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Research

Pachysandra Species and Cultivar Susceptibility to the Boxwood Blight Pathogen, *Calonectria pseudonaviculata*

James A. LaMondia, Chief Scientist, The Connecticut Agricultural Experiment Station Valley Laboratory, Windsor, CT 06095

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Abstract

This research was conducted to answer grower questions regarding potential differences among *Pachysandra* species and cultivars in susceptibility to the boxwood blight pathogen, *Calonectria pseudonaviculata*. Five cultivars of *P. terminalis*, one cultivar of *P. axillaris*, and one selection of *P. procumbens* were evaluated using whole plants and detached leaves. *Pachysandra* species and cultivars differed somewhat in susceptibility to boxwood blight, with more significant differences observed between species and cultivars using whole plants than with detached leaf assays. All *Pachysandra* species and cultivars were susceptible to the pathogen and sporulation occurred on lesions; therefore, all of these cultivars may serve as inoculum reservoirs for the boxwood blight pathogen. Best management practices will need to take this into account in landscapes, garden centers, and nurseries to prevent additional spread of the pathogen.

Calonectria pseudonaviculata (Crous et al.) L. Lombard et al., the pathogen that causes boxwood blight, has also been experimentally shown to cause leaf and stem disease on other genera in the *Buxaceae*, including sweet box *Sarcococca* spp. (Henricot et al. 2008), Japanese spurge *Pachysandra terminalis* (LaMondia et al. 2012), and Allegheny spurge *Pachysandra procumbens* (LaMondia and Li 2013). Natural infection of *Pachysandra terminalis* in the landscape was first demonstrated in Connecticut in 2012 (Douglas 2012) in close proximity to diseased boxwood plants. Since best management practices were instituted in Connecticut in 2013, boxwood blight has not been detected in nurseries in the state, and diseased *Buxus* plants have only been observed in the landscape. Multiple instances of diseased *Pachysandra* associated with diseased boxwood in the landscape have been recorded in Connecticut. Recently, *C. pseudonaviculata* leaf spot of *Pachysandra* was also documented on symptomatic plants underneath and surrounding boxwood infected by the same pathogen in a landscape in Virginia (Kong et al. 2017). To date, *Pachysandra* infection has only been found in landscapes in association with infected boxwood; no isolated plants have been documented with this disease and no infected plants from nursery, retail sales locations, or production nurseries have been suspected or detected (J. A. LaMondia, *unpublished*).

Differences in disease susceptibility exist within *Buxus* species and cultivars (Ganci et al. 2013; Guo et al. 2016; Henricot et al. 2008; LaMondia 2015; Shishkoff et al. 2015). The goal of this research was to answer grower and landscaper questions and concerns regarding potential differences in susceptibility in *Pachysandra* species and cultivars.

Plants Evaluated

Healthy *P. terminalis* 'Common', 'Green Sheen', and 'Variegated' were obtained from O'Hara's Nursery, Monroe, CT; *P. terminalis* 'Crinkled Green Sheen' and 'Green Carpet' were purchased from JW-Pachysandra, Hopewell Junction, NY; and *P. axillaris* 'Windcliff Fragrant' plants were provided by Monrovia Nursery, Granby, CT. Healthy *Pachysandra procumbens* plants used in detached leaf assays had been previously obtained from Imperial Nurseries, Granby, CT, and maintained in the greenhouse. All plants were transplanted into 11-cm-diameter square plastic pots containing 500 cm^3 Sunshine #3 plant growth medium (SunGro Horticulture, Bellevue, WA) and grown under greenhouse conditions.

Pathogen

Two anamorphic isolates of *Calonectria pseudonaviculata* (syn ≡ *Cylindrocladium pseudonaviculatum* Crous, J.Z. Groenew. & C.F. Hill, = *Cylindrocladium buxicola* Henricot) from Connecticut, Cps-CT-L1 (ATCC MYA-4891) and Cps-CT-S1 (ATCC MYA-4890) were used in these experiments. Inocula consisted of conidial suspensions prepared from cultures grown on half-strength potato dextrose agar (LaMondia 2015).

Plant Assays

Conidial suspensions were adjusted to 1.0×10^5 conidia per ml and *Pachysandra* foliage were inoculated to wet using a hand-held hand-pump spray bottle on a coarse setting. Two sprays per plant (0.75 ml per spray) from two directions were used to uniformly inoculate plants. There were 10 replicate plants of each cultivar. Humidity was maintained after inoculation by suspending white polyethylene over plants for 24 h. Plants were then grown in the greenhouse and overhead watered by hand twice per day. Discrete lesions typical of blight on leaves and stems were counted after two weeks. The experiment was conducted twice. An additional experiment was conducted with 20 plants per cultivar, 10 each with or without application of 0.18 kg Spectro 90 WDG fungicide (Cleary Chemicals LLC, Alsip IL) per 100 liters water applied to foliage to runoff. Plants were inoculated as described above and lesions counted after two weeks.

Detached Leaf Assays

Detached leaf experiments were performed five times. Mature leaves were collected and surface sterilized in 0.5% NaOCl solution

Corresponding author: James A. LaMondia; E-mail: James.LaMondia@ct.gov

for 30 s, then rinsed with tap water for 30 s. Leaves were air dried and placed on labeled glass sides over bent glass rods in glass Petri dishes (9-cm diameter) with wet filter paper in the bottom to maintain high humidity. There were four leaves per Petri dish replicated six times per cultivar. Inoculum was added to the adaxial surface for experiments 1 and 2. For experiments 3 to 5, there were three replicate dishes per cultivar that were inoculated on either the adaxial or abaxial surface. Inoculum was prepared as before and adjusted to 3,000 conidia per drop (0.04 ml). One drop was placed on the surface of each leaf. Petri dishes were held at 22 to 25°C, and numbers of diseased leaves determined 11 to 12 days after inoculation.

Statistics

Data concerning *Pachysandra* plant susceptibility to *C. pseudonaviculata* 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 ($P = 0.05$). An additional experiment to determine whether there was an interaction between *Pachysandra* cultivar susceptibility and use of the fungicide Spectro 90 WDG was analyzed by two-way ANOVA after log transformation to stabilize variance. Means were separated by LSD ($P = 0.05$). Percent diseased detached *Pachysandra* leaves after inoculation with *Calonectria pseudonaviculata* on the adaxial or abaxial surface 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 ($P = 0.05$).

Susceptibility of *Pachysandra* Species and Cultivars to *C. pseudonaviculata*

Pachysandra terminalis Common had greater numbers of lesions than *P. terminalis* Crinkled, Green Carpet, or *P. axillaris* Windcliff Fragrant when evaluated as plant assays (Table 1). *Pachysandra terminalis* Variegated and Green Sheen were intermediate in susceptibility. The pathogen was reisolated from symptomatic lesions on *P. axillaris* Windcliff Fragrant and cultured on half-strength potato dextrose agar.

Fungicide application prior to inoculation reduced lesion development by approximately 85% and there was no interaction between cultivar and fungicide application (Table 2). Disease was again greatest for *P. terminalis* Common than the other cultivars in both instances.

TABLE 1
Susceptibility of *Pachysandra* plants to *Calonectria pseudonaviculata*

	Number of lesions per plant [a]	
Pachysandra	Exp. 1	Exp. 2
P. terminalis Common	31.7 A [b]	32.0 A
P. terminalis Crinkled	5.5 CD	11.1 BC
P. terminalis Green Carpet	6.5 CD	6.5 C
P. terminalis Green Sheen	15.8 B	15.8 B
P. terminalis Variegated	13.5 BC	26.5 A
P. axillaris Windcliff	4.3 CD	13.8 BC
P =	0.0001	

[a] Ten replicate plants per cultivar. Lesions were counted 2 weeks after inoculation.

[b] 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
Susceptibility of *Pachysandra* plants treated with fungicide or not to infection by *Calonectria pseudonaviculata*

	Number of lesions per plant	
Pachysandra	Fungicide[a]	Nontreated
P. axillaris Windcliff	10.1 B	143.1 B
P. terminalis Common	74.6 A[b]	340.3 A
P. terminalis Crinkled	10.6 B	67.9 B
P. terminalis Green Carpet	9.4 B	50.7 B
P. terminalis Green Sheen	16.7 B	98.4 B
P. terminalis Variegated	19.3 B	137.3 B
P =		
Variety	0.0001	
Fungicide	0.0001	
Interaction	0.22	

[a] Ten replicate plants per cultivar treated with Spectro 90 WDG fungicide at 0.18 kg/100 liter water or not treated (water alone). Lesions were counted 2 weeks after inoculation.

[b] Data were analyzed by ANOVA after log transformation to stabilize variance and means were separated by LSD, means within columns followed by the same letter are not significantly different ($P = 0.05$).

TABLE 3
Percent diseased detached *Pachysandra* leaves after inoculation with *Calonectria pseudonaviculata* on the adaxial or abaxial surface

	Percent leaves with lesions or sporulation on lesions			
	Exp. 1 to 2[a]	Exp. 3 to 5		
Pachysandra	Adaxial	Abaxial	Adaxial	Sporulation[c]
P. procumbens	62.5 A[b]	100.0 A	–	100.0 A
P. axillaris Windcliff	55.0 AB	42.5 B	10.0 AB	4.0 B
P. terminalis Common	7.5 B	47.5 B	10.0 AB	70.0 A
P. terminalis Crinkled	35.0 AB	47.5 B	22.5 A	73.3 A
P. terminalis Green Carpet	30.0 AB	47.5 B	2.5 B	70.0 A
P. terminalis Green Sheen	25.0 AB	47.5 B	17.5 A	78.5 A
P. terminalis Variegated	7.5 B	40.0 B	0.0 B	70.0 A
P =	0.0001	0.001	0.0001	0.0001

[a] Five experiments were conducted, two with inoculation of a conidial suspension of 3,000 conidia in a single drop to the adaxial surface, and an additional three inoculated to either abaxial and adaxial surfaces, 24 leaves per cultivar per experiment. Leaves were evaluated 11 or 12 days after inoculation.

[b] 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 ($P = 0.05$).

[c] Percent of abaxial leaf lesions with *C. pseudonaviculata* sporulation present.

Susceptibility to disease was also measured by the percent of inoculated detached leaves which developed symptoms and sporulation of the pathogen within 11 to 12 days (Table 3). While it was difficult to consistently determine significant differences in susceptibility between plant types, more leaves developed disease

when inoculated on the bottom of the leaf than on the top, and *P. procumbens* was generally more susceptible than the other *Pachysandra* species evaluated. The pathogen sporulated on all of the *Pachysandra* species tested, but sporulation was much less common on *P. axillaris* Windcliff Fragrant than on the other species.

Conclusions

Pachysandra species and cultivars differed somewhat in susceptibility to the boxwood blight pathogen *C. pseudonaviculata*. We observed more significant differences between species and cultivars using whole plants than with detached leaf assays. Fungicidal control of disease with a combination product containing a systemic active ingredient (thiophanate-methyl) and a protectant active ingredient (chlorothalonil) was equally effective for all of the *Pachysandra* tested. Our results are consistent with our previous observation that *P. procumbens* was very susceptible to *C. pseudonaviculata*, and plants can be killed when lesions girdle the stem (LaMondia and Li 2013). *Pachysandra procumbens* is a native plant that is already listed as endangered in some natural habitats in Florida and Indiana https://plants.usda.gov/core/profile?symbol=papr7.

Pachysandra axillaris Windcliff Fragrant is a recently introduced plant in the nursery trade. *Calonectria pseudonaviculata* has not previously been reported causing disease on this *Pachysandra* species. All *Pachysandra* species and cultivars tested to date could be infected, could sporulate to some extent, and may therefore serve as inoculum reservoirs for the boxwood blight pathogen. Best management practices will need to take this into account in landscapes, garden centers, and nurseries to prevent additional spread of the pathogen.

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