Indications of Susceptibility to *Calonectria pseudonaviculata* in Some Common Groundcovers and Boxwood Companion Plants

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Abstract

Knowing the host range of a pathogen is critical to developing and implementing effective disease management programs. *Calonectria pseudonaviculata* (*Cps*) is known to attack a number of species, varieties, and cultivars in the genus *Buxus* as well as three *Pachysandra* species (*Pachysandra terminalis*, *Pachysandra procumbens*, and *Pachysandra axillaris*) and several *Sarcococca* species, all in the Buxaceae family. The objective of this study was to evaluate non-Buxaceae groundcovers and companion plants commonly associated with boxwood plantings for their susceptibility to *Cps*. Twenty-seven plant species belonging to 21 families were exposed to different levels of inoculum: 50 to 300 conidia per drop for detached leaf assays and 30,000 to 120,000 conidia per 1 ml for whole-plant assays. Inoculated plants were incubated in humid environments for at least 48 h to facilitate infection. *Cps* infection and

sporulation were observed on 12 plant species: *Alchemilla mollis*, *Arctostaphylos uva-ursi*, *Brunnera macrophylla*, *Epimedium* × *youngianum*, *Galium odoratum*, *Geranium sanguineum*, *Phlox subulata*, *Tiarella cordifolia*, *Callirhoe involucrata*, *Iberis sempervirens*, *Mazus reptans*, and *Vinca minor*. These results suggest that there may be more hosts of *Cps* commonly grown in nurseries and landscapes. If corroborated by observations of natural infection, these findings have implications for the Boxwood Blight Cleanliness Program instituted by the National Plant Board and for planning disease mitigation at production and in the landscape.

Keywords: box blight, *Cylindrocladium buxicola*, *Cylindrocladium pseudonaviculatum*, disease mitigation, host range

Boxwood blight caused by *Calonectria pseudonaviculata* (*Cps*) poses an increasing threat to new and established gardens in the United States and other affected countries (Daughtrey 2019; Hong 2019a). It was first reported as a new disease in New Zealand (Ridley 1998) and the United Kingdom (Henricot et al. 2000). This disease has since been documented for most countries in continental Europe, North America, and Iran (Ganci 2014; Gehesquière 2014; LeBlanc et al. 2018; Palmer and Shishkoff 2014; Vitale et al. 2013). In the United States, boxwood blight was first observed in North Carolina and Connecticut (Ivors et al. 2012); this pathogen has reached 28 states in 2019 (Hong 2019b).

To safeguard boxwood crops and plantings in the United States, the National Plant Board, a nonprofit organization of state plant pest regulatory agencies (<https://nationalplantboard.org/>), instituted the Boxwood Blight Cleanliness Program, whereas several states have also enacted quarantines against *Cps*. The Cleanliness Program utilizes a systems approach to prevent the spread of boxwood blight via nursery trade. This is accomplished through a close partnership between production nurseries and their State Departments of Agriculture. Nurseries participating in this voluntary program agree to use best management practices developed by the National Plant Board and the horticulture industry (<https://hortknowledgecenter.org/getattachment/7068c31f-fee0-4541-bf4a-ac89350be97b/BoxwoodBlightBMPs2017.pdf?lang=en-US>). These nurseries also are inspected and audited by the Office of Plant Industry Services as exemplified by the Commonwealth of Virginia (<http://www.vdacs.virginia.gov/plant-industry-services-boxwood-blight.shtml>). Full quarantine protocol against *Cps* has been

enacted in the states of Pennsylvania (Anonymous 2016) and Tennessee (Anonymous 2017b). In recognition of its importance, boxwood blight was also declared a nuisance in the State of Illinois (Anonymous 2017a).

Although both the Cleanliness Program and state quarantines have helped slow *Cps* spread nationally and locally, their effectiveness depends on correct information regarding the host range of this pathogen. Henricot et al. (2008) demonstrated through detached stem assays that *Cps* can infect many *Buxus* species and a *Sarcococca* sp. Others have demonstrated that *Cps* infects multiple boxwood species, varieties, and cultivars: in fact, all that have been evaluated (Brand 2005; Ganci 2014; Gehesquière 2014; LaMondia and Shishkoff 2017; Shishkoff et al. 2015). Variations in boxwood cultivar susceptibility (Ganci 2014; Shishkoff et al. 2015) and differences in inoculum level necessary for infection of different cultivars have been noted (Avenot et al. 2017). This pathogen also has been observed to attack *Pachysandra terminalis* (Kong et al. 2017b; LaMondia et al. 2012), *Pachysandra procumbens* (LaMondia and Li 2013), and *Pachysandra axillaris* (LaMondia 2017) as well as *Sarcococca hookeriana* var. *humilis* (Kong et al. 2017a; Malapi-Wight et al. 2016) and several additional *Sarcococca* species (Ryan et al. 2018). *Pachysandra* and sweet box must be taken into consideration when developing and implementing best management practices for boxwood blight. These plants must not be left in the landscape when gardeners are attempting to eradicate boxwood blight because they may be a reservoir for the pathogen.

In addition to *pachysandra* and sweet box, many other annuals and perennials are often planted with boxwood to increase the aesthetic value of the landscape (Batdorf 2005; Saunders 2017). It was not known whether *Cps* could infect any groundcovers and/or companion crops outside of the Buxaceae family. The objective of this study was to bridge this knowledge gap by evaluating 27 non-Buxaceae species commonly used adjacent to boxwood in landscape plantings for their susceptibility to *Cps*.

Materials and Methods

Groundcover and companion plants. A total of 27 plant species belonging to 21 families were selected for this study after consulting with horticulturists, landscapers, and gardeners in New York and Virginia (Table 1). *P. terminalis* and two *Buxus* cultivars (*Buxus* × ‘Green Velvet’ and *Buxus microphylla* var. *japonica* ‘Winter

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Gem') were included as positive controls from within the family Buxaceae. Liners or bare root plants were obtained from multiple suppliers. On receipt of stock plants, they were transplanted into 9-cm square containers with MetroMix 830 potting mix and maintained in a greenhouse until use.

Inoculum preparation. Stock cultures of *Cps* isolate 11D05, recovered from boxwood, were maintained on Difco potato dextrose agar (PDA; Fisher Scientific) at 25°C. Conidia were produced as described previously (Kong et al. 2017a, b). Briefly, a 5- to 6-cm² mycelial mass was scraped from the surface of a 2- to 3-week-old stock culture with a cotton swab and added to 20 ml of fresh potato dextrose broth in a 10-cm petri dish. The swab was swirled vigorously to evenly distribute mycelial fragments. The liquid cultures were incubated at 25°C in the dark for 5 to 7 days until the entire dish area was covered with a thick mycelial mass. Cultures were removed from the incubator and flushed with sterile deionized water (SDW), and their surfaces were brushed gently with a cotton swab. The liquid was decanted followed by two additional washes with SDW. Cultures were then placed under fluorescent light at room temperature for 3 to 5 days. After this period, 10 ml of 0.01% Tween 20 (Fisher Scientific) was added to each dish, and conidia were dislodged by rubbing gently with a glass rod. This suspension and two additional washes with 0.01% Tween 20 were decanted and pooled into a 50-ml screw cap conical tube or 500-ml bottle depending on the inoculum volume required. The suspension was vortexed for 1 min, and conidial concentration was determined using a hemacytometer. Dilutions for each inoculum concentration were prepared in 0.01% Tween 20.

Detached leaf assays. Twelve detached leaves were collected from each of the 23 groundcover plant species and the three Buxaceae controls (the genus *Pachysandra*, Green Velvet, and Winter Gem boxwood), washed three times in deionized water, and then, blotted dry. Clear plastic boxes (Pioneer Plastics #295C 31.3 × 23.0 × 10.2 cm)

were prepared by lining the bottom with three paper towels saturated with SDW. Plastic mesh was placed on top of the towels, and three rows of four leaves from each plant were arranged abaxial side up on top of the mesh. Two inoculum concentrations of 5,000 and 30,000 conidia per 1 ml in 0.01% Tween 20 were prepared in 2-ml microcentrifuge tubes. After vortexing briefly, for each test species one 10-μl drop of selected inoculum was placed on the abaxial leaf surface of four leaves to deliver 50 conidia per drop and on four leaves with 300 conidia per drop. One 10-μl drop of 0.01% Tween 20 was placed on the remaining four leaves of each test species as controls. Boxes were lidded and kept on the laboratory bench at room temperature (approximately 23°C). The four remaining species (*Asarum canadense*, *Pulmonaria saccharata*, *Iris cristata*, and *Ophiopogon japonicus*) were inoculated with 300 conidia per 10-μl drop only because no symptoms developed on the 23 species tested previously, except for *Alchemilla* at 50 conidia per drop. This experiment was repeated once on all species using 300 conidia per 10-μl drop of inoculum and *P. terminalis* as a positive control.

Inoculated leaves were assessed for disease symptoms 3 days after inoculation and weekly thereafter for 4 weeks using a scale of 0 to 5 as follows: 0 = no symptoms, 1 = faint or spotty discoloration, 2 = full localized necrosis, 3 = spreading necrosis, 4 = necrosis with sporulation, and 5 = entire leaf necrotic and/or covered with mycelia. Symptomatic areas were examined microscopically for fungal mycelium and conidium production; if present, they were photographed and recorded.

Two approaches were taken to reisolate the pathogen. To isolate by collecting conidia produced on the leaf surface, a 10-μl drop of 0.01% Tween 20 was pipetted directly onto conidiophores and then collected again into a pipette tip. Several 1- to 2-μl droplets were then transferred onto acidified PDA. If no conidia were observed, 5- to 6-mm² pieces were cut from necrotic leaf areas and plated on acidified PDA medium.

Table 1. Groundcover/companion crops evaluated for their susceptibility to *Calonectria pseudonaviculata* (Cps) as indicated by presence (+) or absence (–) of Cps sporulation on inoculated tissues

Habitat	Family	Species	Cultivar	Common name	Sporulation
Shade	Aristolochiaceae	<i>Asarum canadense</i>		European wild ginger	–
Shade	Boraginaceae	<i>Brunnera macrophylla</i>	Jack Frost	Siberian bugloss	+
Shade	Asteraceae	<i>Chrysogonum virginianum</i>		Golden knee	–
Shade	Berberidaceae	<i>Epimedium</i> × <i>youngianum</i>	Niveum	Bishop's hat	+ ^a
Shade	Rubiaceae	<i>Galium odoratum</i>		Sweet woodruff	+
Shade	Ericaceae	<i>Gaultheria procumbens</i>	Cherry Berries	Creeping wintergreen	–
Shade	Liliaceae	<i>Liriope muscari</i>	Majestic	Big blue lilyturf	–
Shade	Rubiaceae	<i>Mitchella repens</i>		Partridge berry	–
Shade	Asparagaceae	<i>Ophiopogon japonicus</i>		Dwarf mondo grass	–
Shade	Boraginaceae	<i>Pulmonaria saccharata</i>	Margery Fish	Lungwort	–
Shade	Saxifragaceae	<i>Tiarella cordifolia</i>		Foam flower	+
Partial shade	Lamiaceae	<i>Ajuga reptans</i>	Bronze	Common bugleweed	–
Partial shade	Rosaceae	<i>Alchemilla mollis</i>		Lady's mantle	+
Partial shade	Ericaceae	<i>Arctostaphylos uva-ursi</i>	Massachusetts	Bearberry	+
Partial shade	Geraniaceae	<i>Geranium sanguineum</i>	Max Frei	Bloody geranium	+
Partial shade	Iridaceae	<i>Iris cristata</i>		Dwarf crested iris	–
Partial shade	Asparagaceae	<i>Liriope spicata</i>		Creeping liriope	–
Partial shade	Mazaceae	<i>Mazus reptans</i>		Mazus	+ ^a
Partial shade	Polemoniaceae	<i>Phlox stolonifera</i>	Sherwood purple	Creeping phlox	–
Partial shade	Apocynaceae	<i>Vinca minor</i>	Bowles	Dwarf periwinkle	+ ^a
Sun	Malvaceae	<i>Callirhoe involucrata</i>		Purple poppy mallow	+
Sun	Aizoaceae	<i>Delosperma cooperi</i>		Ice plant	–
Sun	Caryophyllaceae	<i>Dianthus gratianopolitanus</i>	Firewitch	Carnation	–
Sun	Brassicaceae	<i>Iberis sempervirens</i>	Purity	Candytuft	+
Sun	Polemoniaceae	<i>Phlox subulata</i>	Emerald Blue	Moss phlox	+ ^a
Sun	Crassulaceae	<i>Sedum spurium</i>	Fuldaglut	Sedum	–
Sun	Lamiaceae	<i>Thymus serpyllum</i>	Pink Chintz	Thyme	–
Control	Buxaceae	<i>Buxus microphylla</i> var. <i>japonica</i>	Winter Gem	Japanese boxwood	+
Control	Buxaceae	<i>Buxus sempervirens</i> 'Suffruticosa' × <i>Buxus sinica</i> var. <i>insularis</i>	Green Velvet	Boxwood hybrid	+
Control	Buxaceae	<i>Pachysandra terminalis</i>		Pachysandra	+

^a Detached leaf only.

Whole-plant assays. Seven plant species were initially selected for the whole-plant inoculation assay based on the responses observed in the detached leaf assay. These included *Alchemilla mollis*, *Phlox subulata* ‘Emerald Blue’, *Dianthus gratianopolitanus* ‘Firewitch’, *Epimedium × youngianum* ‘Niveum’, *Gaultheria procumbens* ‘Cherry Berries’, *Geranium sanguineum* ‘Max Frei’, and *Iberis sempervirens* ‘Purity’. Two inoculum concentrations of 30,000 and 60,000 conidia per 1 ml in 0.01% Tween 20 were prepared in 1-liter and 500-ml bottles, respectively. The genus *Pachysandra* and the two boxwood cultivars were included as positive controls in the 30,000-conidia per 1 ml inoculation only. Four plants of each species were arranged in plastic storage containers in an enclosed room and inoculated with a total of 40 ml: a calibrated hand sprayer was used to pump 10 ml twice onto the upper surface and twice onto the undersurface of the leaves. Four additional plants of each species were sprayed with 40 ml of 0.01% Tween 20 and kept in separate containers. To maintain high humidity, all containers were lidded for 48 h immediately after inoculation. Lights were kept on during daytime hours, and temperature was recorded with a data logger; a portable heater ran intermittently to keep room temperature at 23°C. Plants were monitored for 6 weeks.

Whole-plant inoculations were later made on the remaining 20 plant species at 60,000 conidia per 1 ml, and a third trial used 120,000 conidia per 1 ml on all 27 plant species using the same procedures described above. The 120,000-conidia per 1 ml inoculation of all plant species was repeated once. The genus *Pachysandra* was included in all inoculations as the positive control.

Disease symptom severity was assessed at 3 to 5 days after inoculation and then weekly for 6 weeks on a scale of 0 to 10 (0 = no symptoms; values 1 to 10 represent the percentage of symptomatic leaves in increments of 10 up to 100). Symptomatic leaf or stem tissue was sampled at 2, 3, and 6 weeks. Samples were initially examined with a stereomicroscope for conidia. If no conidia were observed, each sample was then washed in deionized water, surface sterilized in 10% bleach for 1 min, and blotted dry. Some tissue was plated onto acidified PDA, and the remainder of the sample was placed into a plastic bag with a moist paper towel. After a few days, the isolation dishes and samples were examined microscopically for the formation of conidia on lesions. If observed, conidia were isolated as described above using 0.01% Tween 20. Resultant colonies were subcultured onto acidified PDA for identification.

Data analysis. Disease severity ratings were compared among the non-Buxaceae plants on which *Cps* sporulation was observed. Because the data from both detached leaf and whole-plant assays generally were in accord with *Geranium sanguineum* having the highest disease rating, data analysis was only done for the whole-plant assays. Disease rating data from repeated experiments were converted to the midpoint of the corresponding range and then subjected to a homogeneity test before being pooled together for further analysis. Analysis of variance (ANOVA) was conducted using a statistical analysis software (SAS Institute; version 9.4) to determine the level of difference in disease severity among inoculum concentrations of 30,000, 60,000, and 120,000 conidia per 1 ml. Because disease severity rating appeared to increase with increasing inoculum concentration, ANOVA was performed to further determine the level of difference among the 12 non-Buxaceae plants plus the genus *Pachysandra* at the highest inoculum concentration of 120,000 conidia per 1 ml, and mean ratings were separated according to the least significant difference at $P = 0.05$.

Results

Detached leaf assays. Necrosis at the inoculation site developed within 3 days after inoculation on all three susceptible controls. Aerial mycelium and conidiophore production on the leaf surface were observed on all boxwood and most of the genus *Pachysandra* leaves by 7 days postinoculation (Table 1).

Distinct necrotic areas at the inoculation site (rated at levels 2 to 5) were observed on six species. Brown spots appeared on *Alchemilla mollis* within 3 days and *Epimedium × youngianum* and *Arctostaphylos uva ursi* at 5 days postinoculation. *Galium odoratum* developed

necrotic areas after 2 to 3 weeks. *Cps* conidiophores were observed on the leaf surface of *Alchemilla mollis*, *Epimedium × youngianum* (Fig. 1), and *Galium odoratum*, and *Cps* was recovered from *Alchemilla mollis*.

All *Geranium sanguineum* and *Callirhoe involucrata* leaves and most of the *Phlox subulata* leaves (both uninoculated and inoculated) became severely necrotic and rotted before the end of the observation period. Prior to this, however, *Cps* conidiophores were produced on inoculated *Phlox subulata*, and a *Cps* isolate was recovered (Fig. 1 and Table 1).

Minor hypersensitive response was observed on seven species: *Brunnera macrophylla*, *Iberis sempervirens*, *Mazus reptans*, *Phlox stolonifera*, *Tiarella cordifolia*, and *Vinca minor*. Brown mycelium was produced at the inoculation site on the leaf surface of *Phlox stolonifera*, *T. cordifolia*, and *V. minor*. A few conidiophores were observed on *Mazus reptans* and *Vinca minor* (Fig. 1); *Cps* was not successfully reisolated from any tissue.

Brown mycelium without a necrotic response was also observed on the leaf surface at the inoculation site on three additional species: *Dianthus gratianopolitanus*, *Iris cristata*, and *Ophiopogon japonicus*. *Cps* conidiophores or conidia were never observed at these inoculation sites. When the brown mycelium was plated onto agar, *Cps* was not recovered.

No symptoms developed on eight species: *Ajuga reptans*, *Chrysogonum virginianum*, *Delosperma cooperi*, *Liriope muscari*, *Liriope spicata*, *Mitchella repens*, *Sedum spurium*, and *Thymus serpyllum*.

The only plant showing a difference in symptoms at the two inoculum densities was *Epimedium × youngianum*, on which disease ratings were 2.0 versus 0 for 300 and 50 conidia per drop, respectively. The most severe symptoms of necrosis were seen on detached leaves of *Geranium sanguineum* followed by *Iberis sempervirens*, *Phlox subulata*, *Epimedium × youngianum*, *Alchemilla mollis*, *Arctostaphylos uva-ursi*, *Brunnera macrophylla*, *Galium odoratum*, *Vinca minor*, *Tiarella cordifolia*, and *Mazus reptans*.

Whole-plant assays. The susceptible controls (boxwood and pachysandra) became symptomatic within 3 to 4 days of inoculation with 30,000 conidia per 1 ml and showed extensive leaf necrosis (rated 9 or 10) within 1 to 2 weeks. The seven other plant species inoculated at 30,000 conidia per 1 ml remained asymptomatic.

More severe symptoms developed at the higher inoculum concentration on some plants, including *Galium odoratum* ($P = 0.0251$), *Geranium sanguineum* ($P = 0.0024$), and *Mazus reptans* ($P = 0.0045$). At 120,000 conidia per 1 ml, the highest disease severity of necrotic symptoms was observed on *Geranium sanguineum* followed by *Phlox subulata*, *Callirhoe involucrata*, *Brunnera macrophylla*, and *Tiarella cordifolia* (Fig. 2). Conidiophore production was observed on the leaves and/or plated tissue of *Brunnera macrophylla*, *Callirhoe involucrata*, *Geranium sanguineum*, and *Tiarella cordifolia* (Fig. 1). *Cps* was reisolated from *Geranium sanguineum* and *Tiarella cordifolia*. By the end of the trial, *Geranium sanguineum*, *Phlox subulata*, and *Pulmonaria saccharata* succumbed to Botrytis blight or other fungal infection.

Minor symptoms of yellowing and tip necrosis (rated 1 or lower) were observed on eight varieties after 2 to 3 weeks; these included *Alchemilla mollis*, *Arctostaphylos uva-ursi*, *Epimedium × youngianum*, *Galium odoratum*, *Iberis sempervirens*, *Mazus reptans*, and *Vinca minor*. Conidiophore production was observed on the leaves or plated tissue after surface sterilization, and *Cps* was reisolated from five species: *Alchemilla mollis*, *Arctostaphylos uva-ursi*, *Epimedium × youngianum*, *Galium odoratum*, and *Iberis sempervirens* (Fig. 1).

No symptoms were observed on 13 species: *Ajuga reptans*, *Asarum canadense*, *Chrysogonum virginianum*, *Delosperma cooperi*, *Dianthus gratianopolitanus*, *Iris cristata*, *Liriope muscari*, *Liriope spicata*, *Mitchella repens*, *Ophiopogon japonicus*, *Phlox stolonifera*, *Sedum spurium*, and *Thymus serpyllum*.

Discussion

Cps is currently known to infect only plants in the family Buxaceae: boxwood, pachysandra, and sweet box (Ganci 2014; Kong

et al. 2017a, b; LaMondia 2017; LeBlanc et al. 2018; Malapi-Wight et al. 2016; Palmer and Shishkoff 2014; Shishkoff et al. 2015). This study demonstrates for the first time that *Cps* infected 12 plants outside of the Buxaceae family under controlled conditions. These included *Alchemilla mollis*, *Arctostaphylos uva-ursi*, *Brunnera macrophylla*, *Epimedium x youngianum*, *Galium odoratum*, *Geranium sanguineum*, *Phlox subulata*, *Tiarella cordifolia*, *Callirhoe involucrata*, *Iberis sempervirens*, *Mazus reptans*, and *Vinca minor*. These potential *Cps* hosts belong to 12 different plant families. These results have several practical implications.

Cps may have a much broader host range than currently known, and the results of this study should be a useful guide when looking

for natural infection of non-Buxaceae plants by this pathogen. *Cps* was initially known to attack only boxwood until Henricot et al. (2008) demonstrated that this pathogen could infect detached stems of *Sarcococca* sp. The publication of that study facilitated the discovery of natural infection of *Sarcococca hookeriana* var. *humilis* (Kong et al. 2017a; Malapi-Wight et al. 2016) and *Pachysandra terminalis* by *Cps* (Kong et al. 2017b), and it also stimulated the identification of *Pachysandra procumbens* (LaMondia and Li 2013), *Pachysandra axillaris* (LaMondia and Shishkoff 2017), and several other *Sarcococca* species (Ryan et al. 2018) as potential hosts. Similarly, the susceptibility studies at the U.S. Department of Agriculture Agricultural Research Service containment facility in Fort Detrick, Maryland

Fig. 1. Characteristic disease symptoms and sporulation of *Calonectria pseudonaviculata* on 12 non-Buxaceae, with lady's mantle, phlox, epimedium, mazus, and vinca sporulation photos from inoculations of detached leaves and remaining photos from whole-plant inoculations.

(Shishkoff 2012; Tooley and Browning 2009; Tooley and Kyde 2007) stimulated and facilitated the identification of many natural hosts of *Phytophthora ramorum*, a high-impact quarantine pathogen. It is worth noting that in this study a range of inoculum levels from 50 to 300 conidia per drop and from 30,000 to 120,000 conidia per 1 ml were tested for detached leaf and whole-plant assays, respectively. This range was chosen to reflect the inoculum load variations in nature. The highest concentration of 120,000 conidia per 1 ml used in this study was comparable with that used in the etiology studies of *Sarcococca* blight (Kong et al. 2017a) and *Pachysandra* leaf spot (Kong et al. 2017b), but it was much lower than those used in the studies on susceptibility to *Cps* of *Pachysandra terminalis* (LaMondia et al. 2012) and *Pachysandra procumbens* (LaMondia and Li 2013). The lowest concentration of 30,000 conidia per 1 ml used in this study was slightly greater than that (10,000 conidia per 1 ml) normally used in inoculating susceptible boxwood for boxwood blight epidemiology (Avenot et al. 2017; Marine et al. 2017) and management studies (Kong 2019; Kong and Hong 2017; Yang and Hong 2017, 2018).

While looking for natural infection of the plants identified in this study, it is advisable to add these 12 species along with pachysandra and sweet box to the watch list as potential carriers of *Cps* in the Boxwood Blight Cleanliness Programs (<http://www.vdacs.virginia.gov/plant-industry-services-boxwood-blight.shtml>). Our findings are important for production nurseries, retailers, and landscapers as well as public and private gardeners to be aware of as they undertake efforts to mitigate the risk of accidental *Cps* introduction. These 12 plant species are not as susceptible as boxwood, pachysandra, or sweet box to *Cps*. Disease symptoms do not develop as fast, and *Cps* sporulation on diseased tissues is not as conspicuous as it is on susceptible boxwood under the same environmental conditions. Thus, such as with pachysandra and sweet box (Kong and Hong 2019), if any of these 12 plants should become infected, it will be more likely than boxwood to escape the inspection process and serve as a *Cps* reservoir or carrier to new locales.

It is also advisable to take these 12 non-Buxaceae species into consideration when developing integrated disease management plans for boxwood blight. It would be advisable not to plant any of these 12 plants near boxwood crops and plantings. Garden managers for historic sites and other established public and private gardens are urged not to introduce any of these plants to areas adjacent to boxwood plantings. Landscapers and home gardeners are advised to exclude these 12 plants from the vicinity of new plantings of boxwood. Likewise, growers and retailers are recommended to avoid mingling any of these potential host plants with boxwood, pachysandra, and sweet box in production areas or sales yards. Before being placed in gardens containing boxwood, the plants identified here as potential hosts of *Cps* should be placed in isolated

areas without any fungicide protection and monitored for at least 4 weeks. If any disease symptoms are noted during this quarantine period, samples should be taken and sent to the state Plant Pathology Laboratory or university Plant Disease Clinic for analysis and verification. In the meantime, access to these holding areas should be restricted until the symptoms are determined not to be caused by the boxwood blight pathogen.

The 15 plant species that were not infected by *Cps* at any of the inoculum levels tested in both assays are safer choices as groundcovers or companion plants for established and new boxwood plantings. Use of these plants rather than those 12 potential hosts will reduce the risk of accidental *Cps* introduction to a new locale and retention of inoculum in a garden after a disease outbreak. Plant species choice is thus important in the vicinity of all boxwood plantings—established and new and whether or not boxwood blight is known to be present. Groundcovers have been considered for use as living mulch to prevent soil inoculum from splashing onto new growth of boxwood at sites of accidental *Cps* introduction (Likins et al. 2019). Only nonhost plant species should be considered for this use.

Koch's postulates have not been fulfilled on the 12 potential new hosts identified in this study, and therefore, this work has only pointed out the possibility of a much larger host range for *Cps* and has not given definitive proof of host status for any non-Buxaceae. Further studies are needed to explore and delineate the interaction of *Cps* and its sister species, *Calonectria henricotiae*, with these and other important ornamental crops. In particular, the reaction of *Arctostaphylos uva-ursi* to inoculations in these experiments indicates that further testing is immediately called for in other members of the Ericaceae because this family includes rhododendrons and azaleas, important garden staples of great value to the nursery industry.

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Fig. 2. Average disease severity rating on 12 non-Buxaceae groundcovers and companion plants along with the genus *Pachysandra* inoculated with *Calonectria pseudonaviculata* at 120,000 conidia per milliliter. Bars topped with the same letter did not differ according to the least significant difference test at $P = 0.05$.

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