Laboratory Assessment of Boxwood Blight Susceptibility of *Buxus* Accessions from the United States National Arboretum©

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INTRODUCTION

Boxwood (*Buxus*) is a very important landscape staple in the Northeastern United States in part because it is an evergreen that is not prone to deer browse. The new disease boxwood blight is caused by *Calonectria pseudonaviculata* (= *C. buxicola*), an invasive pathogen first noticed in the mid-1990s in the United Kingdom (Henricot and Culmam, 2002), spreading through Europe and to New Zealand (Crous et al., 2002) thereafter. The disease was first detected in the United States in 2011 in North Carolina and Connecticut (Ivors et al., 2011; Douglas et al., 2012). It has caused serious concern in the nursery/landscape industry not only because it can weaken and disfigure plants, destroying their aesthetic value, but also because infected leaves and stems contain microsclerotia that might persist in soil and organic debris for years (Weeda and Dart, 2012; Dart and Shishkoff, 2015). The ability of microsclerotia to germinate and produce conidial inoculum years after diseased plants are removed from a site makes replanting of boxwood in contaminated field nurseries or gardens difficult. In addition to boxwood, pachysandra (*Pachysandra terminalis*) has also shown symptoms of *C. pseudonaviculata* infection in the landscape, presumably originating from inoculum produced on diseased boxwood (LaMondia et al., 2012; Douglas, 2012). Learning which species and cultivars of *Buxus* are least susceptible to this new disease will be important information for landscape designers, as the disease has shown itself to be highly destructive in gardens where the pathogen has inadvertently been introduced. For this study, we collected cuttings of 42 boxwood accessions from the US National Arboretum in late July, 2013. Some of these cuttings were propagated for planting at different sites in Connecticut, North Carolina, New Jersey, and New York, where they will be either inoculated or exposed to natural infection by *C. pseudonaviculata*. Two sets of unrooted cuttings were promptly tested in vitro for their susceptibility to *C. pseudonaviculata* in a dip inoculation, and these results are reported here.

MATERIALS AND METHODS

A representative *C. pseudonaviculata* isolate (cbs114417) from the United Kingdom was used. Microsclerotia were produced by placing culture plugs of the pathogen onto the surface of autoclaved cellophane sheets (Biorad GelAir cellophane support, Bio-Rad Laboratories, Inc.) covering the surface of glucose-yeast extract-tyrosine (GYET) agar plates. After 1-2 months of incubation at 20°C, the surface of the cellophane was covered with microsclerotia. The cellophane could then be peeled from the surface of the culture and placed on fresh GYET agar, which caused the microsclerotia to produce copious numbers of conidia. These were collected in water and adjusted to 2000 spores/ml.

Each cutting was immersed in the spore suspension and then the cut end was placed in a 50-ml centrifuge tube filled with water. In each of the two consecutive trials, four cuttings from each cultivar were inoculated, and one was immersed in water alone to serve as a negative control. Cuttings were placed in a mist tent overnight exposed to the fog

produced from a model DK625 ultrasonic fogger. Cuttings were then placed in the greenhouse at 25°C and misted every 10 min. Symptoms of boxwood blight were observed and recorded at 7 days and 11 days after inoculation. At 7 days, the number of infected leaves and the number of leaves total per cutting was counted, along with the number of spots per leaf. Any fallen leaves were also rated and counted. At 11 days, the number of infected leaves and fallen leaves were recorded, as was the number of lesions per stem. These data were analyzed using General Linear Models for significance of the variables and Fisher's Least Significant difference to look for differences in susceptibility among cultivars.

RESULTS AND CONCLUSION

Many of the cuttings developed black leaf lesions that were evident within a week; leaf abscission followed in most instances and stem lesions were also noted (Table 1). As expected based on earlier research and observations, the English boxwood, *B. sempervirens* 'Suffruticosa,' was one of the most susceptible of the accessions. A number of American boxwood cultivars also proved highly susceptible in this detached-cutting assay, with some showing as much leaf spotting as English boxwood. Although laboratory studies are sometimes misleading, the relative performance of a number of these accessions in our study was found to be similar to field results reported by Ganci et al. (2012). The additional planned field trials will add more to our knowledge of the relative susceptibility of different *Buxus* species and cultivars, by including factors related to plant form — and under less conducive environmental conditions. This study has, however, identified a number of plants with the potential to show less susceptibility than English boxwood (and certain American boxwood cultivars) to this highly damaging new boxwood disease.

Table 1. Susceptibility of cuttings of 42 accessions of boxwood to *Calonectria pseudonaviculata*.

No.[a]	*Buxus* species and cultivar	Diseased leaves (%)[b]		Spots/ leaf[c]	Lesions/ stem[d]	Fallen leaves (%)[e]	
9548*H	*sempervirens* 'Scupi'	80.9	A	2.75	10.63	12.2	
59820*H	*sempervirens* 'Pendula'	76.4	AB	2.33	0.63	1.3	
29703*H	*sempervirens* 'Suffruticosa'	74.2	AB	1.99	1.50	6.5	
36365*J	*sempervirens*	71.5	ABC	2.22	2.75	14.3	
35494*H	*sempervirens* 'Rotundifolia'	70.4	ABC	1.74	6.88	34.7	AB
34196*H	*sempervirens* 'Denmark'	67.5	ABCD	2.83	3.38	15.2	CDEF
4233*H	*sempervirens* 'Handsworthiensis'	63.0	ABCDE	1.81	2.38	18.3	CDE
51910*H	*sempervirens* 'Northland'	62.1	ABCDE	1.47	5.38	21.5	BCD
31793*H	*sempervirens* 'Arborescens'	59.2	BCDEF	2.48	5.00	17.2	CDEF
29701*H	*sempervirens* 'Northern New York'	59.5	BCDEF	1.88	1.75	15.7	CDEF
18834*H	*harlandii*	52.5	CDEFG	3.93	1.88	20.8	CD
29694*H	*sempervirens* 'Marginata'	52.5	DEFG	1.19	1.25	4.2	
54327*H	*sempervirens* 'Newport Blue'	49.2	DEFGH	1.04	2.13	10.5	
57953*H	*sempervirens* 'Arborescens'	48.4	EFGHI	2.88	12.50	40.4	A
51907*H	'Green Velvet'	48.1	EFGHIJ	2.25	3.00	5.4	
68631*H	*sempervirens* 'Dee Runk'	46.5	EFGHIJK	2.65	3.88	22.3	BC
33789*H	*sempervirens* 'Graham Blandy'	46.6	FGHIJK	2.93	7.25	6.6	F
35487*H	*sempervirens* 'Edgar Anderson'	44.0	FGHIJKL	1.97	2.63	8.2	EF
29224*H	*microphylla* 'Grace Hendrick Phillips'	42.9	FGHIJKLM	2.51	1.75	9.0	
51905*H	'Green Mountain'	41.5	GHIJKLMN	1.67	1.63	16.8	CDEF
34198*H	*sempervirens* 'Myrtifolia'	41.5	GHIJKLMN	0.96	1.88	9.6	DEF
7025*H	*microphylla* var. *japonica* 'National'	40.4	GHIJKLMN	2.06	3.13	26.8	ABC
33810*H	*microphylla* 'John Baldwin'	39.8	GHIJKLMN	1.22	1.25	9.1	

Table 1. Continued.

No.[a]	*Buxus* species and cultivar		Diseased leaves (%)[b]	Spots/ leaf[c]	Lesions/ stem[d]	Fallen leaves (%)[e]	
72213*H	*microphylla* var. *japonica* 'Jim Stauffer'	37.4	GHIJKLMNO	1.70	0.63	7.1	
52423*H	*bodinieri*	36.2	HIJKLMNOP	2.29	0.75	13.9	
51904*K	'Green Gem'	34.8	HIJKLMNOPQ	1.91	0.38	7.3	
68273*H	'Glencoe'	33.3	IJKLMNOPQ	1.81	2.88	7.6	
51896*H	*wallichiana*	31.7	JKLMNOPQ	1.16	1.00	6.8	
6395*H	*sempervirens* 'Vardar Valley'	31.8	KLMNOPQR	0.98	1.88	3.0	
69558*H	*sempervirens* 'Ohio'	31.8	KLMNOPQR	1.50	3.25	0.0	
78079*H	*microphylla* var. *japonica* 'Gregem' , Baby Gem™ boxwood	28.5	LMNOPQRS	1.89	2.38	0.9	
71429*H	'Krazgreen', Green Ice® boxwood	28.6	MNOPQRS	1.06	5.00	0.0	
17078*H	*sempervirens* 'Decussata'	26.4	NOPQRS	2.56	3.63	16.2	CDEF
37772*H	*sinica* var. *insularis* 'Wintergreen'	23.8	OPQRS	1.14	4.50	8.9	
57950*H	*Buxus* sp.	21.6	PQRS	2.11	3.63	0.5	
51906*H	'Green Mound'	20.4	QRST	1.00	1.50	1.3	
51900*H	*sinica* var. *insularis* 'Winter Beauty'	17.5	RST	1.66	4.25	3.7	
51898*H	*sinica* var. *insularis* 'Pincushion'	16.6	ST	1.10	0.25	5.7	
54326*H	*microphylla* var. *japonica* 'Winter Gem'	7.3	T	0.63	1.88	4.6	
4899*CH	*microphylla* 'Compacta'	14.1		0.13	2.63	0.0	
4227*R	*microphylla* var. *japonica*	19.3		0.73	2.88	9.3	
60705*H	*sinica* var. *aemulans*	6.3		0.33	1.13	4.7	

[a] Accession number for the U.S. National Arboretum collection.

[b] The percentage of diseased leaves 11 days after inoculation. Numbers followed by the same letter do not differ significantly by General Linear models with LSD. Data not followed by a letter had to be excluded from the dataset because of excessive zeros preventing the normalization of the dataset.

[c] Spots counted on infected leaves 7 days after inoculation.

[d] Lesions counted on each stem piece 11 days after inoculation.

[e] The percentage of leaves that had dropped off over the 11-day period after inoculation. Numbers followed by the same letter do not differ significantly by General Linear models with LSD. Data not followed by a letter had to be excluded from the dataset because of excessive zeros preventing the normalization of the dataset.

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