between fungicide treatments. The level of disease control was good, averaging from 95 to 100% control. No phytotoxicity was observed, although it was noted that new growth on plants that were treated with propiconazole had shorter internodes by the end of the experiment. Plants

Table 3.1. Efficacy of fungicides applied 3 times at 2-week intervals against boxwood blight caused by *Calonectria pseudonaviculata* in 5- to 6-year-old *Buxus sempervirens* × *B. sinica* var. *insularis* ‘Green Velvet’ grown in no. 3 containers in a container nursery area, Fall 2012

Systemic fungicide a.i. ^x	Protectant (or local systemic) fungicide a.i.	Applications	Lesions per plant ^y
None	None	NA	123.1 ^z
Thiophanate-methyl	None	3	89.4
Thiophanate-methyl	Pyraclostrobin	3	53.6
Thiophanate-methyl	Fludioxonil	3	14.2
Thiophanate-methyl	Chlorothalonil	3	19.1
Propiconazole	None	3	109.8
Propiconazole	Pyraclostrobin	3	68.4
Propiconazole	Fludioxonil	3	24.9
Propiconazole	Chlorothalonil	3	4.9
Thiophanate-methyl	Pyraclostrobin	1	3.3
Propiconazole	Fludioxonil	1	
Kresoxim-methyl	Chlorothalonil	1	

^x Ten treatments included water only or three applications of the systemic fungicides thiophanate-methyl (3336 50% WP, 90 g/100 liters) or propiconazole alone (ProConZ, 14.3L, 90 ml/100 liters) or each in combination with the locally systemic/translaminar fungicide pyraclostrobin (Insignia 20% WG, 60 g/100 liters) and protectant fungicides, fludioxonil (Medallion 50% WG, 23 g/100 liters), or chlorothalonil (Daconil 54%F Weatherstik, 170 ml/100 liters). An additional treatment regime consisted of thiophanate-methyl plus chlorothalonil followed by propiconazole plus pyraclostrobin and finally the locally systemic/translaminar fungicide kresoxim-methyl (Cygnus 50 WG, 25 g/100 liters) plus fludioxonil.

^y Leaf and stem lesions per plant were counted at the end of the experiment 2 weeks after the third spray.

^z Data were analyzed by ANOVA and means were separated by single degree-of-freedom linear contrasts.

Table 3.2. Significance of fungicides applied 3 times at 2-week intervals against boxwood blight lesions caused by *Calonectria pseudonaviculata* in 5- to 6-year-old *Buxus sempervirens* × *B. sinica* var. *insularis* ‘Green Velvet’ grown in no. 3 containers in a container nursery area, Fall 2012, as determined by linear contrasts

Linear contrasts	(P=)
Fungicide versus none	0.001
Systemic fungicides alone vs. none	0.05
Thiophanate-methyl vs. propiconazole	ns
Systemic alone vs. systemic plus protectant	0.001
Rotation (3 different treatments) vs. all other systemic plus protectants	0.01

grown in spring experiments had newly produced leaves in addition to fully expanded hardened-off leaves.

Experiment 2: Spring 2012. The highest number of boxwood blight lesions on liners occurred after the first inoculation in the untreated control plants, and while they still remained relatively high, lesion numbers decreased over time as lesions expanded and heavily diseased leaves dropped (Table 2). The proportion of stem lesions increased relative to leaf lesions over time (data not shown). The fungicides most effective at reducing leaf and stem lesions over the course of the experiment were propiconazole plus pyraclostrobin, kresoxim-methyl, chlorothalonil, and myclobutanil or myclobutanil plus pyraclostrobin. Etridiazole resulted in similar lesion numbers, but etridiazole plus pyraclostrobin did not, so results were not consistent.

Experiment 3: Fall 2012. All of the fungicides evaluated, whether the systemics alone or the combination of systemic plus locally systemic/translaminar or protectant fungicides, resulted in

significantly fewer leaf and stem lesions than the untreated control at the end of the experiment 2 weeks after the third and final spray (Table 3.1). There were no differences between the fungicide treatments that included thiophanate-methyl and those that included propiconazole as the systemic fungicide. However, the combination of systemic plus locally systemic or protectant fungicides resulted in superior disease control compared to the use of any systemic fungicide used alone (Table 3.2). Finally, using linear contrasts of the means, the treatment that consisted of a single spray each of three different systemic or local systemic fungicides combined with one of three different protectants was significantly better than all other systemic plus protectant combinations evaluated as a group for statistical analysis.

Experiment 4: Fall 2012. All treatments of systemic alone or systemic plus protectant fungicides applied to six cultivars of boxwood resulted in fewer leaf and stem lesions and fewer dropped diseased leaves than untreated controls (Tables 4.1 and 4.2). There

Table 4.1. Efficacy of fungicides applied at 2-week intervals against boxwood blight caused by *Calonectria pseudonaviculata* in six *Buxus* cultivars or species grown as 2-year-old rooted cuttings in a container nursery area, Fall 2012

Systemic fungicide a.i. ^x	Protectant fungicide a.i.	Applications	Lesions ^y	Dropped leaves
None	None	NA	22.4 ^z	28.3
Thiophanate-methyl	None	3	1.3	3.5
Thiophanate-methyl	Pyraclostrobin	3	0.6	1.7
Thiophanate-methyl	Fludioxonil	3	0.7	2.0
Thiophanate-methyl	Chlorothalonil	3	0.5	1.7
Propiconazole	None	3	1.4	10.0
Propiconazole	Pyraclostrobin	3	0.4	3.5
Propiconazole	Fludioxonil	3	0.1	2.2
Propiconazole	Chlorothalonil	3	0.6	1.6
Thiophanate-methyl	Pyraclostrobin	1	0.6	2.0
Propiconazole	Fludioxonil	1		
Kresoxim-methyl	Chlorothalonil	1		

^x Ten treatments included water only or three applications of the systemic fungicides thiophanate-methyl (3336 50% WP, 90 g/100 liters) or propiconazole alone (ProConZ, 14.3L, 90 ml/100 liters) or each in combination with the locally systemic/translaminar fungicide pyraclostrobin (Insignia 20% WG, 60 g/100 liters) and protectant fungicides, fludioxonil (Medallion 50% WG, 23 g/100 liters), or chlorothalonil (Daconil 54%F Weatherstik, 170 ml/100 liters). An additional treatment regime consisted of thiophanate-methyl plus chlorothalonil followed by propiconazole plus pyraclostrobin and finally the locally systemic/translaminar fungicide kresoxim-methyl (Cygnus 50 WG, 25 g/100 liters) plus fludioxonil.

^y Lesions and dropped diseased leaves per plant were counted at the end of the experiment 2 weeks after the third spray.

^z Data were analyzed by ANOVA and means were separated by single degree-of-freedom linear contrasts.

Table 4.2. Significance of fungicides applied at 2-week intervals on boxwood blight caused by *Calonectria pseudonaviculata* in six *Buxus* cultivars or species grown as 2-year-old rooted cuttings in a container nursery area in Fall 2012, as determined by linear contrasts

Linear contrasts	(P=)	
	Lesions	Dropped leaves
Fungicide vs. none	0.0001	0.0001
Systemic fungicides alone vs. none	0.0001	0.0001
Thiophanate-methyl vs. propiconazole	ns	0.05
Systemic alone vs. systemic plus protectant	ns	0.001
Rotation (3 different treatments) vs. all other systemic/protectants	ns	ns

Table 4.3. Boxwood blight severity in six *Buxus* cultivars or species with or without fungicide protection grown as 2-year-old rooted cuttings in a container nursery area, Fall 2012

Cultivar or species	Lesions ^x		Dropped leaves	
	Untreated control	All fungicides ^y	Untreated control	All fungicides
Common	20.8	2.3 b ^z	36.4 ab	7.5 b
Green Mountain	20.4	2.3 b	15.4 bc	2.9 c
Green Velvet	18.4	4.1 a	30.8 ab	5.1 bc
Korean	21.0	2.3 b	4.4 c	1.0 d
True Dwarf	25.2	2.9 b	76.4 a	16.3 a
Winter Gem	28.8	3.3 b	6.4 c	1.2 d
(P=)	ns	0.0001	0.0001	0.0001

^x Lesions and dropped diseased leaves per plant were counted at the end of the experiment 2 weeks after the third spray.

^y Data were averaged over all fungicide treatments. Treatments included three applications of the systemic fungicides thiophanate-methyl (3336 50% WP, 90 g/100 liters) or propiconazole alone (ProConZ, 14.3L, 90 ml/100 liters) or each in combination with the locally systemic/translaminar fungicide pyraclostrobin (Insignia 20% WG, 60 g/100 liters) and protectant fungicides, fludioxonil (Medallion 50% WG, 23 g/100 liters), or chlorothalonil (Daconil 54%F Weatherstik, 170 ml/100 liters). An additional treatment regime consisted of thiophanate-methyl plus chlorothalonil followed by propiconazole plus pyraclostrobin and finally the locally systemic/translaminar fungicide kresoxim-methyl (Cygnus 50 WG, 25 g/100 liters) plus fludioxonil.

^z Data were analyzed by ANOVA. Means within columns followed by the same letter are not significantly different ($P = 0.05$).

were no differences in lesion numbers between the fungicide treatments that included thiophanate-methyl and those that included propiconazole as the systemic fungicide. However, numbers of dropped leaves were higher for treatments that included propiconazole in comparison to thiophanate-methyl. Systemic fungicides plus protectants were superior to systemic fungicides alone for reducing numbers of dropped leaves, but not numbers of boxwood blight lesions. There were no differences between treatments that consisted of a single spray each of three different systemic or local systemic fungicides combined with three different protectant or local systemic/translaminar fungicides compared to three repeated sprays of all other systemic plus protectant or local systemic combinations.

There were no significant differences between cultivars for numbers of lesions in controls not treated with fungicides, perhaps due to the reduced number of replicates (Table 4.3). When all fungicide treatments were combined, Green Velvet boxwood had significantly

higher lesion numbers. The number of dropped diseased leaves was quite high, perhaps accounting for the lower lesion numbers as diseased leaves were removed, but differences in dropped diseased leaves were significant whether plants were treated with fungicide or not. The most severe disease as measured by dropped diseased leaves was in True Dwarf, common boxwood, and Green Velvet. Korean and Winter Gem had the least measurable disease.

Experiment 5: Winter 2012–2013. All treatments of systemic alone or systemic plus protectant fungicides applied to six cultivars of boxwood in the greenhouse resulted in fewer leaf and stem lesions and fewer dropped diseased leaves than untreated controls (Tables 5.1 and 5.2). There were no differences in lesion numbers or dropped leaves between the fungicide treatments that included thiophanate-methyl and those that included propiconazole as the systemic fungicide. Systemic fungicides plus protectants or a local systemic fungicide were superior to systemic fungicides alone for

Table 5.1. Efficacy of fungicides applied at 2-week intervals against boxwood blight caused by *Calonectria pseudonaviculata* in six *Buxus* cultivars or species grown as 2-year-old rooted cuttings in the greenhouse, Winter 2012–2013

Systemic fungicide a.i. ^a	Protectant fungicide a.i.	Applications	Lesions ^b	Dropped leaves
None	None	NA	23.7 ^z	12.8
Thiophanate-methyl	None	3	1.4	1.3
Thiophanate-methyl	Pyraclostrobin	3	0.5	1.0
Thiophanate-methyl	Fludioxonil	3	0.6	1.0
Thiophanate-methyl	Chlorothalonil	3	0.3	0.3
Propiconazole	None	3	4.7	4.4
Propiconazole	Pyraclostrobin	3	0.4	0.8
Propiconazole	Fludioxonil	3	0.5	0.7
Propiconazole	Chlorothalonil	3	0.3	0.3
Thiophanate-methyl	Pyraclostrobin	1	0.3	0.0
Propiconazole	Fludioxonil	1		
Kresoxim-methyl	Chlorothalonil	1		

^a Ten treatments included water only or three applications of the systemic fungicides thiophanate-methyl (3336 50% WP, 90 g/100 liters) or propiconazole alone (ProConZ, 14.3L, 90 ml/100 liters) or each in combination with the locally systemic/translaminar fungicide pyraclostrobin (Insignia 20% WG, 60 g/100 liters) and protectant fungicides, fludioxonil (Medallion 50% WG, 23 g/100 liters), or chlorothalonil (Daconil 54%F Weatherstik, 170 ml/100 liters). An additional treatment regime consisted of thiophanate-methyl plus chlorothalonil followed by propiconazole plus pyraclostrobin and finally the locally systemic/translaminar fungicide kresoxim-methyl (Cygnus 50 WG, 25 g/100 liters) plus fludioxonil.

^b Lesions and dropped diseased leaves were counted at the end of the experiment 2 weeks after the third spray.

^z Data were analyzed by ANOVA and means were separated by single degree-of-freedom linear contrasts.

Table 5.2. Significance of fungicides applied at 2-week intervals on boxwood blight caused by *Calonectria pseudonaviculata* in six *Buxus* cultivars or species grown as 2-year-old rooted cuttings in a container nursery area in Winter 2012–2013, as determined by linear contrasts

Linear contrasts	(P=)	
	Lesions	Dropped leaves
Fungicide versus none	0.0001	0.0001
Systemic fungicides alone vs. none	0.0001	0.0001
Thiophanate-methyl vs. propiconazole	ns	ns
Systemic alone vs. systemic plus protectant	0.001	0.005
Rotation (3 different treatments) vs. all other systemic/protectants	ns	ns

Table 5.3. Boxwood blight severity in six *Buxus* cultivars or species with or without fungicide protection grown as 2-year-old rooted cuttings in a container nursery area, Winter 2012–2013

Cultivar or species	Lesions ^a		Dropped leaves	
	Untreated control	All fungicides ^b	Untreated control	All fungicides
Common	26.8	2.0 a ^z	20.3 a	2.6 a
Green Mountain	23.5	0.9 b	6.5 b	0.5 bc
Green Velvet	18.3	0.6 b	12.8 a	0.8 b
Korean	16.8	0.3 b	4.8 b	0.0 c
True Dwarf	30.8	1.4 b	26.3 a	1.7 ab
Winter Gem	26.0	0.6 b	6.0 b	0.8 b
(P=)	ns	0.005	0.01	0.0001

^a Lesions and dropped diseased leaves per plant were counted at the end of the experiment 2 weeks after the third spray.

^b Data were averaged over all fungicide treatments. Treatments included three applications of the systemic fungicides thiophanate-methyl (3336 50% WP, 90 g/100 liters) or propiconazole alone (ProConZ, 14.3L, 90 ml/100 liters) or each in combination with the locally systemic/translaminar fungicide pyraclostrobin (Insignia 20% WG, 60 g/100 liters) and protectant fungicides, fludioxonil (Medallion 50% WG, 23 g/100 liters), or chlorothalonil (Daconil 54%F Weatherstik, 170 ml/100 liters). An additional treatment regime consisted of thiophanate-methyl plus chlorothalonil followed by propiconazole plus pyraclostrobin and finally the locally systemic/translaminar fungicide kresoxim-methyl (Cygnus 50 WG, 25 g/100 liters) plus fludioxonil.

^z Data were analyzed by ANOVA. Means within columns followed by the same letter are not significantly different ($P = 0.05$).

both blight lesions and dropped leaves. There were no differences between treatments that consisted of a single spray each of three different systemic or local systemic combined with three different protectants or three sprays of all other systemic plus protectant combinations. There were no differences between treatments that consisted of a single spray each of three different combinations of systemic or local systemic fungicides paired with three different protectant or local systemic fungicides compared to three repeated sprays of all other systemic plus protectant or local systemic combinations.

There were no significant differences between cultivars for numbers of lesions in controls not treated with fungicides, perhaps due to reduced replicates (Table 5.3). When all fungicide treatments were combined, common boxwood had significantly higher lesion numbers. Fungicide treatments resulted in significantly fewer dropped diseased leaves. The most severe disease as measured by dropped diseased leaves was in True Dwarf, common boxwood, and Green Velvet. Korean boxwood had the least amount of disease.

Discussion

These results confirm that a number of fungicides shown to have activity against *C. pseudonaviculata* conidial germination, mycelial growth, or both in vitro also have efficacy against boxwood blight on a number of boxwood species and cultivars under greenhouse and container nursery conditions. Products containing propiconazole, myclobutanil, thiophanate-methyl, fludioxonil, pyraclostrobin, kresoxim-methyl, and chlorothalonil all had significant activity against boxwood blight in previous in vitro studies (2,11,15) and in these greenhouse and container nursery experiments. While some of these fungicides may have curative activity against certain pathogens, potential curative activity was not evaluated in these experiments, as all of these fungicides were evaluated as protectants, applied 2 days prior to inoculation with the pathogen. Henricot and Wedgwood (11) concluded that the most effective fungicides against blight disease in boxwood in UK studies were epoxiconazole plus kresoxim-methyl plus pyraclostrobin, prochloraz, boscalid plus pyraclostrobin, and chlorothalonil. Epoxiconazole and prochloraz are not currently registered in the United States, but they are members of FRAC group 3 and appear to have similar activity against *C. pseudonaviculata* to propiconazole and myclobutanil, which are also in FRAC 3. The results of the present study, conducted with similar fungicides, agree with the conclusion that fungicide combinations were most effective in preventing boxwood blight.

Management of *Calonectria/Cylindrocladium* diseases in other ornamentals or perennial plants has focused on some of the same fungicides. Aiello et al. (1) investigated disease control for several *Calonectria* species in bottlebrush (*Callistemon*) and feijoa (*Acca sellowiana*). They concluded that thiophanate-methyl, tebuconazole, prochloraz, fosetyl-Al, and copper hydroxide consistently showed efficacy. Haralson et al. (8) determined that fludioxonil resulted in the best management of *Cylindrocladium* root rot of blueberry. Thiophanate-methyl and triflumizole also had activity but required more than a single application to suppress disease.

Many of the fungicides that have activity against the target pathogen are also at risk for the development of fungicide resistance. Gehesquiere et al. (6) separated European isolates of *C. pseudonaviculata* into G1 and G2 clades, identified using AFLP markers. They determined that there was little variation within clades and that both G1 and G2 had similar pathogenicity. However, G2 isolates were uniformly less sensitive to both kresoxim-methyl (FRAC 11) and tetraconazole (FRAC 3) fungicides than G1 isolates. All Connecticut isolates of *C. pseudonaviculata* tested, including the Cps-CT-L1 and Cps-CT-S1 isolates used in this study, have been identified as G1 isolates (R. Marra, *personal communication*), but the potential for the development of fungicide resistance is very real. It is noteworthy that these results demonstrate that combinations of systemic plus protectant or local systemic/translaminar fungicides result in significantly better disease

control than the use of systemic fungicides alone. For two of three trials, there were no differences between treatments that consisted of a single spray each of three different systemic or local systemic fungicides combined with three different protectant or local systemic fungicides compared to three repeated sprays of all other systemic or local systemic and protectant combinations. Rotation of a number of different fungicides with different modes of action may still be desirable, even if efficacy is not substantially increased, as a means of combating potential fungicide resistance development.

These results also demonstrate that boxwood cultivars differ in susceptibility to boxwood blight. Korean and Winter Gem (*B. sinica* var. *insularis*) were the least susceptible of the cultivars evaluated, True Dwarf and common boxwood (*B. sempervirens*) were the most susceptible, and Green Mountain and Green Velvet (*B. sempervirens* × *B. sinica*) were intermediate. These data suggest that *B. sinica* var. *insularis* may carry some level of resistance to boxwood blight. A wider range of cultivars within these boxwood species should be evaluated to test this hypothesis.

Management of boxwood blight will rely on integrated best management practices that include inspection of incoming plant material, sanitation, cultural controls including level of susceptibility or tolerance of different cultivars to infection, and fungicide application. The level of control demonstrated in these experiments was quite high, but the tolerance for this disease in boxwood nurseries or landscape plantings is extremely low. The fungicides shown to have activity in these experiments may be best applied as protectants prior to pathogen infection. Continual application of fungicides is expensive and impractical, so research to determine disease epidemiology and risk of pathogen spread as well as the environmental conditions necessary for infection periods needs to be conducted.

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Literature Cited

1. Aiello, D., Cirvilleri, G., Polizzi, G., and Vitale, A. 2013. Effects of fungicide treatments for the control of epidemic and exotic *Calonectria* diseases in Italy. *Plant Dis.* 97:37-43.
2. Brand, T. 2006. *In vitro*-Wirkung fungizider Wirkstoffe auf Konidien-keimung und Myzelwachstum von *Cylindrocladium buxicola*. *Nachrichtenbl. Dtsch. Pflanzenschutzdienst* 58:117-121.
3. Dart, N., Hansen, M. A., Bush, E., and Hong, C. 2011. Boxwood blight: A new disease of boxwood found in the eastern U.S. Retrieved 3 February 2014 from http://pubs.ext.vt.edu/PPWS/PPWS-4/Boxwood_Blight_pdf.pdf
4. Douglas, S. M. 2012. Boxwood Blight - A new disease for Connecticut and the U.S. Retrieved 3 February 2014 from www.ct.gov/caes/lib/caes/documents/publications/fact_sheets/plant_pathology_and_ecology/boxwood_blight_a_new_disease_for_connecticut_and_the_u.s._12-08-11.pdf
5. Elmhirst, J. F., and Auxier, B. E. 2013. First report of box blight caused by *Cylindrocladium pseudonaviculatum* (*C. buxicola*) in British Columbia, Canada. *Plant Dis.* 97:559.
6. Gehesquiere, B., Rys, F., Maes, M., Gobin, B., Van Huylenbrowe, J., and Heungens, K. 2012. Genotypic and phenotypic variation in *Cylindrocladium buxicola*. (Abstr.) *Commun. Agric. Appl. Biol. Sci.* 77:95-96.
7. Hagan, A. K., and Conner, K. 2013. Boxwood blight - a new disease of boxwood in the nursery and landscape in Alabama. Retrieved 30 May 2014 from <https://sites.aces.edu/group/timeinfo/Documents/2013%20Boxwood%20Blight%20TI%20Updated.pdf>
8. Haralson, J. C., Brannen, P. M., NeSmith, D. S., and Scherm, H. 2013. Chemical control of *Cylindrocladium* and *Rhizoctonia* root rots in blueberry. *Crop Prot.* 44:1-5.
9. Henricot, B., and Culham, A. 2002. *Cylindrocladium buxicola*, a new species affecting *Buxus* spp., and its phylogenetic status. *Mycologia* 94:980-997.
10. Henricot, B., Gorton, C., Denton, G., and Denton, J. 2008. Studies on the control of *Cylindrocladium buxicola* using fungicides and host resistance. *Plant Dis.* 92:1273-1279.
11. Henricot, B., and Wedgwood, E. 2013. Evaluation of foliar fungicide sprays

- for the control of boxwood blight, caused by the fungus *Cylindrocladium buxicola*. Plant Health Progress. doi:10.1094/PHP-2013-1024-01-RS
12. Ivors, K. 2013. Beating boxwood blight. American Nurseryman. Retrieved 3 February 2014 from <http://www.amerinnursery.com/article-9178.aspx>
 13. Ivors, K. L., Lacey, L. W., and Ganci, M. 2013. Evaluation of fungicides for the prevention of boxwood blight, 2012. Plant Dis. Manag. Rep. 7:OT014. Online publication. doi: 10.1094/PDMR07
 14. Ivors, K., Lacey, W., Milks, D., Douglas, S. M., Inman, M. K., Marra, R. E., and LaMondia, J. A. 2012. First report of boxwood blight caused by *Cylindrocladium pseudonaviculatum* in the United States. Plant Dis. 96:1070.
 15. LaMondia, J. A. 2014. Fungicide efficacy against *Calonectria pseudonaviculata*, causal agent of boxwood blight. Plant Dis. 98:99-102.
 16. LaMondia, J. A., and Li, D. W. 2013. First report of *Cylindrocladium pseudonaviculatum* causing leaf spot and stem blight of *Pachysandra procumbens*. Plant Health Progress. doi:10.1094/PHP-2013-0226-01-BR
 17. LaMondia, J. A., Li, D. W., Marra, R. E., and Douglas, S. M. 2012. First report of *Cylindrocladium pseudonaviculatum* causing leaf spot of *Pachysandra terminalis*. Plant Dis. 96:1069.
 18. Malapi-Wight, M., Hebert, J. B., Buckley, R., Daughtrey, M. L., Gregory, N. F., Rane, K., Tirpak, S., and Crouch, J. A. First report of boxwood blight caused by *Calonectria pseudonaviculata* in Delaware, Maryland, New Jersey, and New York. Plant Dis. 98:698.