Ranking Resistance of *Buxus* Cultivars to Boxwood Blight – an Integrated Analysis[1]

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Abstract

Boxwood (*Buxus* L. spp., Buxaceae) are popular woody landscape shrubs grown for their diverse forms and broad-leaved evergreen foliage, with an estimated $126 million economic impact in the U.S. alone. Boxwood plants grown in temperate zones worldwide are now threatened by a destructive blight disease caused by the ascomycete fungi, *Calonectria pseudonaviculata* and *C. henricotiae*. While the disease can be mitigated somewhat through cultural practices and fungicides, the most sustainable long-term solution is the development of disease-resistant boxwood cultivars. Hundreds of boxwood accessions from the National Boxwood Collection at the U.S. National Arboretum were screened for resistance using a lab-based, detached-leaf assay. Initial comparisons of our results with those of multiple other disease resistance assays found inconsistent ranking of cultivar resistance among studies. We used a meta-analysis approach on compiled data from six studies and were able to produce a consistent ordering of cultivars sorted by their susceptibility to boxwood blight, despite the diversity in materials and methods of the studies.

Index words: Boxwood, *Calonectria pseudonaviculata*, *Cylindrocladium buxicola*, meta-analysis, plant breeding, resistance screening.

Species used in this study: *Buxus bodinieri* H. Lev.; *B. harlandii* Hance; *B. microphylla* Seibold & Zucc.; *B. sempervirens* L.; *B. sinica* var. *insularis* (Nakai) M. Cheng; *B. wallichiana* Baill.; *Calonectria pseudonaviculata (Crous*, *J.Z. Groenew. & C.F. Hill) L. Lombard*, *M.J. Wingf. & Crous*, *2010*.

Significance to the Horticulture Industry

Boxwood is a valuable nursery commodity, with more than 11 million plants sold in the United States each year at a market value of $126 million. However, boxwood plants are threatened by boxwood blight, a destructive disease cause by a fungal pathogen that leads to defoliation and plant death in nurseries and established landscapes. The best long-term solution to combat this pathogen is to develop resistant cultivars. Multiple studies have been conducted to screen for resistance among cultivars; however, the results of these studies are sometimes inconsistent as to which cultivars are the most disease resistant. We compiled and evaluated data from several studies to produce a list of cultivars sorted by their susceptibility to boxwood blight. Results will enable further development of consistent and accurate resistance screening protocols and indicate the most promising taxa for developing more resistant cultivars.

Introduction

Boxwood (*Buxus* L. spp., Buxaceae) are popular woody landscape shrubs grown for their diverse forms and broad-leaved evergreen foliage. Each year, more than 11 million boxwood plants are sold in the United States, with an annual market value of $126 million (USDA-NASS 2015). Boxwood plants grown in temperate zones are threatened by a destructive blight disease caused by the ascomycete fungi, *Calonectria pseudonaviculata* and *C. henricotiae*. The disease was first identified in the United Kingdom in 1994 (Henricot and Culham 2002) and it has spread throughout continental Europe, parts of western Asia, New Zealand, and into North America. (Daughtrey 2019, Douglas 2012, Elmhirst et al. 2013, Gehesquière et al. 2013, Hagan and Conner 2013, Henricot and Culham 2002, Ivors et al. 2012, LeBlanc et al. 2018, Malapi-Wight et al. 2014). It causes dark lesions on leaves/stems and severe defoliation, leading to plant death in nurseries and established landscapes, hence the need to identify blight-resistant or tolerant boxwood cultivars. The National Boxwood Collection at the U.S. National Arboretum contains more than 700 *Buxus* accessions, making it one of the most complete collections in the world and a valuable genetic resource for developing blight-resistant varieties. In 2015-2017, we screened these accessions using a lab-based detached leaf assay, and found variability in susceptibility, but no complete resistance. However, we also found that in some cases our results were inconsistent with those of previous resistance screening assays, which were also inconsistent with each other regarding which cultivars were the least susceptible to boxwood blight. The objective of this study was to compile and analyze resistance screening data from multiple studies, including our own unpublished results, to determine which cultivars or taxa are consistently most disease resistant across assays and environments.

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Table 1. **Boxwood blight susceptibility studies used for meta-analysis. Letters are used to designate which data sets contributed to which cultivar ranking data in Table 2.**

Reference or name of study used	Reference in Table 2	Number of trials per cultivar available for use in meta-analysis (actually used)
Shishkoff et al. 2014	A	12 (10)
Ganci 2014	B	6 (6)
Shishkoff unpublished	C	6 (4)
LaMondia and Shishkoff 2017	D	5 (1)
Guo unpublished (unwounded inoculations)	E	3 (1)
Guo unpublished (wounded inoculations)	F	3 (3)

A common approach in meta-analysis is to use a mixed model, where the origin of each data set (study) is considered to be a random (or block) effect in a hierarchical model. This works well when the treatments (here, cultivars) are common to most studies, the number of treatments (cultivars) is small, and the method of evaluation is the same in all studies. We could not use this approach because we had a large number of cultivars, with the pairwise overlap of cultivars between two trials potentially small or zero, and the use of different kinds of evaluations among trials. However, the meta-analysis method we used, described below, works well for these kinds of data sets.

Materials and Methods

Resistance assays. Beginning in 2015, we screened 289 taxa in the National Boxwood Collection at the U.S. National Arboretum for relative susceptibility to boxwood blight, using the methods and experimental design of the leaf assays developed by Guo et al. (2016). We then selected 65 of the most resistant taxa for further replicated assays. The first study (data "E" in Table 1) followed our previously published methods. For our second study (data "F" in Table 1), we followed the same methods except that we wounded each leaf with a sterile pipette tip prior to inoculation. We found that this additional step greatly reduced the variability of symptom expression across replications. For both studies, 6-10 detached leaves from each boxwood accession were inoculated with 3 μl of a mycelium suspension of *Calonectria pseudonaviculata* strain NCBB-1 prepared as described in Guo et al. (2016) and placed in a covered water-agar petri plate to maintain humidity. Lesion sizes were measured using Assess2.0 (APS Press, St. Paul, MN) when infection on the susceptible control (leaves *of B. sempervirens* 'Suffruticosa') reached 75-100% of the leaf area, usually in approximately one week (Guo et al. 2015).

Data sources. For the meta-analysis, we incorporated data from our two studies described above and data from four other boxwood resistance studies, published or unpublished (Table 1). Data from Shishkoff et al. (2014), LaMondia and Shishkoff (2017), and Shishkoff (USDA-ARS, Frederick, MD, unpublished) were based on inoculation of stem cuttings from the National Boxwood Collection. Data from Ganci (2014) were based on inoculated plants in outdoor containers, as well as detached branches in a humidity chamber.

Data analysis. We used R code (R Core Team 2019) developed previously (Ehlenfeldt et al. 2010, Simko et al. 2012) to create a composite score based on principal components (PC) and resampling. The basic R code can be found in Simko et al. (2012) or is available from the authors.

Before creating the PC matrices, most of the trial variables used in the meta-analysis were transformed to provide, across their ranges, a more even distribution and more homogeneous variances for resistant and susceptible cultivars, as measured by that variable. For proportion/percent data, we used a logit transformation after subtracting a small number from 100% or adding a small number to 0% to avoid taking logs of zero. For other kinds of data, we looked at optimal Box-Cox transformations, based on models with the resistance score as the dependent variable and the cultivar as the independent variable. In most cases, log (X) or log (X + 1) were acceptable. All transformed scores were standardized to mean 0, variance 1.

A matrix was created with the results of each cultivar (131 rows) and trial variable (35 columns) combination (with many blank entries). In our context, we are using the word "trial" to mean the trials in one of the six studies in Table 1. Each trial in each study typically was done with several replications for each cultivar. We calculated means if we had the raw data available to represent the cultivar from that trial; otherwise we used the values reported in the study. Thus, the actual number of plant measures (as well as which cultivars were present) varied among trials. Note that some measures are inherently less accurate (e.g. percents) than others (e.g. measured lesion size), so the number of measures going into each value in the matrix does not necessarily translate into some values being more accurate than others. Also, a less accurate measure may actually better represent true resistance than a carefully measured value that is less correlated with true resistance.

A primary reason for carrying out this analysis was the documented inconsistencies among studies in determining blight resistance. We therefore expected some trials to be problematic in the analysis; they do not provide useful information since they can only be meshed arbitrarily with the other trials. In fact, the mean pairwise correlation between trials was only 0.17 (SD = 0.51), and approximately a third of the pairwise correlations were negative. Ten of the 35 trials were deemed too problematic to use, based on examining the distribution of pairwise correlations and were removed before proceeding. The median correlation for these ten trials with other trials was < 0.1. However, all sources contributed at least one trial to the final composite score. In other studies using this method (Ehlenfeldt et al. 2010, Simko et al. 2012), some cultivars also had to be removed, typically because they were extremes in trials with few cultivars. In this study, that was not necessary.

The algorithm for the analysis can be summarized as follows: To begin, two trials were randomly selecting and merged using those cultivars in common by creating a principal components (PC) axis; the unique cultivars (i.e., those not shared) in these two trials were then projected onto the PC axis using regression. This was repeated with other randomly selected pairs of trials sufficiently for all trials and cultivars to be represented in the combined scores on the resulting population of 1st order PC axes. This same process was then used for pairs of these first order PC axes, to build a population of 2nd order PC axes. The process was then iterated (pairs of 2nd order PC axes were used to build a population of 3rd order PC axes, pairs of 3rd order PC axes were used to build a population of 4th order PC axes, etc.) until all the resulting high order PC axes (holding a composite score for each cultivar) were essentially identical. This could occur in as few as 50 iterations or as many as 500. The entire process, starting with pairs of trials, was repeated 30 times, yielding 30 composite scores (30 high order PC axes) for each cultivar. From these 30 PC axes, we calculated, for each cultivar, their mean, mean rank, and estimates of uncertainty (SD of the 30 composite scores or their ranks).

Results and Discussion

Our dataset included results from studies that used diverse methods to evaluate resistance. For example, different studies used different isolates of the pathogen, took place in different environments (lab, greenhouse, field), utilized different plant parts (leaves, stems, whole plants), used different estimates of susceptibility (e.g. leaf drop, percent diseased leaf area, number of diseased leaves), occurred during different times of the year, and utilized different cultivars. Previous studies (Guo et al. 2016) found that in controlled experiments, inoculation methods did not significantly impact the relative resistance scores for cultivars. Specifically, using detached leaves gave comparable results to using whole plants, and inoculating at different times of the year did not affect relative expression of symptoms. Likewise, the use of mycelium vs. spores did not change relative symptom ratings among cultivars. Therefore, the reason for the variation seen in results among different resistance trials across multiple labs is not clear. Despite the diversity in materials and methods of the studies that were included in this analysis, our method of combining and analyzing many trials was successful in finding a consistent ordering of the boxwood cultivars. Table 2 lists the composite data for 131 boxwood taxa that were analyzed in this study. Taxa in Table 2 are listed by their estimate of relative susceptibility, with the least susceptible at the top; these have negative scores because scores are based on standardized variables with mean $= 0$, SD $= 1$.

It should be noted that although we use the term "resistant" in this study, there have been no reports to date of truly blight-resistant boxwood genotypes. Hence, this and other studies refer to a continuum of comparative resistance or susceptibility, rather than absolute resistance. It is because of this subtle distinction that we refer to cultivars at the top of Table 2 as "least susceptible" rather than "most resistant".

As with previous studies, this meta-analysis did not reveal clear trends of species or taxa that are more susceptible than others. While some of the most resistant cultivars represent species of *Buxus microphylla* and *B. sinica* and some of the most susceptible cultivars are *B. sempervirens* (Table 2), there are clearly exceptions to this trend. Thus, although generalizations about species resistance can be made based on this and other studies (Daughtrey 2019; Ganci 2014), screening of individual genotypes from diverse species and hybrids will continue to be necessary to identify the best genotypes, rather than relying on species identity to predict resistance.

Transforming the data for each trial prior to performing the analysis created more homogeneous variances and a more even distribution of values across the trial. This is important because, within the same trial, different cultivars can have different precisions, simply due to their resistance. For example, on a percent scale, a highly susceptible cultivar such as 'Suffruticosa' might have a lower percent (with accompanying lower variance, as is typically seen in biological data, where variances are smaller for smaller means) than a cultivar that falls in the middle range of susceptibility, with a higher percent (and a higher accompanying variance). While differences in precision were ignored during the creation of the composite score, they were at least partly remedied by using variance stabilizing transformations.

The uncertainty of the mean composite score of each of the 131 cultivars using this method is small enough to reliably rank the cultivars in relation to each other; that is, those deemed more resistant were consistently found to be more resistant in all 30 iterations of creating a composite score. This does not imply that the ordering is exact; rather, it means that one would expect similar orderings with different subsets of these trials.

These results will enable further development of consistent and accurate resistance screening protocols and indicate the most suitable material for developing more disease resistant cultivars. The meta-analysis resistance scores can ultimately be used to determine what factors (phenotypic and genotypic) affect resistance.

Table 2. Composite data for 131 boxwood taxa, including an estimate of relative susceptibility to boxwood blight, the standard error of that estimate, the mean rank, and the standard deviation (SD) of the rank. Taxa that are least susceptible are at the top. The number of trials used for each cultivar for the estimate and rank is also indicated, along with a list of which studies the data points came from. Cultivar names or designations are as they appeared in the original data. When known, species designations were added or modified to be consistent with current taxonomy (USDA-ARS 2019).

Cultivar/Accession	Susceptibility estimate	SE (estimate)	Mean Rank	SD (rank)	# trials used	Data source (Table 1)
B. sinica var. *insularis* 60705	-3.96	0.10	1.03	0.18	11	ADE
B. microphylla 'Little Missy'	-3.54	0.15	2.47	0.57	4	C
B. microphylla var. *japonica* 'Winter Gem'	-3.53	0.08	2.50	0.51	14	ABDE
B. SB17	-3.13	0.11	4.30	0.53	4	C
B. microphylla 'Compacta'	-3.03	0.11	5.13	0.86	11	ADE
B. microphylla var *japonica* 'Green Beauty'	-2.91	0.10	6.23	0.97	5	B
B. sempervirens 43877	-2.80	0.09	7.03	1.13	4	EF
B. microphylla 'Northern Emerald'	-2.80	0.06	7.30	0.65	5	B
B. microphylla var. *japonica* 4227	-2.42	0.09	9.20	0.48	10	AE
B. sinica var. *insularis* 'Wee Willie'	-2.27	0.08	10.20	0.71	2	B
B. sinica var. *insularis* 'Nana'	-2.17	0.09	11.10	0.88	6	B
B. harlandii 'Richard'	-2.07	0.12	12.20	1.52	10	BC
B. sinica var. *insularis* 'Pincushion'	-2.02	0.05	13.17	1.02	11	ADE
B. microphylla var. *japonica* 'Golden Dream'	-1.96	0.06	13.90	0.84	3	B
B. microphylla var. *japonica* 'Jim Stauffer'	-1.89	0.12	15.03	1.94	18	ABDEF
B. microphylla var. *japonica* 'Wedding Ring'	-1.74	0.06	16.90	1.18	5	B
B. 'Green Mound'	-1.74	0.08	16.90	1.45	15	ABDE
B. sinica var. *insularis* 'Winter Beauty'	-1.71	0.06	17.37	1.07	11	ADE
B. 57950	-1.62	0.10	19.17	1.58	12	ADE
B. sempervirens 'Ohio'	-1.61	0.09	19.40	1.30	11	ADE
B. sinica var. *insularis* 'Franklins Gem'	-1.54	0.04	20.50	0.90	5	B
B. microphylla 'Hohmans Dwarf'	-1.34	0.05	22.50	0.82	5	B
B. 'Green Ice'	-1.26	0.11	24.00	1.98	11	ADE
B. 'Verdant Hills'	-1.24	0.09	23.77	1.36	4	EF
B. microphylla 'John Baldwin'	-1.17	0.06	25.23	1.38	14	ABDE
B. sinica var. *insularis* 'Wintergreen'	-1.12	0.06	26.47	1.28	15	ABE
B. sempervirens 'Cliffside'	-1.12	0.09	26.50	1.80	4	EF
B. wallichiana 51896	-1.02	0.10	28.17	1.80	15	ADEF
B. 'Green Gem'	-1.01	0.07	28.63	1.45	14	ABDE
B. bodinieri 52423	-0.95	0.11	29.53	1.91	15	ADEF
B. microphylla v. *japonica* 'Gregem' baby gem	-0.89	0.07	30.93	0.94	11	AD
B. microphylla v. *japonica* 'Morris Midget variegated'	-0.86	0.07	31.30	1.02	2	B
B. sempervirens (K-019) 9425	-0.67	0.04	33.30	0.75	4	EF
B. sempervirens 'Myrtifolia'	-0.62	0.05	35.13	1.20	11	ADE
B. sempervirens 'Newport Blue'	-0.61	0.07	35.37	1.75	11	ADE
B. sempervirens 'Fastigiata'	-0.59	0.05	35.77	1.30	5	BEF
B. sempervirens (K-107) 9509	-0.55	0.04	36.93	1.23	4	EF
B. sinica var. *insularis* 34083	-0.47	0.08	39.73	2.53	4	EF
B. sempervirens 'Vardar Valley'	-0.47	0.07	39.73	2.70	18	ABCDE
B. harlandii 18834	-0.45	0.13	40.70	4.27	15	ADEF
B. sempervirens 'Highlander'	-0.44	0.05	40.20	1.88	2	B
B. sempervirens 'Washington Missouri'	-0.40	0.05	42.03	2.01	4	EF
B. microphylla 'Helen Whiting'	-0.39	0.05	42.50	2.10	1	E
B. sempervirens 'Abilene'	-0.35	0.09	43.80	2.91	7	BEF
B. 79112	-0.34	0.05	44.70	2.02	1	E
B. sempervirens 'North Star'	-0.31	0.04	45.53	2.00	2	B
B. sempervirens 'Woodland'	-0.27	0.08	47.10	2.44	6	BEF
B. sempervirens 'Decussata'	-0.26	0.08	47.43	2.76	12	ADE
B. sempervirens 'Unraveled'	-0.21	0.06	48.97	2.08	2	B
B. sempervirens 'Marginata'	-0.20	0.04	49.60	1.22	12	ABDE
B. sempervirens 'Pier Cove'	-0.16	0.07	50.37	1.83	1	B
B. sempervirens 'Holland'	-0.11	0.04	52.23	1.30	2	B
B. sempervirens 'Myosotidifolia'	-0.09	0.07	52.97	1.97	1	B
B. sinica var. *insularis* 'Sprinter'	-0.02	0.07	55.90	2.83	1	B
B. sempervirens 4212	-0.01	0.05	56.13	2.44	1	E
B. sempervirens (K-040) 9444	-0.01	0.05	56.13	2.44	1	E
B. sempervirens 'Angustifolia'	0.03	0.06	57.63	3.00	9	BEF
B. sempervirens 'Undulifolia'	0.07	0.07	59.40	3.16	4	EF
B. 'Glencoe' Chicagoland green	0.07	0.08	59.90	3.46	12	ABDE
B. sempervirens 'Edgar Anderson'	0.08	0.06	60.13	3.31	15	ADEF
B. sempervirens 'Ransom'	0.12	0.10	62.10	4.93	4	EF
B. sempervirens 'Handworthiensis'	0.13	0.12	63.03	6.04	13	ABDE
B. 'Conrowe'	0.13	0.05	62.93	2.60	1	B
B. microphylla 'Green Pillow'	0.13	0.05	62.93	2.60	1	B

Table 2. Continued.

Cultivar/Accession	Susceptibility estimate	SE (estimate)	Mean Rank	SD (rank)	# trials used	Data source (Table 1)
B. sempervirens 82675	0.14	0.05	62.87	2.98	1	E
B. microphylla (seedling selection) 33811	0.19	0.05	65.60	3.39	1	E
B. sempervirens 'Ashville'	0.22	0.07	66.07	3.51	1	B
B. sempervirens 'Rochester'	0.26	0.07	69.47	3.49	2	B
B. sempervirens 'Latifolia Aurea Maculata'	0.28	0.08	70.27	3.33	1	B
B. sempervirens 'Welleri'	0.28	0.04	69.67	2.56	2	B
B. sempervirens 'Longwood'	0.29	0.11	70.90	5.76	4	B
B. sempervirens (K-100) 9502	0.33	0.05	72.70	3.25	1	E
B. sempervirens (K-146) 9543	0.33	0.05	72.70	3.25	1	E
B. sempervirens 'Aurea Maculata'	0.33	0.05	73.33	3.17	3	B
B. sempervirens 'Natchez'	0.34	0.03	73.60	1.83	3	BE
B. sempervirens 'Pullman'	0.37	0.04	76.03	2.08	2	B
B. microphylla var. *japonica* 'Sunnyside'	0.38	0.06	76.30	2.88	1	E
B. sempervirens (K-094) 9498	0.42	0.06	78.97	2.55	1	E
B. sempervirens 'Route 50'	0.44	0.08	79.33	3.99	1	B
B. 'Meyer Columnar'	0.47	0.04	80.57	1.61	2	B
B. microphylla var. *japonica* 'Morris Midget'	0.47	0.08	80.33	3.80	3	B
B. Thomas Jefferson	0.47	0.12	79.87	5.87	4	C
B. sempervirens 'Black American'	0.50	0.07	81.97	2.28	2	B
B. sempervirens 'Memorial'	0.55	0.04	83.83	1.32	2	B
B. sempervirens 'Berlin'	0.63	0.05	86.67	1.84	2	B
B. sempervirens 'Pendula'	0.63	0.11	87.27	4.35	11	ADE
B. 'Green Mountain'	0.64	0.08	87.23	2.99	16	ABDEF
B. sempervirens 'Northern New York'	0.64	0.09	87.50	3.77	13	ABDE
B. microphylla var. *japonica* 'National'	0.68	0.09	88.77	3.41	11	ADE
B. sempervirens 'Bob Dunn'	0.70	0.04	90.00	1.72	2	B
B. sempervirens (K-146) 9543	0.71	0.06	89.60	2.87	1	E
B. sempervirens 'Hermann von Schrank'	0.72	0.04	91.20	1.90	2	B
B. sempervirens (K-089) 9494	0.76	0.06	92.40	2.91	1	E
B. sempervirens 18608	0.81	0.06	94.73	3.04	1	E
B. sempervirens 'Mary Gamble'	0.84	0.03	95.83	1.34	3	BE
B. microphylla var. *japonica* 'Morris Dwarf'	0.87	0.06	96.60	2.50	1	B
B. sempervirens 36365	0.91	0.11	98.90	4.83	11	ADE
B. sempervirens 'Halifax American'	0.93	0.07	99.57	2.80	2	B
B. sinica var. *insularis* 'Justin Browers'	0.94	0.07	99.93	3.22	6	BC
B. 'Green Velvet'	0.95	0.09	100.50	4.12	20	ABCDE
B. sempervirens 'Henry Shaw'	0.95	0.16	100.40	6.59	2	BE
B. sempervirens 'Jensen'	0.98	0.05	101.37	2.34	2	B
B. sempervirens 'Graham Blandy'	1.00	0.08	102.67	3.46	11	ADE
B. sempervirens 'Morrison Garden'	1.02	0.05	103.53	1.94	2	B
B. sempervirens 'Dee Runk'	1.02	0.07	103.80	3.35	18	ABCDE
B. sempervirens 'Latifolia Macrophylla'	1.04	0.07	104.93	3.77	4	BE
B. sempervirens 'Russian Blue'	1.07	0.05	106.50	2.24	1	B
B. sempervirens 'Ipek'	1.12	0.09	108.70	4.51	2	BE
B. sempervirens 'Argentea'	1.13	0.05	109.87	2.29	1	B
B. sempervirens 'American'	1.13	0.07	109.67	3.08	3	B
B. sempervirens 'Fineline'	1.14	0.05	110.00	2.24	2	B
B. sempervirens (K-065) 9470	1.19	0.07	111.47	3.27	1	E
B. sempervirens 'Elegantissima'	1.19	0.07	112.07	2.64	1	B
B. sempervirens 'Liberty'	1.24	0.05	114.07	1.44	1	B
B. microphylla 'Grace Hendrick Phillips'	1.25	0.11	113.17	3.13	14	ABD
B. microphylla var. *japonica* 4223	1.28	0.07	115.03	2.01	1	E
B. sempervirens 'West Ridgeway'	1.34	0.06	116.33	1.15	1	B
B. sempervirens 'Northland'	1.56	0.09	118.50	0.94	15	ADEF
B. sempervirens (K-099) 9501	1.67	0.08	119.37	0.78	1	E
B. microphylla var. *japonica* 33902	1.67	0.08	119.37	0.78	1	E
B. sempervirens 'Denmark'	1.85	0.08	121.00	0.64	14	ABDE
B. sempervirens 'Rotundifolia'	1.94	0.11	122.07	0.78	17	ABDE
B. sinica var. *insularis* 'Miss Jones'	2.09	0.10	123.33	1.15	1	E
B. sempervirens 'Scupi'	2.16	0.10	123.97	1.19	11	ADE
B. sempervirens 'Arborescens'	2.27	0.09	125.23	1.04	12	AEBD
B. harlandii 36672	2.39	0.11	126.70	1.32	3	F
B. sempervirens 'Suffruticosa'	2.39	0.13	126.50	1.11	21	ABDEF
B. sempervirens 'Pendula'	2.42	0.13	126.97	1.22	2	ADE
B. sinica var. *insularis* 'Tall Boy'	2.81	0.12	128.97	0.18	1	E
B. sempervirens 'Salicifolia'	2.86	0.12	131.00	0	1	E
B. microphylla var. *japonica* 35485	3.10	0.13	131.00	0	1	E

Literature Cited

Daughtrey, M.L. 2019. Boxwood blight: Threat to ornamentals. Ann. Rev. Phytopathol. 57:189–209.

Douglas S.M. 2012. Boxwood blight - A new disease for Connecticut and the U.S. The Connecticut Agricultural Experiment Station. https://portal.ct.gov/-/media/CAES/DOCUMENTS/Publications/Fact_Sheets/Plant_Pathology_and_Ecology/BOXWOODBLIGHTANEWDISEASEFORCONNECTICUTANDTHEUS120811pdf.pdf?la=en. Accessed September 30, 2019.

Ehlenfeldt, M.K., J.J. Polashock, A.W. Stretch, and M. Kramer. 2010. Ranking cultivated blueberry for mummy berry blight and fruit infection incidence using resampling and principal components analysis. HortScience 45:1205–1210.

Elmhirst, J.F., B.E. Auxier, and L.A. Wegener. 2013. First report of box blight caused by *Cylindrocladium pseudonaviculatum* (*C. buxicola*) in British Columbia, Canada. Plant Dis 97:559.

Ganci, M.L. 2014. Investigation of host resistance in Buxus species to the fungal plant pathogen *Calonectria pseudonaviculata* (=*Cylindrocladium buxicola*), the causal agent of boxwood blight and determination of overwinter pathogen survival. MS Thesis, NC State University, Raleigh, NC. 124 p.

Gehesquière, B., S. D'Haeyer, K.T.K. Pham, A.J. Van Kuik, M. Maes, M. Höfte, and K. Heungens. 2013. qPCR assays for the detection of *Cylindrocladium buxicola* in plant, water and air samples. Plant Dis 97:1082–1090.

Guo, Y.H, R.T. Olsen, M. Kramer, and M. Pooler. 2015. Effective bioassays for evaluating boxwood blight susceptibility using detached stem inoculations. HortScience 50:268–271

Guo, Y.H., R.T. Olsen, M. Kramer, and M. Pooler. 2016. Use of mycelium and detached leaves in bioassays for assessing resistance to boxwood blight. Plant Dis. 100:1622–1626.

Hagan A.K. and K. Conner. 2013. Boxwood blight - A new disease of boxwood in the nursery and landscape in Alabama. https://sites.aces.edu/group/timelyinfo/Documents/2013%20Boxwood%20Blight%20TI%20Updated.pdf. Accessed September 30, 2019.

Henricot B. and A. Culham. 2002. *Cylindrocladium buxicola*, a new species affecting *Buxus* spp., and its phylogenetic status. Mycologia 94:980–997.

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

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

LeBlanc, N., C. Salgado-Salazar, and J.A. Crouch. 2018. Boxwood blight: an ongoing threat to ornamental and native boxwood. Appl. Microbiology and Biotechnology 102:4371–4380.

Malapi-Wight M, J.B. Hébert, R. Buckley, M.L. Daughtrey, N.F. Gregory, K. Rane, S. Tripak, and J.A. Crouch. 2014. First report of boxwood blight caused by *Calonectria pseudonaviculata* in Delaware, Maryland, New Jersey and New York. Plant Dis 98:698.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/. Accessed September 30, 2019.

Shishkoff, N., M. Daughtrey, S. Aker, and R.T. Olsen. 2014. Evaluating boxwood susceptibility to *Calonectria pseudonaviculata* using cuttings from the National Boxwood Collection. Plant Health Progress doi:10.1094/PHP-RS-14-0033.

Simko, I., R.J. Hayes, and M. Kramer. 2012. Computing integrated ratings from heterogeneous phenotypic assessments: a case study of lettuce postharvest quality and downy mildew resistance. Crop Sci. 52:2131–2142.

USDA-ARS, National Plant Germplasm System. 2019. Germplasm Resources Information Network (GRIN-Taxonomy). National Germplasm Resources Laboratory, Beltsville, Maryland. https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearch.aspx. Accessed September 30, 2019.

USDA – NASS. 2015. Census of Agriculture - Census of Horticultural Specialties (2014). https://www.nass.usda.gov/Publications/AgCensus/2012/Online_Resources/Census_of_Horticulture_Specialties/. Accessed September 30, 2019.