Rooting Response of Boxwood Cultivars to Hot Water Treatment and Thermal Sensitivity of *Calonectria henricotiae* and *C. pseudonaviculata* in Diseased Boxwood (*Buxus* spp.)[1]

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Abstract

Boxwood blight is caused by *Calonectria henricotiae* (Che) and *C. pseudonaviculata* (Cps). Unrecognized symptoms on *Buxus* cuttings used for propagation could potentially serve as a source of inoculum and result in pathogen spread. In this study, cuttings of boxwood (*Buxus* spp.) cultivars 'Justin Brouwers', 'Nana', 'Green Beauty', and 'Green Velvet' were assessed for root production after exposure to 45 C (113 F) or 47.5 C (118 F) water for 0 to 60 minutes in 5 minute increments. The number of roots greater than 1 cm (0.4 in) in length produced by cuttings of all cultivars three months after treatment in 45 C water for up to 60 minutes was not statistically different from the non-immersed control. A similar response was observed for cuttings of all cultivars treated in 47.5 C water for up to 60 minutes, except for cv. 'Nana,' which produced fewer roots than the non-immersed control after 35 minutes of exposure to heated water. Experiments conducted on diseased, detached boxwood leaves of susceptible cultivar 'Justin Brouwers' at 47.5 C, 50 C (122 F) or 52.5 C (127 F) showed significantly reduced production of conidia and viability of Che and Cps after 25 to 30 minute exposure to 47.5 C water (44%) or 12 minute exposure to 50 C water (22%). After 8 minutes of exposure to 52.5 C water, little or no sporulation was observed for either pathogen.

Index words: Boxwood blight, *Calonectria pseudonaviculata*, *Calonectria henricotiae*, *Buxus*, hot water treatment, rooting, cuttings, plant propagation.

Species used in this study: Boxwood blight [*Calonectria henricotiae* Gehesquière, Heungens and J.A. Crouch and *C. pseudonaviculata* (Crous J.Z. Groenewald & C.F. Hill) L. Lombard, M.J. Wingf & Crous], Boxwood [*Buxus sempervirens* L. 'Justin Brouwers'; *Buxus sinica* (Rehder & E. H. Wilson) M. Cheng var. *insularis* (Nakai) M. Cheng 'Nana'; *B. sempervirens* 'Suffruticosa'×*B. sinica* var. *insularis* 'Green Beauty', and *B. sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis* 'Green Velvet'].

Significance to the Horticulture Industry

The production of healthy boxwood cuttings is important for reducing the introduction and spread of boxwood blight disease to nurseries and the landscape. In this study, we investigated the potential use of hot water as a cost-effective and non-chemical way to reduce the occurrence and spread of boxwood blight disease to growers, homeowners and the nursery trade in the United States. Our approach involved determining the time and water temperature needed to kill the boxwood blight fungus without causing damage and injury to boxwood cuttings. We determined that cuttings of four commonly grown boxwood cultivars were able to withstand treatment in 47.5 C (117 F) water for 1 hour without damaging leaves or the ability of cuttings to form roots to a standard level accepted by growers. After 25-30 minutes of treatment in heated water at this temperature, we found that fungal growth and spore production were greatly reduced on boxwood branches and leaves. We conclude that with careful attention to the boxwood cultivar being treated, it may be possible to include hot water immersion in an integrated pest management system for propagating cuttings to produce healthy boxwood plants.

[1]Received for publication September 25, 2020; in revised form November 23, 2020. This research was partially supported by the USDA-APHIS-CPHST through the FY2013, FY2014, FY2015, and FY2016 Farm Bills received in collaboration with Cristi Palmer, IR-4 Project and Gregory Parra USDA APHIS PPQ Science and Technology. We would like to thank Bennett Saunders for providing boxwood plants for our experiments. We thank Elaine Cerchin, Bianca Hoch, and Rachel Norris for technical assistance and Margery Daughtrey and Jerry Weiland for helpful review. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture (USDA). The USDA is an equal opportunity employers and provider.

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Introduction

Boxwood (*Buxus* sp.) is a popular and widely grown woody ornamental that is deer resistant, mostly evergreen, and low maintenance with a relatively long lifespan in the absence of boxwood blight disease. Boxwood is commonly used in the landscape as hedges, specimen plants and topiary, while cut greenery are sold as boxwood holiday wreaths (part of the $46 million dollars in sales for cut greenery in 2015 (USDA National Agricultural Statistics Service 2017). Annual sales of boxwood in the United States (US) in 2012 were approximately $126 million (USDA National Agricultural Statistics Service 2014). Boxwood blight disease is caused by *Calonectria pseudonaviculata* (Crous, J.Z. Groenewald & C.F. Hill) L. Lombard, M.J. Wingf & Crous. (syn=*Cylindrocladium pseudonaviculatum* Crous, J.Z. Groenewald & C.F. Hill =

Cylindrocladium buxicola Henricot) and *Calonectria henricotiae* Gehesquière, Heungens and J.A. Crouch, a second species that occurs in Europe not currently found in the US (Gehesquière et al. 2015). *C. pseudonaviculata* (Cps) was first reported in the US in Connecticut and North Carolina in 2011 (Ivors et al. 2012) and has since spread to 28 other states (Daughtrey 2019, Hong 2019). These 28 states account for 95% of the total boxwood production in the US (USDA National Agricultural Statistics Service 2014). Leaf symptoms are typically characterized by brown to black, circular lesions with a necrotic center surrounded by a yellow halo (LeBlanc et al. 2018). Eventually, the entire leaf becomes necrotic, resulting in blighting and defoliation (Henricot and Culham 2002). The pathogen can also cause elongated, irregular-shaped black stem cankers (Weeda and Dart 2012). Leaf and stem symptoms caused by *C. henricotiae* (Che) are similar (Gehesquière et al. 2015).

Management of boxwood blight requires integrated use of cultural practices, preventive fungicides and host plant resistance strategies. Recent investigations suggest that all commercial boxwood cultivars are susceptible to blight disease (Ganci 2014, Shishkoff and Olsen 2015, Miller et al. 2016, LaMondia and Shishkoff 2017). *Buxus sempervirens* 'Suffruticosa', or common English boxwood, is highly susceptible and severely affected by the disease, but cultivars and hybrids with less susceptibility have been identified (Ganci 2014, Shishkoff and Olsen 2015, Miller et al. 2016, LaMondia and Shishkoff 2017). In general, Asiatic species of boxwood (e.g., *Buxus harlandii, B. microphylla,* and *B. sinica*) are less susceptible to boxwood blight disease than cultivars within the European species *B. sempervirens* (such as 'Justin Brouwers' and 'Vardar Valley'). The hybrid cultivars 'Nana' (*B. sinica* var. *insularis*), 'Green Beauty' (*B. sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis*), and 'Green Velvet' (*B. sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis*) are less susceptible than *B. sempervirens* (Ganci 2014).

The increased incubation period (time required from application of fungal spores to leaf symptom development) and delay in the subsequent production of another successive generation of asexual spores (latent period) on less susceptible boxwood cultivars decreases progression of boxwood blight disease over time (Ganci 2014). Ganci (2014) demonstrated under controlled environmental conditions that spore production on the abaxial leaf surface of less susceptible cultivars is delayed by up to 7 days compared to more susceptible cultivars. Disease symptoms on cultivars with less susceptibility are often manifested as small inconspicuous dark necrotic spots. The inconspicuous nature of disease symptoms on less susceptible cultivars creates challenges for disease scouting, especially on boxwood cuttings during propagation. Boxwood is often propagated by placing dormant vegetative cuttings in soilless potting media maintained at high moisture content and relative humidity. When collecting cuttings for propagation, disease symptoms in the form of small lesions can easily be ignored, resulting in movement of infected plants into propagation houses that provide a conducive environment for disease development. Because frequent application of preventive fungicides is often cost-prohibitive, growers are interested in an alternative strategy for managing boxwood blight disease during propagation. A method to thermally kill the pathogen in plant tissue would help growers prevent or reduce disease spread through commercial channels. Movement of infected plant stock without readily recognizable symptoms can lead to outbreaks in new geographic production areas. Treatment of boxwood cuttings during propagation could potentially minimize the risk of disease occurrence and spread.

Cuttings have been treated in various ways to reduce the likelihood for introducing pathogens during plant propagation. Morgan and Colbaugh (1983) suggested that the benefits of disinfesting potting media are negated if a pathogen is introduced on cuttings. Hot water treatment, a type of thermotherapy, has been used to mitigate diseases caused by bacteria, fungi, nematodes, and viruses in corms, fruits, seeds, tubers, and vegetables for more than 75 years (Baker 1962). Treatment of woody propagative material with hot water has been previously shown to eliminate or reduce plant pathogenic bacteria from pecan [*Carya illinoinensis* (Wangenh.) K.Koch] , pear (*Pyrus* spp.), and apple (*Malus domestica* Borkh.) scions, and grape (*Vitus vinifera* L.) vine cuttings (Sanderlin and Melanson 2008, Burr et al. 1989, Goheen et al. 1973).

However, studies investigating the response of woody plants to hot water treatment and its potential for eliminating plant pathogenic fungi in woody propagative material are limited. The fungal pathogens *Phaeomoniella* chlamydospora (W. Gams, Crous, M.J. Wingf. & Mugnai) Crous & W. Gams 2000 and *Phaeoacremonium* spp., which cause Petri disease of grape, were successfully eliminated from grapevines treated in hot water at 50 C for 30 minutes (Fourie and Halleen 2004). However, grape cultivars varied in sensitivity to hot water treatment (Waite and Morton 2007). Morello et al. (2015) evaluated cuttings of olive to prevent spread of *Verticillium dahliae*, a microsclerotial pathogen of olive cultivars that can survive for years in soil. The pathogen was detected in nurseries in Spain and Italy and suspected of being transported on propagated material into previously disease-free orchards. Heat treatment seemed promising because of the combination of a heat-sensitive pathogen and a heat-insensitive host. Five olive cultivars were evaluated as whole plants and classified according to their thermotolerance and an optimized treatment was developed to eliminate the pathogen from olive (*Olea europaea* L.) plants with temperatures of 42–44 C (108-111 F) for 6-12 hours. However, the rooting ability of the olive cuttings exposed to heat was severely affected. The treatment of azalea (*Rhododendron* spp.) cuttings artificially inoculated with *Rhizoctonia* anastomosis group U (AG-U, teleomorph=*Ceratobasidium* sp. formerly AG-P) in 50 C water for 21 minutes prevented development and spread of the pathogen and reduced occurrence of Rhizoctonia web blight disease without reducing rooting of cuttings (Copes and Blythe 2009, 2011). According to Baker (1962), cuttings of woody ornamental plants may be more amenable to hot water treatment due to their relatively low moisture content. Before hot water treatment of

Table 1. Designation, plant host species, isolation date, and geographic location for isolates of *Calonectria henricotiae* and *C. pseudonaviculata* used for thermal inactivation studies conducted at the USDA-ARS, Foreign Disease-Weed Science Research Quarantine Facility, Ft. Detrick, MD.

Fungal species	Designation	Plant host species	Isolation date	Geographic location
Calonectria henricotiae	55 (JKI 2106)	*Buxus* sp.	2007	Germany
	78-che (NL009)	*B. sempervirens*	2011	The Netherlands
	182-che (CB045)	*B. sempervirens*	2009	Belgium
	209-che (bg209a)	*Buxus* sp.	-	The Netherlands
Calonectria pseudonaviculata	BB5b	*B. sempervirens* 'Suffruticosa'	2011	North Carolina, USA
	BD6c	*B. semperviren* 'Suffruticosa'	2014	North Carolina, USA
	T (CBS114417)	*B. sempervirens* 'Suffruticosa'	2001	Belgium
	CT-1	*B. sempervirens* 'Suffruticosa'	2013	Connecticut, USA

boxwood cuttings infected with Cps is deployed, however, a comprehensive investigation of the rooting response of boxwood cuttings after heat treatment is required. To our knowledge, there are no studies of the effect of heat on rooting for *Buxus*.

The objective of this study was to assess the rooting response of boxwood cuttings of four commonly grown cultivars to hot water treatment. We tested the hypotheses that 1) treating boxwood cuttings in 45 C or 47.5 C water for 30 minutes will not reduce root production, 2) root production will vary among boxwood cultivars treated for more than 30 minutes in 45 C or 47.5 C water, and 3) treating boxwood cuttings in 47.5 C water for 30 minutes will reduce Che and Cps viability and asexual spore (conidium) production in diseased leaves and branches. Prior to conducing our studies, the baseline *in vitro* thermal sensitivity of Che and Cps conidia and microsclerotia was documented (Miller et al. 2018), but the relative *in planta* response of hot water treatment on pathogen viability and production of conidia in diseased boxwood leaves and stems was not yet known. It was also known that exposure to air temperatures >50 C for 24 h or longer, and exposure in a composting system for 48 h or longer at 40 C would kill the microsclerotia of boxwood blight (Harvey et al. 2019).

Materials and Methods

Isolates and conidia production. Four monoconidial isolates each of *Calonectria pseudonaviculata* (BB5b, BD6c CT-1, and T and *Calonectria henricotiae* (55-che, 78-che, 182-che, and 209-che) were used in thermal sensitivity experiments (Table 1). To produce conidia, 8-cm (3.1 in) cellophane disks (Biorad GelAir cellophane support, Bio-Rad Laboratories, Inc.) covering the surface of glucose-yeast extract-tyrosine medium (GYET; Hunter 1992) in 9-cm (3.5 in) diameter Petri dishes were inoculated and incubated for at least one month to form microsclerotia on the cellophane. To produce conidia from microsclerotia, cellophane was peeled from the surface of the culture and placed on fresh GYET medium, which stimulated production of conidia after 4-6 days. Conidia were collected in water with 0.1% v/v Tween 20 and adjusted to 2000 conidia/mL using a hemocytometer.

Source and maintenance of boxwood cultivars. Four boxwood cultivars ''Justin Brouwers' (*Buxus sempervirens*), 'Nana' (*B. sinica* var. *insularis*), 'Green Beauty' (*B. sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis*), and 'Green Velvet' (*B. sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis*), ranging in age from 3-to 5-years old, were obtained in 1 gal pots from hoop houses at Saunders Brothers Nursery in Piney River, VA in mid-December 2015 and transported to hoop houses at the North Carolina State University (NCSU) Horticultural Field Laboratory. Prior to conducting experiments, potted plants were moved from the field facility and placed in a greenhouse maintained at 18 C (64 F) at NCSU for 6 to 10 days.

Hot water treatment experiments to assess boxwood cultivar sensitivity. To examine sensitivity of boxwood cultivars to hot water treatment, cuttings were taken from the top 15-cm (6 in) of the mother plant and leaves were removed from the bottom 7.5 cm (3 in) of each cutting. For each cultivar, two cuttings were placed in 50 mL Falcon tubes (Corning, Inc., Tewksbury, M.A.) with six 1-cm diameter holes to facilitate water movement through the tube (Fig. 1). Cuttings from each cultivar were individually treated at 45 C and 47.5 C in a randomized complete block design in a 19-L circulating water bath (Model 289; Precision-Fisher Scientific, Hampton, NH). Due to limited space in the two water baths, cuttings were treated at two time intervals in separate batches for 0-30 and 35-60 minute intervals at 45 and 47.5 C, with tubes removed at 5 min intervals during each treatment period. A non-immersed time zero and room temperature (immersed in 23 C (73 F) water for 30 and 60 minutes) control was included in each experiment. For each time point during the 0-30 and 35-60 minute exposure intervals, there were three replicate tubes containing two cuttings (subsamples). Boxwood cuttings were dipped in C-mone K+ rooting hormone (1% K-I Indole-3-butyric acid + 0.5% naphthalene acetic acid) solution (Coor Farm Supply, Smithfield, NC) for 3 seconds immediately following treatment and planted in soilless peat-based potting media (Farfard 2P, Sun Gro Horticulture, Agawam, MA) in a 32-cell tray in a randomized complete block design. Each experiment was conducted twice: with experiment 1 (cuttings were exposed for 0 to 30 minutes) and experiment 2 (cuttings exposed for 35-60 minutes) conducted on 3 March 2016 and experiments 3 and 4 (repeating 1 and 2) conducted on 14 March 2016.

Growth conditions and root assessment. Treated boxwood cuttings were placed in a PVC-framed, plastic-covered propagation house at the Horticulture Field

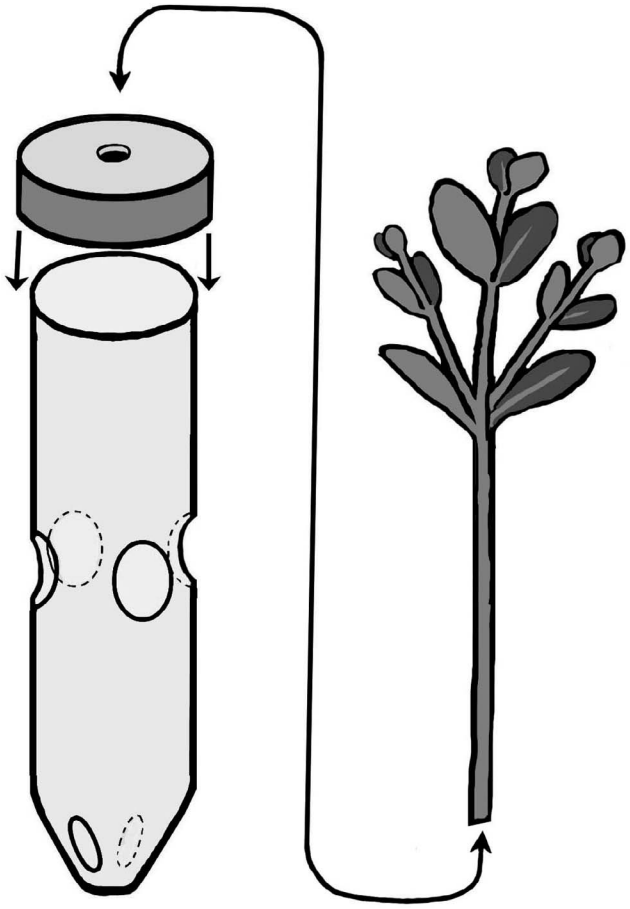

Fig. 1. To expose cuttings to hot water, cuttings were placed in 50 mL Falcon tubes modified with six 1-cm diameter holes to facilitate water movement through the tube.

Laboratory at NCSU, with the floor heated to 21 C (70 F), and fog misted for 6 s every 8 min for the duration of the experiment. Ambient daily average air temperature inside the propagation house was 20 C in March, 22 C in April, and 25 C (77 F) in May. Beginning on 7 June 2016 for experiments 1 and 2, and 13 June 2016 for experiments 3 and 4, cuttings were removed from the trays and the number of roots greater than 1 cm in length was counted.

Thermal inactivation of Calonectria henricotiae and C. pseudonaviculata in diseased boxwood leaves and stems. An experiment was conducted to determine the thermal sensitivity of Che and Cps in diseased, detached leaves and branches exposed to temperatures of 47.5, 50 and 52.5 C. Three-year-old boxwood plants (*Buxus sempervirens* 'Justin Brouwers') were artificially inoculated with a conidial suspension (final concentration 2000 spores/mL) combining equal volumes of either four isolates of *C. henricotiae* (55, 182, 78, and G2) or four isolates of *C. pseudonaviculata* (BB5b, BD6c, T, and CT1). Inoculated plants were placed in a dew chamber at 20 C for 2 d, and then placed in a mist tent in a greenhouse at 22 ± 2 C. As symptomatic leaves abscised from plants, they were collected on a bed of moist sand and misted for 3 weeks to promote development of microsclerotia. At the end of 3 weeks symptomatic branches remaining on the plant after diseased leaves had fallen were cut into 1-cm lengths and collected. For immersion in hot water, detached leaves and branches were placed in 90×20 mm plastic mesh bags (25 µm mesh size, 100% polyamide Sefar Nitex bags, Sefar Inc., 111 Calumet St. Depew NY 14043). Twelve detached leaves or six detached branches were placed in each bag. Time zero controls were bags dipped in room temperature water. Remaining samples were suspended in a water bath (Polyscience model WB02) at 47.5, 50 or 52.5 C, and 2-3 bags per treatment were removed at each sampling time and immediately dipped in room temperature water to stop the heat treatment. At 47 C, the sample exposure times were 0, 5, 10, 15, 20, and 25 minutes (also 30 minutes for the second experiment); at 50 C, sample exposure times were 0, 2, 4, 6, 8 and 10 minutes; at 52.5 C, were 0, 2, 4, 6 and 8 minutes. At the conclusion of heat treatment, Nitex bags were split at a seam, and detached leaves and branches placed on the surface of 9-cm diameter plastic Petri plates containing GYET medium. Within 4-5 days, asexual fruiting bodies (sporodochia) bearing spores (conidia) were visible on plant tissue and the number of leaves with conidia and characteristic mycelium of Che or Cps on each GYET plate was recorded. The experiment was conducted twice for temperatures 47.5, 50 and 52.5 C. Two bags per treatment were taken for each experiment at 47.5 C (two bags of detached leaves and two bags of detached branches infected with Cps or Che). Two bags per treatment were taken at each sample time for the first experiment at 50 C and 2-3 bags for the second experiment. Two bags of detached branches and three bags of detached leaves were taken at each sample time for both experiments at 52.5 C.

Statistical analysis. Prior to data analysis for heat-treated boxwood cutting samples, two subsamples in each replicate were averaged to provide the mean number of roots at each time point. Analysis of variance (ANOVA) was performed for each temperature, cultivar, and 0-30 and 35-60 min time periods separately, by fitting a generalized linear mixed model using the PROC GLIMMIX procedure in SAS (Version 9.3, Cary, N.C.), where the duration of exposure to each temperature was a fixed effect and block was a random effect, denoted by the RANDOM statement in SAS. Dunnett's Multiple Comparison Analysis was performed to determine significant differences between the time zero and room temperature (RT) controls and cuttings exposed to hot water. Data for survival of the pathogen in detached leaves and branches were recorded as number of infected leaves/total leaves and transformed using the arcsine of the square root to normalize the data. General Linear Models analysis was done using SAS to analyze the effect of variables "fungal species", "experiment", "time", and interaction effects, with Least Squares Means tests done to compare survival of Che and Cps at each sample time. Leaf samples at 52.5 C and all detached branch sample data could not be normalized and comparisons between species were done using Proc Npar1way in SAS with a Wilcoxon Two Sample test.

Table 2. Mean root number produced per cutting of boxwood cultivars 'Green Beauty', 'Green Velvet', 'Justin Brouwers', and 'Nana' after treatment in 45 and 47.5 C water temperatures (Temp) for 0-30 and 35-60 minutes of exposure in experiments 1 and 2 conducted on 3 March 2016.

Experiment 1

		Exposure time (minutes)								
Temp	**Cultivar[z]**	**0[y]**	**5**	**10**	**15**	**20**	**25**	**30**	**30RT[y]**	**Pr>F[x]**
45 C	Green Beauty	28.3[w]	13.5	16.8	16.7	24.2	17.5	18.0	13.7	0.066
	Green Velvet	35.0	40.0	31.8	28.3	23.5	19.5	23.8	22.3	0.564
	Justin Brouwers	21.5	26.7	24.2	24.0	26.7	23.5	22.2	15.5	0.581
	Nana	29.2	27.0	35.0	17.5	23.5	23.2	21.7	33.3	0.595
47.5 C	Green Beauty	28.8	13.5	16.8	16.7	24.2	17.5	19.0	13.7	0.152
	Green Velvet.	24.7	34.8	35.5	28.5	22.3	22.2	20.3	13.8	0.284
	Justin Brouwers	21.3	26.5	25.8	31.3	21.7	27.7	16.5	30.2	0.066
	Nana	20.7	35.5*	31.0	17.3	21.3	19.2	14.0	31.8	0.001

Experiment 2

		Exposure time (minutes)								
Temp	**Cultivar[z]**	**0[y]**	**35**	**40**	**45**	**50**	**55**	**60**	**60RT[y]**	**Pr>F[x]**
45 C	Green Beauty	20.8[w]	19.5	11.3	21.0	11.4	21.7	12.5	0.0*	0.0005
	Green Velvet.	36.0	21.2	15.5	24.3	23.8	23.3	24.8	20.3	0.268
	Justin Brouwers	28.8	21.7	26.2	27.7	19.2	30.5	26.8	27.8	0.754
	Nana	37.8	18.3	25.5	25.2	28.0	20.3*	17.0*	28.0	0.017
47.5 C	Green Beauty	13.7	11.7	7.5	12.7	8.5	10.8	8.5	13.7	0.434
	Green Velvet	26.3	28.7	32.7	17.7	22.2	13.2	14.7	24.3	0.649
	Justin Brouwers	25.5	16.8	20.2	11.8	17.8	6.2	12.7	21.8	0.144
	Nana	28.8	13.5	16.8	16.7	24.2	17.5	19.0	13.7	0.152

[z]Boxwood cultivars evaluated included; 'Green Beauty' (*Buxus sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis*), 'Green Velvet' (*B. sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis*), 'Justin Brouwers' (*B. sempervirens*), and 'Nana' (*B. sinica* var. *insularis*).

[y]Control treatments included in each experiment consisted of a non-immersed time zero control (0); 30RT (Room Temperature) control = cuttings submerged in 23 C water for 30 min and 60RT (Room Temperature) control = cuttings submerged in 23 C water for 60 minutes.

[x]Probability based on Type III sum of squares from the GLIMMIX procedure in SAS 9.3 of exposure time at each temperature affecting root production.

[w]Values represent mean number of roots greater than 1.0 cm in length after 3 months of incubation. Values followed by an asterisk (*) indicate root formation is significantly different (P=0.05) from the non-immersed (time zero) and immersed room temperature (RT) controls for each cultivar, based on Dunnett's Multiple Comparisons test.

Results and Discussion

Hot water treatment experiments to assess boxwood cultivar sensitivity experiment 1. Due to differences (e.g. significant run effect) in root production between experiments 1-4, data from these experiments were analyzed separately and not combined. In experiment 1, no significant differences in rooting (number of roots greater than 1-cm in length) were observed after exposure of boxwood cuttings of cultivars 'Green Beauty', 'Green Velvet', 'Justin Brouwers', and 'Nana' in 45 C water for up to 30 minutes compared to the non-immersed time zero and immersed RT controls (Table 2). However, root production was stimulated (P=0.001) in cultivar 'Nana' after five minutes of exposure in 45 C water compared to the time zero control (Table 2).

Hot water treatment experiments to assess boxwood cultivar sensitivity experiment 2. At 45 C, the number of roots produced by cultivar 'Nana' was reduced (P=0.017) from 37.8 to 20.3 and 17.0 at 55 and 60 minutes, respectively, in experiment 2. For cultivar 'Green Beauty' no differences in rooting were observed after 35-60 minute exposure in 45 C water compared to the non-immersed time zero control (Table 2). Interestingly, no roots formed on cuttings of this cultivar when immersed in 23 C water for 60 minutes (60RTRoom Temperature control, Table 2). Exposure of boxwood cuttings of all cultivars for 35-60 minutes in 47.5 C water did not reduce rooting compared to the non-immersed time zero and immersed RT controls (Table 2).

Hot water treatment experiments to assess boxwood cultivar sensitivity experiment 3. Treatment of cuttings from all cultivars in 45 C water for up to 30 minutes did not significantly reduce root production compared to the time zero and 30 minute RT controls, which produced a similar number of roots in experiment 3 (Table 3). The mean number of roots produced by cultivar 'Nana' was reduced (P=0.010) to 4.2 after a 30 minute exposure to 47.5 C water, in contrast to a mean of 24.8 and 20.7 roots produced by the time zero and RT controls, respectively, for this cultivar (Table 3). All other cultivars produced similar numbers of roots compared to their respective non-immersed time zero and RT controls when exposed to 47.5 C water for 30 minutes (Table 3).

Hot water treatment experiments to assess boxwood cultivar sensitivity experiment 4. At 45 C, root production for cultivars 'Green Beauty', 'Justin Brouwers', and 'Nana' treated for 35-60 minutes did not differ from the time zero and RT controls in experiment 4. However, in experiment 4, cultivar 'Green Velvet' produced fewer (P=0.037) roots

Table 3. Mean root production of boxwood cultivars 'Green Beauty', 'Green Velvet', 'Justin Brouwers', and 'Nana' after treatment in 45 and 47.5 C water temperatures (Temp) for 0-30 and 35-60 minutes of exposure in experiments 3 and 4 conducted on 14 March 2016.

Experiment 3

		Exposure time (minutes)								
Temp	**Cultivar[z]**	**0[y]**	**5**	**10**	**15**	**20**	**25**	**30**	**30RT[y]**	**Pr>F[x]**
45 C	Green Beauty	19.7[w]	16.8	26.0	21.5	13.7	15.8	16.0	11.7	0.367
	Green Velvet	19.0	24.2	17.2	16.7	19.2	17.7	13.2	18.0	0.607
	Justin Brouwers	3.2	18.3	11.0	5.5	17.3	10.2	9.0	7.7	0.531
	Nana	32.7	35.7	31.7	25.5	16.5	26.0	21.3	26.0	0.607
47.5 C	Green Beauty	20.3	9.2	16.2	11.7	12.8	11.7	11.3	19.5	0.132
	Green Velvet.	14.8	21.3	14.0	22.5	28.2	14.0	9.8	19.5	0.064
	Justin Brouwers	26.5	24.0	17.4	18.3	16.3	13.3	13.3	20.2	0.341
	Nana	24.8	21.0	19.2	10.7	14.5	12.0	4.2*	20.7	0.010

Experiment 4

		Exposure time (minutes)								
Temp	**Cultivar[z]**	**0[y]**	**35**	**40**	**45**	**50**	**55**	**60**	**60RT[y]**	**Pr>F[x]**
45 C	Green Beauty	17.2[w]	18.3	13.7	13.5	9.2	17.7	9.3	15.2	0.290
	Green Velvet.	19.2	14.0	10.0	13.2	14.7	5.0*	10.3	19.8	0.037
	Justin Brouwers	5.0	12.0	4.5	4.2	4.7	0.0	1.2	11.8	0.323
	Nana	21.2	17.5	17.3	17.8	11.3	17.0	8.3	27.2	0.073
47.5 C	Green Beauty	14.5	5.8	13.7	6.3	5.2	4.5	3.7*	13.7	0.022
	Green Velvet.	18.0	14.0	13.5	14.2	8.8	4.2	0.1*	23.3	0.005
	Justin Brouwers	3.8	0.0	0.0	0.0	0.0	0.0	0.0	10.8*	<0.0001
	Nana	25.2	8.3*	3.4*	0.0*	0.0*	0.0*	0.0*	29.8	<0.0001

[z]Boxwood cultivars evaluated included; 'Green Beauty' (*Buxus sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis*), 'Green Velvet' (*B. sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis*), 'Justin Brouwers' (*B. sempervirens*), and 'Nana' (*B. sinica* var. *insularis*).

[y]Control treatments included in each experiment consisted of a non-immersed time zero control (0); 30RT (Room Temperature) control = cuttings submerged in 23 C water for 30 min and 60RT (Room Temperature) control = cuttings submerged in 23 C water for 60 minutes.

[x]Probability based on Type III sum of squares from the GLIMMIX procedure in SAS 9.3 of exposure time at each temperature affecting root production.

[w]Values represent mean number of roots greater than 1.0 cm in length after 3 months of incubation. Values followed by an asterisk (*) indicate root formation is significantly different (P=0.05) from the non-immersed (time zero) and immersed room temperature (RT) controls for each cultivar, based on Dunnett's Multiple Comparisons test.

after exposure for 55 minutes than the non-immersed time zero and RT controls (Table 3). Boxwood cuttings of cultivar 'Justin Brouwers' treated in 45 C water for 55 minutes did not produce roots but was not statistically different from the time zero and RT controls. Root production was reduced (P=0.05) in cultivars 'Green Beauty' and 'Green Velvet' after exposure to 47.5 C water for 60 minutes compared to the non-immersed (time zero) and room temperature (RT) immersed controls in experiment 4. The mean number of roots produced by 'Justin Brouwers' for the time zero and RT controls was 3.8 and 10.8, respectively, but no roots were produced when cuttings were exposed to 47.5 C water for 35-60 minutes. Exposure of cultivar 'Nana' to 47.5 C water for 35-60 minutes reduced (P<0.0001) root production compared to the time zero and RT controls, with no roots produced after 45 minutes of exposure (Table 3).

Thermal inactivation of Calonectria henricotiae and C. pseudonaviculata in diseased detached boxwood leaves and branches. In experiments where diseased leaves were exposed to water at 47.5 C for different times, 88-91% of leaves infected with Che or Cps not exposed to hot water (time zero control) showed evidence of pathogen spore production (sporulation percentage) when leaf samples were plated on GYET medium. After 25-30 minutes of exposure to 47.5 C water, 28-48% of leaves produced asexual fruiting structures with spores (sporodochia conidiomata) (Fig. 2a). A General Linear Models Analysis of data for each experiment at 47.5 C showed the model to be significant at $P < 0.001$, with no significant effect of fungal species or experiment, but there was a significant effect of time ($P < 0.0001$). The interaction of fungal species by time was not significant ($P = 0.40$), suggesting no differences between the two pathogenic species of *Calonectria* in survival after exposure to the heat treatment. Decline was linear and the time by time interaction was not significant. Regression analysis for the model "x= species time time*fungal species" had an R^2 value of 0.65 and no difference in the linear component for the two fungal species. Least Squares Means tests at each sampling time showed no difference in survival of the two fungal species at any time.

In experiments at 50 C, production of conidia decreased from 90-91% of leaves infected with Che or Cps not exposed to hot water (time zero control) to 0-3% sporulation from leaves infected with Cps after 10-12 minutes, and 22-26% sporulation from leaves infected with Che (Fig. 2b). For statistical analysis at 50 C, results of the two experiments differed (an experiment by fungal species interaction of p= 0.03) and results of each experiment were analyzed separately. While the model and independent variable time were significant at P< 0.0001 in both experiments, in experiment 1, the fungal species source of

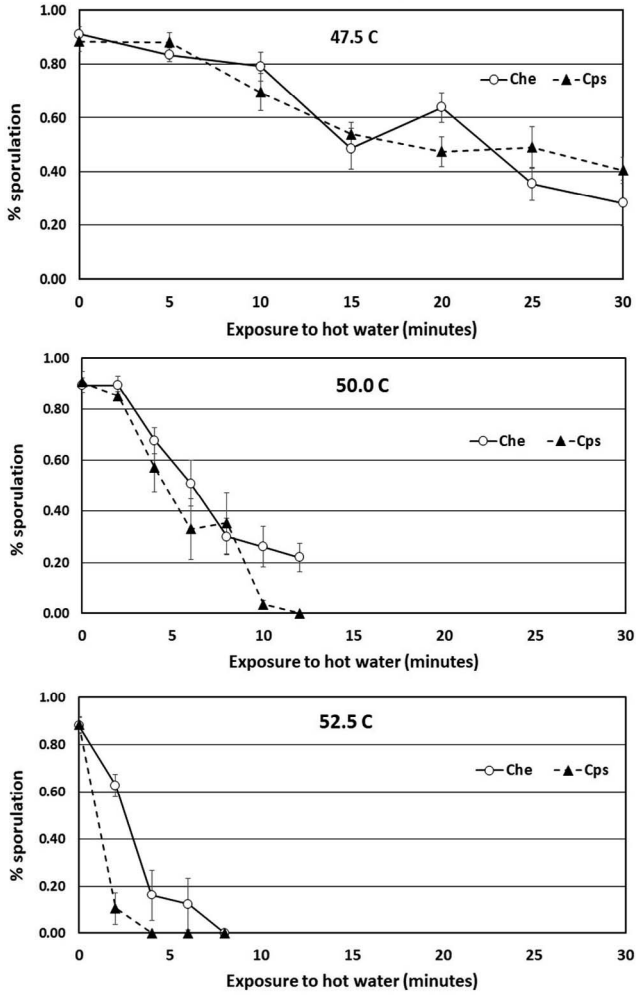

Fig. 2. Viability and spore (conidia) production expressed as proportion sporulation of boxwood blight pathogens *Calonectria henricotiae* (Che) and *C. pseudonaviculata* (Cps) in diseased detached leaves after increasing exposure to hot water at a) 47.5 C, b) 50 C, or c) 52.5 C (average of two experiments with error bars representing standard error).

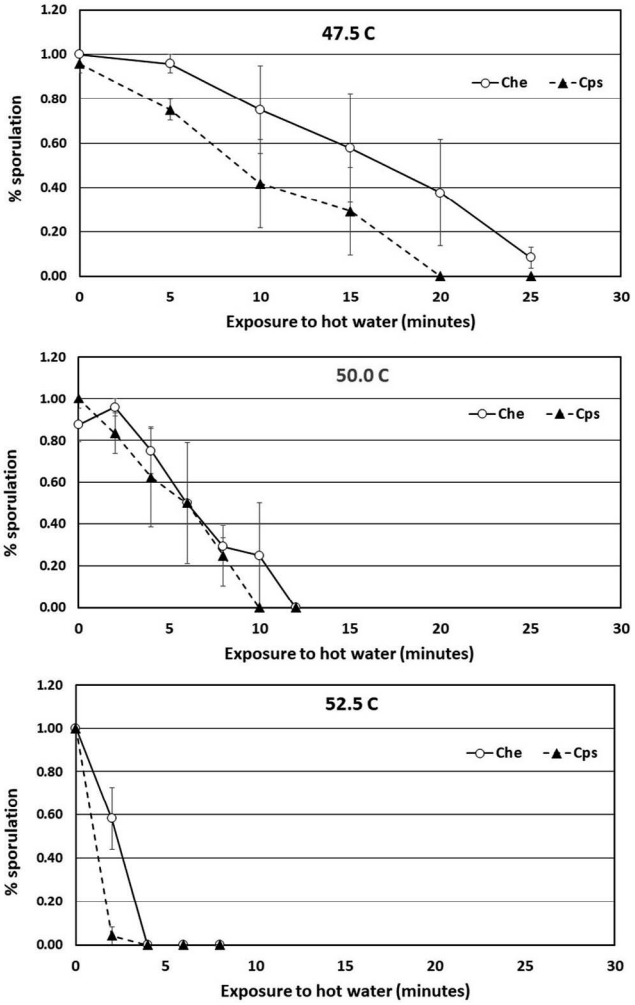

Fig. 3. Viability and spore (conidia) production expressed as proportion sporulation of boxwood blight pathogens *Calonectria henricotiae* (Che) and *C. pseudonaviculata* (Cps) in diseased detached branches after increasing exposure to hot water at a) 47.5 C, b) 50 C, or c) 52.5 C (mean of two experiments with error bars representing standard error).

variation was significant (P=0.003), with significantly greater survival of Che, but in experiment 2, survival did not differ (P= 0.91). In both experiments, there was a significant effect of time ($P < 0.0001$) but not for the time by time or fungal species by time interactions, suggesting no difference in survival with an R^2 value of 0.78-0.80.

In experiments at 52.5 C, an average 88% of leaves infected with Che or Cps not exposed to hot water (time zero control) showed sporulation of the pathogens, but after 4 minutes of exposure sporulation from leaves infected with Cps was reduced to 0%, and after 8 minutes, sporulation from leaves infected with Che was reduced to 0%. (Fig. 2c). Data could not be analyzed by General Linear Model analysis for leaves treated at 52.5 C because data could not be normalized due to a rapid decline to zero, but survival of both fungal species was zero by 8 minutes of exposure to the 52.5 C water and a Wilcoxon Two-Sample Test showed no differences in response to temperature between the two fungal species.

Infected detached branch segments incubated on GYET medium exhibited 88-100% sporulation when segments were not treated with hot water (time zero control), but sporulation was reduced to 0-8% after treatment for 25 minutes at 47.5 C; a Wilcoxon Two-Sample test showed significantly better survival of Che in detached branches (two-sided exact test $p = 0.018$) (Fig 3a). Production of conidia was reduced to 0% after treatment for 12 minutes at 50 C (Fig 3b), and 0% after treatment for 4 minutes at 52.5 C (Fig 3c), with a Wilcoxon Two-Sample Test showing no difference in survival of the two pathogens at either temperature.

The primary objective of our research was to determine whether root production of cuttings from four commonly grown boxwood cultivars was impacted by exposure to hot water, as a prerequisite for deploying this treatment to manage boxwood blight disease during propagation (Grondeau, Samson, and Sands 2011). In this study, the number of roots produced by the non-immersed time zero control was rarely different from the number of roots produced by the immersed room temperature (RT) controls, indicating that reduction in rooting in heat-

treated cuttings was caused by exposure to hot water over time. Root production of treated cuttings was usually greater than the grower standard of 5 roots >1 cm in length following assessment after 3 months of incubation in potting media (Bennett Saunders, Saunders Brothers Nursery, personal communication). Except for the studies of Copes and Blythe (2009, 2011), Morello et al. (2015), and Fourie and Halleen (2004), information related to the response of woody plant cuttings to hot water treatment is limited. In our study, cuttings from all four boxwood cultivars were able to withstand exposure to 45 and 47.5 C for 30 minutes without root production differing from the number of roots produced by cuttings in the time zero and RT controls. These results supported hypothesis 1 that treating boxwood cuttings at 45 and 47.5 C for 30 minutes would not reduce root production. However, after treatment at 47.5 C for 30 minutes, cultivar 'Nana' only produced a mean of 4.7 roots per cutting, which was significantly lower than the non-immersed time zero control, but comparable to the widely accepted grower standard of five roots >1 cm in length. Overall root production by all boxwood cultivars was lower in Experiment 2 than in Experiment 1, which may have been because cuttings were taken from new growth on boxwood for Experiment 2. During periods of new leaf growth, plant water content will increase and shoots having leaves with higher water content often produce fewer roots. Cuttings that weren't heat-treated also produced fewer roots in Experiment 2. While we did not measure the water content of cuttings before heat treatment, boxwood growers in North Carolina and Virginia typically discontinue cutting harvest by mid-March and Experiment 2 was conducted after this date.

Exposure of boxwood cuttings to hot water for longer than 30 minutes resulted in a differential rooting response among the four cultivars tested, supporting hypothesis 2 that root production will vary among cultivars after treatment for 30 minutes in 45 or 47.5 C water. The number of roots produced by cultivars 'Green Beauty' and 'Green Velvet' was not reduced by treatment in 45 C water for 60 minutes. In Experiment 2, treating cuttings from cultivar 'Nana' at 45 C for 55 and 60 minutes significantly reduced the number of roots produced compared to the non-immersed time zero control. However, the mean number of roots produced was 4.1 and 3.4 times greater than the accepted grower standard of five roots >1.0 cm in length. Furthermore, in Experiment 4, the number of roots produced by 'Nana' cuttings treated for up to 60 minutes at 45 C was not significantly lower than the time zero control, indicating that while heat treatment did reduce the number of roots in Experiment 2, the reduction was not great enough to prevent cuttings from being treated for up to 60 minutes at 45 C. Exposure of boxwood cuttings to 45 C water for 60 minutes did not reduce the mean number of roots produced by 'Justin Brouwers' in Experiment 2, but root production in Experiment 4 decreased below the grower standard when cuttings were exposed to 45 C water for 40 minutes. For the 35-60 minute time period treatment at 47.5 C in Experiment 2, root production was significantly reduced only in cultivar 'Nana'. At 50 minutes, this cultivar produced fewer roots than the grower standard of 5 roots >1-cm in length, while all other cultivars were able to withstand treatment for up to 60 minutes without reduced root production. Furthermore, treatment of 'Green Beauty' and 'Green Velvet' cuttings in Experiment 2 at 47.5 C for 55 minutes did not reduce root production compared to the non-immersed and room temperature controls. However, cuttings of these cultivars in Experiments 4 produced fewer roots than the non-immersed and immersed room temperature controls following 55 minutes of exposure to 47.5 C. Additional experiments are probably needed to clarify this.

In Experiment 4, treatment of cuttings of 'Justin Brouwers' in 47.5 C water resulted in decreased root production with no roots produced after 35 minutes of exposure to this temperature. However, in Experiment 2, 'Justin Brouwers' cuttings were able to withstand treatment at 47.5 C for 60 minutes without root production decreasing below the grower standard. Cuttings of all tested cultivars were severely damaged or killed at temperatures of 50 and 52.5 C (data not shown) when exposed to hot water for at least 15 and 5 minutes, respectively. In general, cultivars 'Green Beauty' and 'Green Velvet' were less sensitive to exposure in hot water across the times and temperatures tested than cultivars 'Justin Brouwers' and 'Nana'. Interestingly, 'Green Beauty' and 'Green Velvet' are hybrids with a *Buxus sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis* pedigree, whereas 'Justin Brouwers' and 'Nana' have a *B. sempervirens* and *B. sinica* var. *insularis* ancestry, respectively. Future research studies are needed to test the hypothesis that cuttings of cultivars within a taxonomic species of *Buxus* will have a similar response to hot water exposure but will vary across species boundaries.

In previous research, Miller et al. (2018) found that 90% of Che and Cps conidia at a concentration of 10,000 conidia/ml exposed in a water suspension to heat were killed by treating for 26.8 minutes at 45 C and 15.3 minutes at 47.5 C. Culture-produced microsclerotia of Che and Cps in a water suspension required greater exposure to heated water than conidia (20 minutes at 45 and 47.5 C and 5-10 minutes at 50 and 52.5 C) for thermal inactivation of 90% of these infectious propagules (Miller et al. 2018). Also, treatment of Che and Cps microsclerotia at 45 C for 60 minutes was not effective.

To complement previous thermal inactivation and sensitivity studies of Che and Cps conidia and microsclerotia, we conducted experiments on diseased boxwood cuttings to develop temperature-based metrics for these pathogens in infected boxwood leaves and branches of cultivar 'Justin Brouwers' exposed to 47.5, 50, or 52.5 C water. The formation of microsclerotia in leaves and branches represents a critical stage of fungal ecology, as it is from these structures that infectious propagules (conidia) that serve as sources of primary and secondary inoculum may be generated to initiate and sustain boxwood blight disease development. The viability of Che and Cps in diseased leaves and branches as measured by sporulation, was slightly affected after treatment in 47.5 C water for 20 minutes. These results provided partial support for

hypothesis 3 that treating boxwood cuttings in 47.5 C water for 30 minutes will reduce viability and production of Che and Cps conidia in diseased leaves and branches. These results suggest that while surface contamination (adhering spores or microsclerotia) will be more easily inactivated by heat treatment, greater time exposure beyond 20 minutes at 47.5 C is required to inactivate the pathogens in plant tissue.

At 50 C, there was a smaller percentage of conidia (spores) produced in lesions on diseased leaves after 10 minutes exposure, while no spore production was observed after treatment at 52 C for 5-8 minutes. Higher temperatures (above 47.5 C) are more clearly effective against Cps and Che, but this does not preclude the possibility that sublethal effects of hot water treatment might be observed at 47.5 C. Whiting et al. (2001) observed that while hot water treatment at 51 C of agar plugs containing mycelium of *Phaeomoniella chlamydospora* eventually killed the pathogen, sublethal exposure resulted in reduced hyphal growth rate on potato dextrose agar medium.

Heat treatment at 45 C and 47.5 C has been successfully deployed in other plant pathosystems to inactivate pathogenic fungi *in planta* and *in vitro*. Treating conidia of the soilborne fungal pathogen *Verticillium dahliae* Kleb at 45 C reduced viability to 0.01% (Castejon-Munoz and Bollen 1993). Both mycelial growth and conidial germination of *Colletotrichum musae* (Berk. & M.A. Curtis) Arx 1957 and *Fusarium proliferatum* (Matsush.) Nirenberg 1976, two fungal crown rot pathogens of banana (*Musa* spp.), were inhibited by exposure to 45 and 47.5 C. Furthermore, these temperatures were shown to inactivate these pathogens *in planta* when infected banana fruits were heat treated for 15-30 min (Lopez-Caberra and Marrero-Dominguez 1998). In this study, 47.5 C and 30 minutes appeared to be a cardinal temperature and time for minimizing leaf damage associated with the application of heat to boxwood cuttings and suppressing sporulation of Che and Cps. However, further research is needed to evaluate the effect of shorter exposure treatments at water temperatures ranging from 47.5 to 50 C on rooting of boxwood cuttings, since higher temperature treatments were more successful at reducing viability of Che and Cps in infected boxwood leaves and branches. However, reducing plant associated damage while also suppressing/eliminating the pathogen with temperatures greater than 47.5 C for a specific exposure time represents an intricate balance. Determining non-harmful but effective temperature treatments will likely be dependent on the boxwood cultivar and/or species examined. As our results show, the time of year that cuttings are treated may also have an effect on safety to the treated boxwood, because of differences in the physiological age and state of the plant tissue.

As part of our investigations, we were interested in generating baseline information and knowledge on the potential use of hot water to eliminate Che from boxwood cuttings. This information would provide a potential management option for reducing the pathogenic activity of Che, an invasive pathogen that does not currently occur in the US. In our study, Che showed significantly greater survival in detached leaves and branches in some experiments, but not to a degree that is practically significant with regard to heat treatment. Gehesquière et al. (2015) found slight but significant differences in temperature optima of Che and Cps, with Che exhibiting increased hyphal (vegetative) growth, spore germination and disease-causing activity at higher temperatures (Shishkoff and Stanley 2019). However, it is not known whether these differences are ecologically and epidemiologically significant. For hot water treatment to become a viable management option to prevent development and spread of boxwood blight pathogens, an assessment of a larger and more genetically diverse sample of boxwood cultivars, species and hybrids to determine the relationship of taxonomic affinity with thermal sensitivity is required.

Literature Cited

Baker, K.F. 1962. Thermotherapy of planting material. Phytopathology 52:1244–1255.

Burr, T.J., K. Ophel, B.H. Katz, and A. Kerr. 1989. Effect of hot water treatment on systemic *Agrobacterium tumefaciens* Biovar 3 in dormant grape cuttings. Plant Disease 73:242–245.

Castejon-Munoz, M., and G.J. Bollen. 1993. Induction of heat resistance in *Fusarium oxysporum* and *Verticillium dahliae* caused by exposure to sublethal heat treatments. Netherlands Journal of Plant Pathology 99:77–84.

Copes, W.E., and E.K. Blythe. 2011. Rooting response of azalea cultivars to hot water treatment used for pathogen control. HortScience 46:52–56.

Copes, W.E., and E.K. Blythe. 2009. Chemical and hot water treatments to control *Rhizoctonia* AG-U infesting stem cuttings of azalea. HortScience 44:1370–1376.

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

Fourie, P.H., and F. Halleen. 2004. Proactive control of Petri disease of grapevine through treatment of propagation material. Plant Disease 88:1241–1245.

Ganci, M. 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 survivability. MS Thesis. North Carolina State University, Raleigh, NC.

Ganci, M., D.M. Benson, and K.L. Ivors. 2019. The trojan horse experiment: Understanding reservoirs of boxwood blight inoculum. https://plantpathology.ces.ncsu.edu/wp-content/uploads/2013/05/Ganci-Trojan-Horse-trial-13.pdf?fwd=no. Accessed March 13, 2019.

Gehesquière, B., J.A. Crouch, R.E. Marra, K. Van Poucke, F. Rys, M. Maes, B. Gobin, M. Höfte, and K. Heungens. 2015. Characterization and taxonomic reassessment of the box blight pathogen *Calonectria pseudonaviculata*, introducing *Calonectria henricotiae* sp. nov. Plant Pathology 65:37–52.

Goheen, A.C., G. Nyland, and S.K. Lowe. 1973. Association of rickettsialike organism with Pierce's disease of grapevine and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63:341–345.

Grondeau, C., R. Samson, and D.C. Sands. 2011. A review of thermotherapy to free plant materials from pathogens, especially seeds from bacteria. Critical Reviews in Plant Sciences 13:57–75.

Harvey, R.J., D.D. Davis, N. Shishkoff, and J.A. Pecchia. 2019. Survival of lab grown *Calonectria pseudonaviculata* microsclerotia during small-scale composting. Compost Science and Utilization. https://doi.org/10.1080/1065657X.2018.1536865.

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

Hong, C.X. 2019. Fighting plant pathogens together. Science 365:229.

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 Microbiol Biotechnol. 102:4371–4380.

Lopez-Caberra, J.J., and A. Marrero-Dominguez. 1998. Use of hot water dips to control the incidence of banana crown rot. Acta Hortic. 490:563–569.

Miller, M.E., R.S. Norris, and M.A. Cubeta. 2016. Evaluation of boxwood cultivars for resistance to boxwood blight, 2015. Plant Disease Management Reports 10:1037.

Miller, M.E., N. Shishkoff, and M.A. Cubeta. 2018. Thermal sensitivity of *Calonectria henricotiae* and *Calonectria pseudonaviculata* conidia and microsclerotia, Mycologia 110:546–558.

Miller, M.E., 2016. Investigations into the use of thermotherapy to inactivate the boxwood blight pathogen *Calonectria pseudonaviculata*. MS Thesis, North Carolina State University, Raleigh, NC.

Morello, P., C.M. Diez, M. Codes, L. Rallo, D. Barranco, A. Trapero, and J. Moral. 2016. Sanitation of olive plants infected by *Verticillium dahliae* using heat treatments. Plant Pathology 65:412–421.

Morgan, D.L. and P.F. Colbaugh. 1983. Influence of chemical sanitation treatments on propagation of *Buxus microphylla* and *Peperomia caperata*. Proceedings of the International Plant Propagator's Society 33:600–607.

Sanderlin, R.S. and R.A. Melanson. 2008. Reduction of *Xylella fastidiosa* transmission through pecan scion wood by hot-water treatment. Plant Disease 92:1124–1126.

Shishkoff, N., M. Daughtrey, S. Aker, and R. Olsen. 2015. Evaluating boxwood susceptibility to *Calonectria pseudonaviculata* using cuttings from the National Boxwood Collection. Plant Health Progress. DOI: 10.1094/PHP-RS-14-0033

USDA-NASS, 2012 Census of Agriculture. 2014 CHS - United States Data, (2014). https://www.agcensus.usda.gov.

Weeda, S.M. and N. Dart. 2012. Histological evidence that microsclerotia play a significant role in disease cycle of the boxwood blight pathogen in southeastern United States and implications for disease mitigation. Plant Heath Progress, Online Pub. http://doi.org/10.1094/PHP-2012-0403-01-BR

Waite, H. and L. Morton. 2007. Hot water treatment, trunk diseases and other critical factors in the production of high-quality grapevine planting material. Phytopathologia Mediterranea 46:5–17.

Whiting, E.C., A. Khan, and W.D. Gubler. 2001. Effect of temperature and water potential on survival and mycelial growth of *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. Plant Disease 85:195–201.