Factors affecting boxwood blight spread under landscape conditions[1]

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Abstract

We investigated the spread of boxwood blight in a simulated landscape under conducive natural conditions from 2017 to 2019. We used strict sanitation to greatly reduce or eliminate spread by contact. Movement of the pathogen from an infected source plant was limited to one plant, likely spread by means of water splash. Plants were mulched with composted hardwood chips and mulching likely was primarily responsible for limiting spread to only the adjacent plant. Boxwood (*Buxus* spp.) cultivar susceptibility and fungicide spray programs influenced the incidence of spread and severity of disease; in 2018 and 2019 the more susceptible cultivar had higher disease incidence and severity, respectively, than less susceptible cultivars. Fungicide application only caused a small reduction in disease incidence in 2018. We also demonstrated that spores in clumps could survive extended dry conditions, indicating the importance of sanitation procedures on reducing spread. This experiment demonstrates that boxwood blight can be controlled in a landscape by following best management practices including cultural, sanitation, host susceptibility and fungicide application tactics.

Index words: epidemiology, fungicide management, *Buxus*, chemical disease management, mulch, resistance.

Chemicals used in this study: chlorothalonil (Daconil WeatherStik 54.0% F), fludioxonil plus cyprodinil (Palladium 25% and 37.5% WDG), mancozeb (Manzate 80% WP), metconazole (Tourney 50% WDG), propiconazole (ProCon-Z 14.3 L), pyraclostrobin (Insignia 20 WG), pyraclostrobin plus fluxapyroxad (Orkestra Intrinsic 21.26 SC), tebuconazole (Torque 38.7 SC), thiophanate-methyl (Spectro90 50% WP).

Species used in this study: boxwood (*Buxus* L.), boxwood blight (*Calonectria pseudonaviculata* (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M. J. Wingf. & Crous.

Significance to the Horticulture Industry

Boxwood blight is the most important disease of boxwood in the United States. The *Calonectria pseudonaviculata* pathogen has spread from two states in 2011 to at least 28 states plus the District of Columbia within eight years. In these experiments we demonstrated that spread within a landscape setting can be minimized by sanitation, the use of mulch, planting less susceptible boxwood cultivars and applying fungicides. We also demonstrated that spore clumps could be moved by contact under dry conditions, survive extended periods without moisture that single conidia could not, and germinate when suitable conditions occur.

Introduction

Boxwood blight is the most important disease of boxwood in the United States. The disease has spread from its first detections in Connecticut and North Carolina (Ivors et al. 2012) to at least 28 states plus the District of Columbia (Hong 2019) within eight years. The pathogen, *Calonectria pseudonaviculata* (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M. J. Wingf. & Crous (Crous et al. 2002, 2004) (synonym:*Cylindrocladium pseudonaviculatum* Crous, J.Z. Groenew. & C.F. Hill), is moved over long distances through infected plants (Creswell 2018) and under conducive conditions produces sticky clumps of conidia that are spread locally by contact or by water splash (LaMondia and Maurer 2020). Extended wet and humid environmental conditions are necessary for sporulation and infection (Avenot et al. 2017). The effects of rain on water splash dispersal of fungi has been studied for other pathogens (Madden 1997, Travadon et al. 2007). Significant differences exist in boxwood cultivar susceptibility to blight (Kramer et al. 2020, LaMondia and Shishkoff 2017) and fungicide applications have been demonstrated to reduce disease severity (LaMondia 2020). We initiated an experiment to study the conditions under which boxwood blight spread naturally through a landscape planting and the effects of fungicide applications and boxwood cultivar susceptibility on that spread over a two-year period.

Materials and Methods

We established twenty 3-row landscape boxwood plots consisting of 5 plants per row planted 0.6 m (2 ft) apart within rows on 20 April 2017 (Fig. 1). Rows were placed 1.2 m (4 ft) apart and plots were separated from each other by 3.0 m (9.8 ft). Each row was planted with one of three boxwood cultivars with different susceptibility to blight in a randomized design. In 2017 and 2018, we compared three cultivars, 'Green Velvet', 'Winter Gem' and 'Tide Hill' (moderately high, moderately resistant and lower susceptibility, respectively) (LaMondia 2015, unpublished), to determine spread through a hedge landscape planting with and without fungicide application. Boxwood cultivar was a

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W	G	E	W	E	G	E	W	G	E	G	W	W	E	G
W	G	E	W	E	G	E	W	G	E	G	W	W	E	G
Plot 1			2			3			4			5		

Fig. 1. Plot map for boxwoods within the landscape. Twenty 3 m x 3 m plots established for a repeated block design. The following cultivars were planted: G: Green Velvet, W: Winter Gem, E: Tide Hill in 2018, English Suffruticosa in 2019, MT: Green Mountain, M: Green Mound.

single row subplot treatment in the replicated fungicide program plots. The inoculum source plants were the same cultivar as the other plants in the row. Source plants had been inoculated with 1.5×10^6 conidia per plant in the greenhouse and covered with plastic overnight to maintain humidity. Plants were inoculated on 11 May and planted 12 May 2017 at the west end of each row 0.6 m from target plant #1. The first fungicide applications were to be applied immediately after discovery of boxwood blight spread from source plants to target plant #1 in the row. No fungicides were applied in 2017 as no blight was observed in target plants. All plants were mulched on 31 May with 5 to 8 cm (2-3 in) of aged hardwood chip mulch in a 0.6 m (2 ft) wide strip to help suppress weeds and creeping red fescue grass (*Festuca rubra* L.) was seeded between rows and maintained by mowing.

Infected source plants were replaced on 29 May 2018 with infected ‘Green Mountain’ or ‘Green Mound’ boxwood plants that had been inoculated with 4.7×10^6 conidia on 22 May under greenhouse conditions and had similar levels of disease and sporulation. Source plants were transplanted 0.3 m from the first plant in the row on the western end in all rows (Fig. 2a). Once symptoms were observed on the infected source plants, we initiated spray programs that consisted of currently labeled fungicides, including protectant fungicides alone or different combinations of systemic plus protectant fungicides (Table 1). The first fungicide applications (protectant alone or protectant plus systemics) were made on 31 May or 1 June and at monthly intervals thereafter to October. To investigate whether more frequent fungicide application early after disease discovery had significant impact, two additional treatments consisted of June 1 and June 15 sprays followed by the same calendar-based sprays as above. All plants, both source and target plants, were treated with appropriate spray applications. The fungicide treatments were applied at label rates and untreated control plots did not receive any fungicide applications. Fungicides were applied on 1 June, 15 June, 3 July, 7 August, 31 August, and 3 October 2018. Treatments are listed in Table 1 and consisted of chlorothalonil (Daconil WeatherStik 54.0% F, Syngenta Crop Protection LLC, Greensboro, NC); fludioxonil plus cyprodinil (Palladium 25% and 37.5% WDG, Syngenta Crop Protection LLC, Greensboro, NC); mancozeb (Manzate 80% WP, United Phosphorus Inc., King of Prussia, PA); metconazole (Tourney 50% WDG, Cleary Chemical LLC, Dayton, NJ); propiconazole (ProCon-Z 14.3 L, Loveland Products, Inc., Loveland, CO); pyraclostrobin (Insignia 20 WG, BASF Corp., Ludwigshafen, Germany); pyraclostrobin plus fluxapyroxad (Orkestra Intrinsic 21.26 SC, BASF Corp., Ludwigshafen, Germany); tebuconazole (Torque 38.7 SC, Cleary

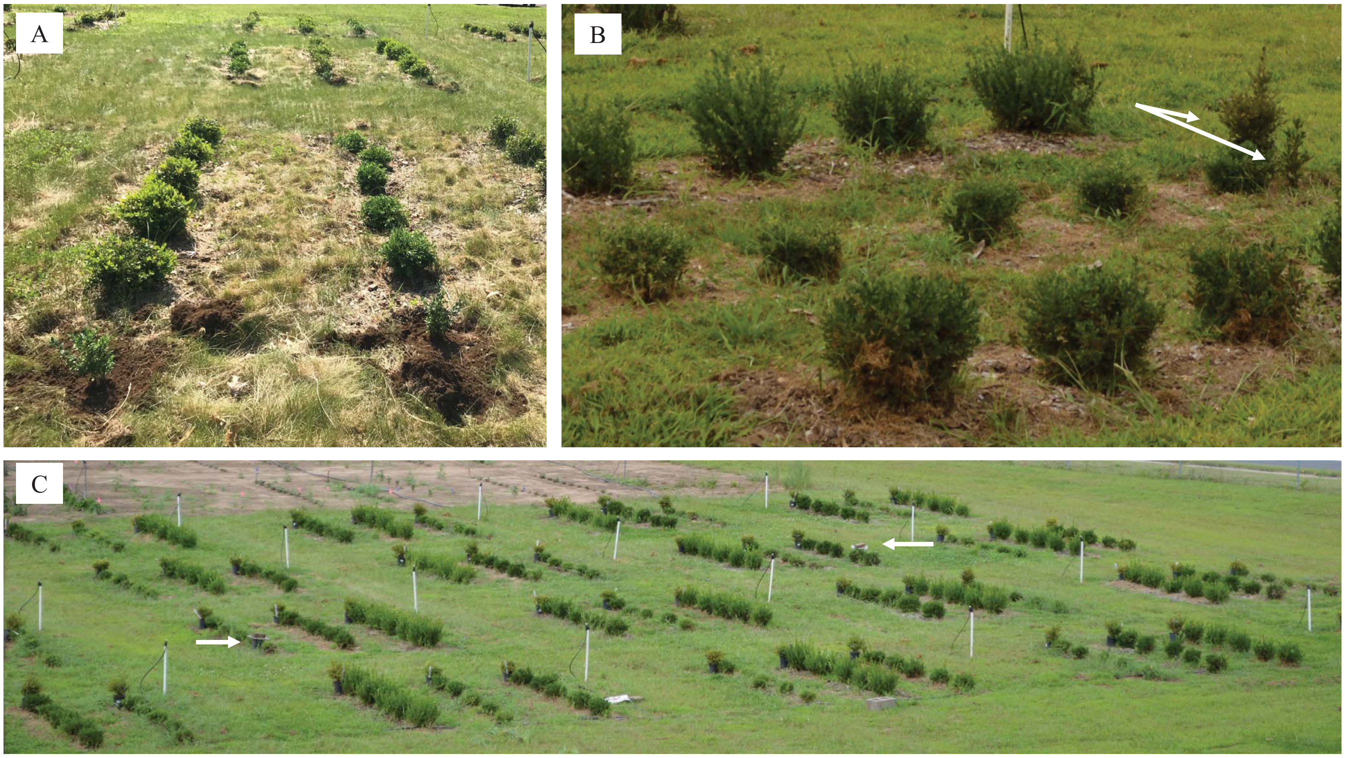

Fig. 2. Landscape boxwood plots A) Newly planted infection source plants in May 2018 at the west end of each row B) Addition of new source plant in liners next to established source plants in 2019, arrow indicates the source plants C) Plots in August 2019, arrows indicate placement of weather stations within plots. Green Velvet source plants in pots adjacent to infected source plants in the ground.

Chemical LLC, Dayton, NJ); thiophanate-methyl (Spectro90 50% WP, Cleary Chemical LLC, Dayton, NJ).

'Tide Hill' plants had significant winter injury over the 2018-2019 winter, and as a result of minimal pathogen spread in the first year, we removed 'Tide Hill' and replanted in the same locations with English boxwood *B. sempervirens* L. 'Suffruticosa' in the spring on 9 May 2019. 'Suffruticosa' is a very susceptible cultivar (Kramer et al. 2020, LaMondia and Shishkoff 2017). The plants were approximately one-quarter the size of the other cultivars (approximately 10 cm (4 in) in height versus 35-40 cm (13.7-15.7 in) in height and width). 'Green Velvet' and 'Winter Gem' plants were trimmed on 16 May 2019, under dry conditions, working from west to east toward the source plants, and disinfesting equipment with 70% isopropanol between each row. New boxwood blight-infected source plants consisting of infected 'Green Velvet' liners in pots were placed at the west end of each row on 29 May 2019 next to the source plants from 2018 (Fig. 2b). Monthly fungicide sprays were initiated on 31 May using the same treatments in the same sequence as 2018. Fungicides were applied on 31 May, 14 June, 1 July, 1 August, 5 September, and 8 October 2019.

Table 1. Fungicide treatments for management of boxwood blight in landscape plots over months with disease-conducive environmental conditions.

Fungicides and Timing	June	Mid-June	July	August	September	October
1. UTC	None	None	None	None	None	None
2. Protectant Fungicide	D	None	I	D	I	D
3. Systemic+Protectant	P + M	None	S	T + I	O	To + Pall
4. Protectant Fungicide	I	D	I	D	I	D
5. Systemic+Protectant	P + M	T + I	S	T + I	O	To + Pall

Fungicides:active ingredient(s)(Frac Group) ***systemic if italicized***

D = Daconil chlorothalonil (M5)
I = Insignia pyraclostrobin (11)
P = ProconZ *propiconazole* (3)
M = Manzate DF mancozeb (M3)
S = Spectro90 *thiophanate-methyl* (1) plus chlorothalonil (M5)
T = Tourney *metconazole* (3)
O = Orkestra *fluxapyroxad* (7) plus pyraclostrobin (11)
To = Torque *tebuconazole* (3)
Pall = Palladium *fludioxonil* (12) plus cyprodinil (9)

Table 2. **Rainfall totals plus supplemental overhead irrigation per month (cm) Windsor Locks CT.**

Month	2017	2018	2019	30-year average
May	4.62	6.32	11.73	6.35
June	6.23	12.80	6.33	7.11
July	3.57	16.87	10.72	7.62
August	6.12	22.91	11.33	6.60
September	2.66	16.08	4.90	4.57
October	8.77	10.21	16.33	8.89

Overhead irrigation consisting of mini wobblers at 0.9 m (3 ft) height and 6 m (20 ft) spacing (Senninger Irrigation, Clermont FL) was set up within the boxwood plots in 2017 and plants were irrigated on 3 occasions in 2017, 5 times in 2018 and 10 times in 2019. Monthly rainfall and irrigation totals are shown in Table 2. Weather conditions; temperature, rainfall, wind speed and direction, were recorded with a nearby [within 30 m (98 ft)]) weather station and with Hobo temperature and humidity recorders (Models U10 and UX100, Onset Computer Corp., Bourne, MA) placed adjacent to plants within rows (Fig. 2c). The data loggers were hung under a small cover open on the sides and bottom to protect them from rain.

All boxwood plants were examined for blight symptoms each week under dry conditions working east to west. Plants with boxwood blight symptoms were scored as positive (1), those without any stem or leaf lesions were scored as negative (0) from July to October 2018 and June to October 2019. In addition, the number of lesions per plant were counted on two dates, June 19 and August 6, 2019. The binomial incidence data consisting of diseased or healthy plants in each location were analyzed using an online Fisher's Exact Test calculator (http://vassarstats.net/fisher2x3.html). Data for the number of boxwood blight lesions per plant were analyzed by GLM Analysis of Variance and means were separated by Fisher's LSD Multiple-Comparison Test.

As a consequence of questions raised by our results, we conducted preliminary experiments to examine survival of conidia singly or in clumps under dry or humid conditions to further understand spread by contact and the importance of sanitation. Conidia from sporulating boxwood leaves were transferred by contact to microscope slide cover slips. These cover slips were placed into two separate moisture chambers, one with water to maintain high humidity and a second chamber without any water. Environmental conditions were monitored by Hobo temperature and humidity recorders. The cover slips infested with spores held in the dry chamber were placed on potato dextrose agar media in petri dishes at 3, 6, 9, 12, and 15 days after removal from boxwood leaves. Spores held under humid conditions were transferred at 1, 2, 3, 4, and 5 weeks of incubation. Three cover slips were prepared for each testing period. The inoculated plates were incubated at ambient laboratory conditions and observed under a dissecting microscope for germination of single spores or spores in clumps. Germination was scored as positive or negative for individual or spore clumps.

Results and Discussion

Weather conditions were drier than normal in 2017 and while disease was present on the inoculated source plants, no boxwood blight spread from source to target plants was observed. However, conditions were very suitable for boxwood blight infection and spread in both 2018 and 2019. Rainfall in May through October 2018 was nearly twice the normal amount, more than 40 cm (16 in) above average and nearly 14 cm (5.5 in) above average in 2019 (Table 2). Temperature and humidity data recorded within rows in the plots indicated that disease conducive conditions (continually above 15 C (59 F) and 90% relative humidity (RH) for at least 8 hours) occurred on 63 occasions and for longer than 12 hours on 29 occasions in 2018 (Fig. 3) as well as 39 and 17 occasions in 2019 (Fig. 4), respectively. In fact, as a result of the extended wet environmental conditions, CAES Plant Disease Diagnostic services in New Haven and Windsor CT received over 400 boxwood sample submissions that were positive for boxwood blight in 2018 and over 150 in 2019 compared to 26, 51 and 16 in 2011, 2012 and 2020, respectively. The 2018 and 2019 samples were almost entirely from landscapes (99%) rather than from nurseries (J. LaMondia, unpublished data and Y. Li, personal communication).

Despite the suitable environmental conditions for disease, we did not observe spread of boxwood blight other than from the infected source plant to the first plant in the row (position number 1) less than 30 cm (1 ft) away in either 2018 or 2019. Plant position was very significant as the only plants infected by spread from the inoculated source plants were in the #1 position adjacent to the source. Not all plants in that position became infected, however. Data from the #1 position plant from 2018 and 2019 are presented in Tables 3 and 4. Binomial incidence data was scored as 1 (positive) or 0 (negative) for individual plants. Data from 2018 (Table 3) represents the proportion of plants infected for that treatment across all replicate plots. If all replicates were infected, incidence was 1.0. Because infected leaves defoliated, the proportion of infected plants changed over time and sometimes declined. For that reason, data was collected for the number of plants which had been scored as positive at any time from July to October 2018. Statistical analyses were only conducted for plants in the #1 position. Blight incidence was affected by cultivar (Table 3); 'Green Velvet' consistently had more infected plants resulting from spread from the adjacent source than either 'Tide Hill' or 'Winter Gem', both of which have been demonstrated to be less susceptible to infection than 'Green Velvet' (LaMondia 2015, LaMondia and Shishkoff 2017). There were no significant differences between fungicide treatments with one or two applications in June of 2018 or 2019, so data were combined for analyses. Monthly fungicide application had a reduced effect on spread compared to boxwood cultivar. There were no significant differences in June, only protectant fungicides slightly reduced disease in August and there was a trend for the cumulative number of plants with positive finds reduced by the combination of protectant and systemic fungicides, ($P = 0.08$).

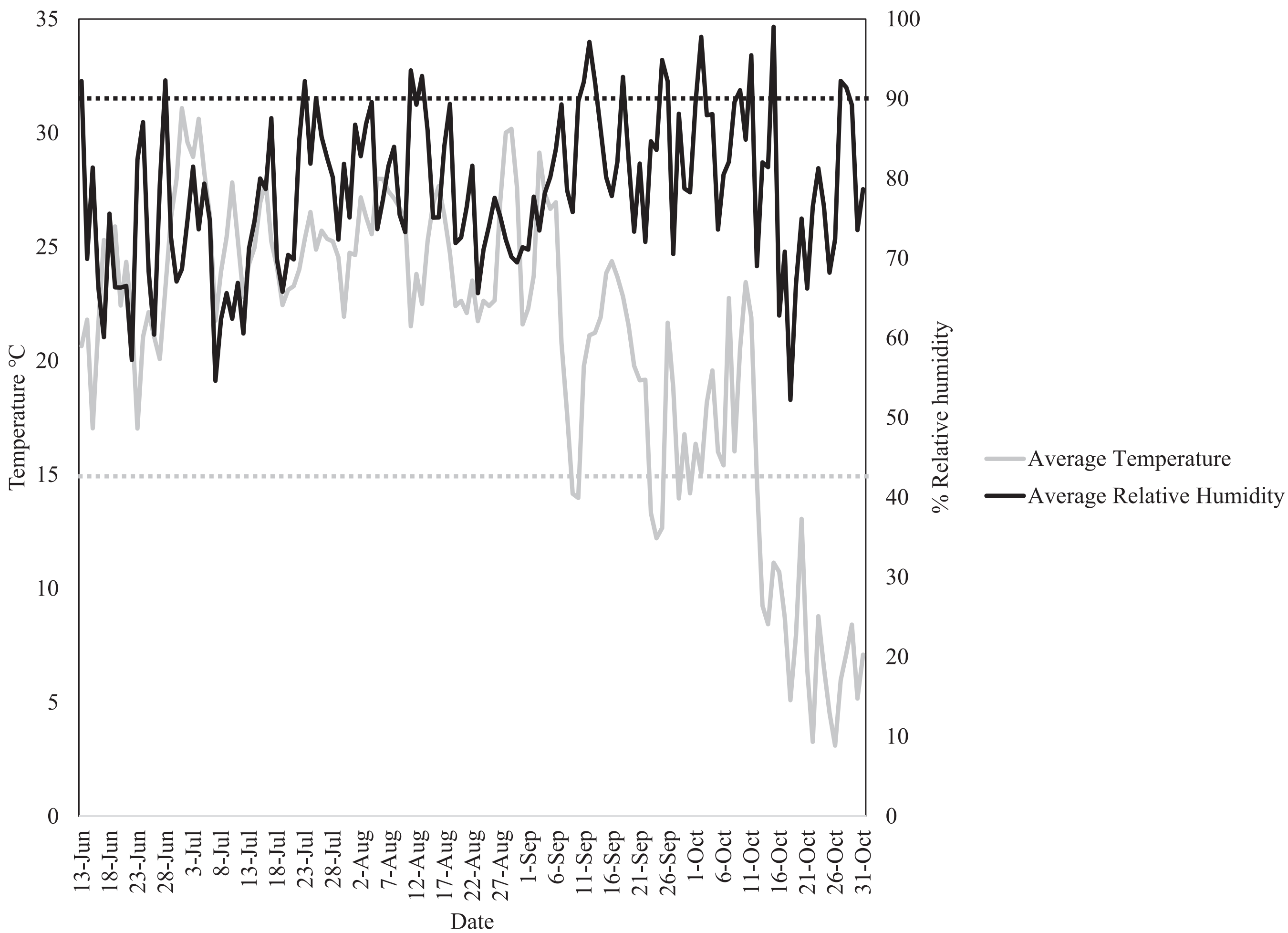

Fig. 3. Temperature and relative humidity for the 2018 study.

Data collected in 2019 included both the binomial presence/absence of disease and the number of boxwood blight lesions per plant for the #1 position plant (Table 4). Incidence represented the number of plants across all replicate plots which had been scored as positive at any time from June to October. 'Tide Hill' plants suffered significant winter injury before spring of 2019, so all Tide Hill plants were replaced with English *B. sempervirens* 'Suffruticosa'. 'Suffruticosa' plants were smaller, approximately 25% of the size of the overwintered 'Green Velvet' and 'Winter Gem' plants and did not have the potential for any infection present to start the season in 2019, unlike the overwintered 'Green Velvet' and 'Winter Gem' plants in the experiment, which had had some disease present in 2018. As a result, there were numerically smaller numbers of lesions on English 'Suffruticosa' boxwood than 'Green Velvet' in June; however, by August there were significant effects of cultivar on disease severity (Table 4). Unlike disease severity, incidence was not different for cultivar or fungicide treatment as 80 to 100 percent of plants in the #1 position in the row had some level of disease after two years. The number of lesions per plant on all cultivars remained very low (1.0 lesions in June and 1.9 in August for untreated controls), especially in comparison to the inoculated source plants which had an average of 84.9 lesions in June and 98.9 lesions in August (data not shown).

The remarkable thing to note from this data is that despite very conducive weather conditions, disease levels in the target plants remained very low and spread was only limited to the boxwood plant in the #1 position of each row over a two-year period, a gap of 0 to 20 cm (0 to 8 in) between leaves of plants, quite different from our observation of boxwood blight in landscapes. There may be a number of factors that contributed to this result. The 0.6 m planting distance between plants and the presence of wood chip mulch likely reduced the ability of the pathogen to spread down rows, although distances between plant foliage were less than distances between the stems at planting, ranging from 10 cm to 45 cm (4 – 18 in) between foliage depending on plant size. *C. pseudonaviculata* is dispersed by contact spread and by water splash of conidia (LaMondia and Maurer 2020). We conducted the experiment using strict sanitation in a manner to restrict any spread by contact. Water splash dispersal of fungal spores occurs along a steep gradient and over short distances (Madden 1997). Splash dispersal of *Leptosphaeria maculans* pycnidiospores exuded in mucilage resulted in 90% of spores remaining within 14 cm (0.5 ft) of the source with few dispersed beyond 30 cm (1 ft) (Travadon et al 2007). Dispersal distance is affected by rain droplet and environmental characteristics such as wind rather than spore morphology (Horberg 2002). Spores are dispersed

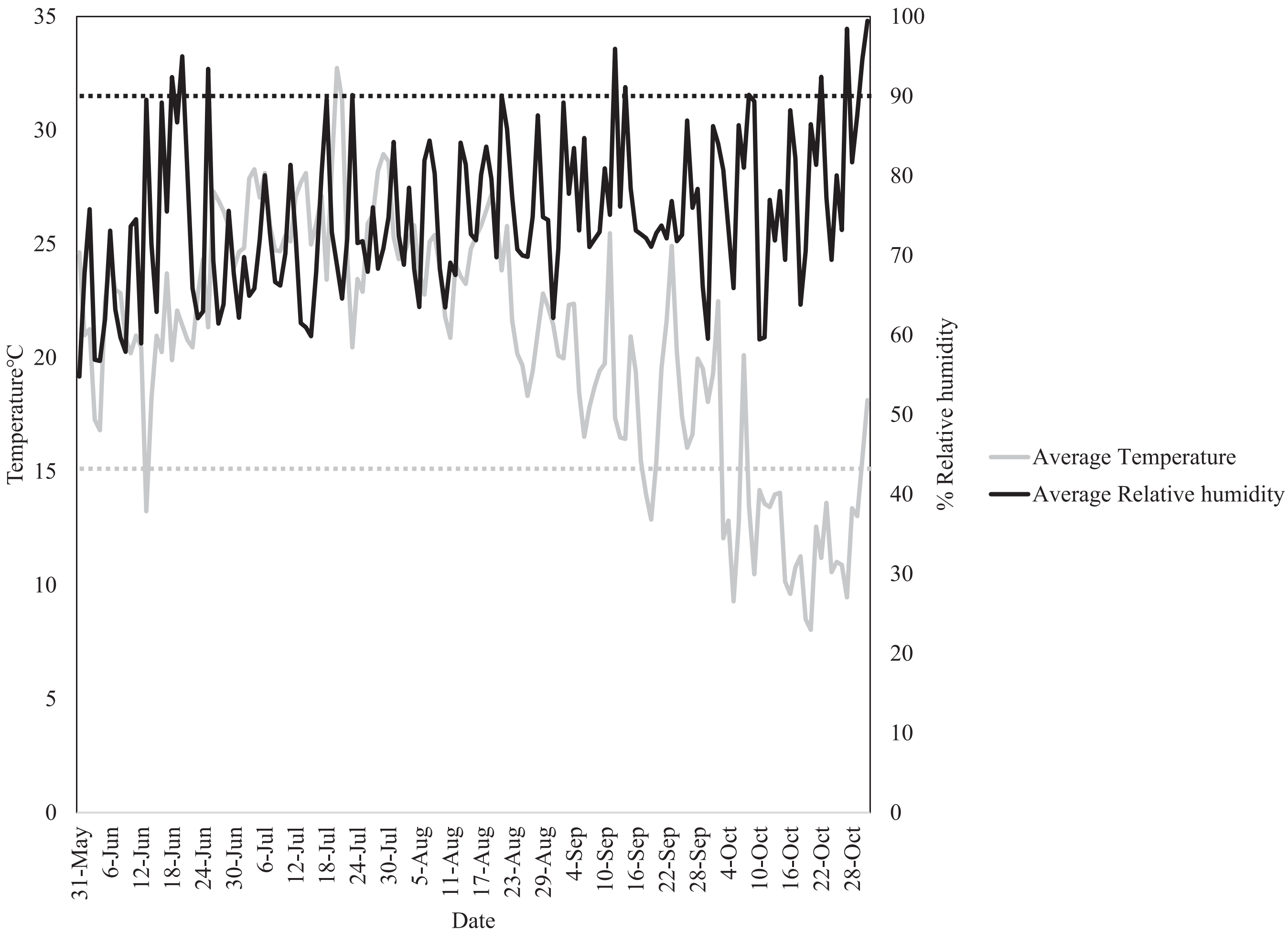

Fig. 4. Temperature and relative humidity for the 2019 study.

greater distances in large rather than small drops (Huber et al. 1996, Geagea et al. 2000) and wind increased the dispersal of drops containing spores (Fitt et al. 1989). Plant density and leaf area index also affect splash dispersal with reduced dispersal distances associated with increased plant density and leaf area index (Boudreau and Madden 1995). Healthy boxwood plants tend to have high leaf area indices which may be favorable for disease development but not for splash dispersal. Dispersal of conidia is also reduced when the inoculum source is within compared to outside the canopy (Boudreau and Madden 1995).

Because splash dispersal gradients are so steep and dispersal distances so short, movement of infected defoliated leaves and secondary splash is important in increasing the distance of spread. Surface topography has a great influence on both leaf movement and secondary splash. A smooth surface resulted in the greatest spread of anthracnose (*Colletotrichum* spp.) disease in strawberry and a rough surface above soil such as straw greatly reduced spread and dispersal (Madden et al. 1993). Likens et al. (2019) have demonstrated that mulching over the top of infected leaf debris provided significant protection of boxwood from blight by reducing infection from an inoculum source in and under the mulch. Our data adds to this knowledge. Mulch reduces plant to plant spread by water splash and appears to reduce disease development over time on infected plants, likely by reducing splash dispersal of conidia back up into the canopy from defoliated leaves which fall into the mulch.

Our data also indicate that boxwood cultivar susceptibility was more important than a monthly fungicide program in reducing spread from an infected plant to an adjacent healthy plant. A four-week interval between fungicide applications may be insufficient to effectively manage disease as was a single extra spray at a 2-week interval when disease was first observed. We have previously shown that fungicide programs applied on two-week intervals were more effective at controlling boxwood blight (98 to 99% control) than when applied at three-week intervals (78 to 87% control) (LaMondia, unpublished). These data underscore the importance of knowing the susceptibility of the boxwood cultivar being grown and the importance of epidemiological models for prediction of disease risk periods (Coop 2020) to better time fungicide applications, especially when using fungicides with curative activity (LaMondia 2020).

Current Best Management Practices (BMPs) note all these tactics (Horticultural Research Institute 2020), as well as pruning and working in boxwoods only under dry conditions. Certainly, pruning when plants are wet is likely to successfully spread the pathogen and allow infection. However, not all cases of spread in a landscape are likely

Table 3. **Boxwood blight incidence (binomial data) on plants adjacent to the source (infected) plant on June 27 and August 17, 2018 and cumulative over 2018 with positive detections.**

Fungicide treatment	Cultivar	Boxwood blight incidence on plant #1 June	August	2018 [Z]
Untreated	Tide Hill	0.0	0.3	0.6
Untreated	Green Velvet	0.3	0.6	1.0
Untreated	Winter Gem	0.0	0.0	1.0
Protectant	Tide Hill	0.0	0.0	0.5
Protectant	Green Velvet	0.4	0.1	1.0
Protectant	Winter Gem	0.3	0.0	0.8
Protectant+Systemic	Tide Hill	0.0	0.3	0.3
Protectant+Systemic	Green Velvet	0.4	0.8	1.0
Protectant+Systemic	Winter Gem	0.0	0.0	0.6

Fungicide treatment	Blight incidence June	August	2018
Untreated	0.1	0.3	0.9
Protectant	0.3	0.1	0.8
Protectant plus systemic	0.1	0.3	0.6

Cultivar	Blight incidence June	August	2018
Tide Hill	0.0	0.2	0.5
Green Velvet	0.4	0.5	1.0
Winter Gem	0.1	0.0	0.8

Fisher Exact Test			
Fungicide	ns	0.03	ns
Cultivar	0.001	0.0001	0.0001

[Z]Proportion of plants with positive detection (July to October 2018).

Table 4. **Boxwood blight incidence and severity on plants adjacent to the source (infected) plant 2019.**

Fungicide treatment	Cultivar	Boxwood blight lesions on plant #1 June 19	Aug 6	Incidence [Z]
Untreated	Suffruticosa	1.0	4.5	0.9
Untreated	Green Velvet	1.5	0.8	1.0
Untreated	Winter Gem	0.5	0.5	1.0
Protectant	Suffruticosa	0.5	0.5	0.8
Protectant	Green Velvet	0.1	0.1	1.0
Protectant	Winter Gem	0.0	0.0	1.0
Protectant+Systemic	Suffruticosa	0.4	1.4	0.9
Protectant+Systemic	Green Velvet	0.9	0.1	1.0
Protectant+Systemic	Winter Gem	0.1	0.1	0.6

Fungicide treatment	Blight lesions June	August	Incidence
Untreated	1.0	1.9	1.0
Protectant	0.5	0.2	0.9
Protectant plus systemic	0.5	0.5	0.8

Cultivar	Blight lesions June	August	Incidence
Suffruticosa	0.5	1.7 A	0.8
Green Velvet	1.0	0.3 B	1.0
Winter Gem	0.2	0.2 B	0.9

AOV			Fisher Exact Test
Fungicide	ns	ns	ns
Cultivar	ns	0.02	ns
Fungicide x Cultivar	ns	ns	ns

[Z]Proportion of plants with positive detection (June to October 2019).

due to non-conformance to BMPs. We hypothesized that conidia in sticky clumps might be moved by contact under dry conditions, survive desiccation until conditions become suitable and subsequently germinate to infect boxwood. In our experiments, spores in sticky clumps held under temperature and humidity conditions of 26 C (79 F) and 16% RH survived and germinated after 9 days. Spore clumps held at 26 C and 99% RH in the moist chamber survived and germinated after at least 3 weeks (Table 5). Interestingly, no germination of single spores from the cover slips was noticed. We have therefore demonstrated that dry clumps of sticky *C. pseudonaviculata* conidia can be transferred and held under dry conditions for an extended time and that growth of the pathogen from clumps may occur when given suitable conditions long after individual conidia become non-viable. Extracellular mucilaginous material surrounding fungal spores may protect them from desiccation (Ali et al. 1999, Dutton et al. 1993, Ramadoss et al. 1985), extreme temperature and osmotic pressure (Qu et al., 2017), ultraviolet radiation (Mondal and Parbery 2005), and perhaps fungicide toxicity (Vesentini et al. 2005, 2007). Mucilage may aid in adhesion to tools and to host plant cuticle (Boucias et al.1988) and may also inhibit conidial germination (Griffiths and Peverett 1980, Louis and Cooke 1985, Louis et al. 1988). The sticky matrix of the spores has been reported to have roles beyond adherence. Proteins from the surface matrix may influence the aggressiveness of the pathogens (Gebremariam et al. 2014). It was also reported that the proteome on the conidial surface may impact the biotic factors of the surrounding environment, as growth competitors. For example, the mutated strain which lack the hydrophobin of the entomopathogenic fungus *Clonostachys rosea* showed increased tolerance to fungal secondary metabolites of the growth competitors (Dubey et al. 2014). We postulate that contact movement of sticky *C. pseudonaviculata* conidial clumps in mucilage under dry conditions by tools, animals etc. may spread clumps of the pathogen which can survive under dry conditions until moisture and humidity create conditions suitable for infection days to weeks later. Our findings indicate that simply pruning or working in infected boxwoods under dry conditions may be insufficient to limit spread. Further measures such as frequent disinfestation of tools with

Table 5. **Germination of *Calonectria pseudonaviculata* conidia over time after incubation on cover slips in dry or humid conditions.**

Days of incubation	Dry chamber (26 C 16% RH) Single spores	Spore clumps	Weeks of incubation	Wet chamber (26 C 99% RH) Single spores	Spore clumps
3 days	- [a]	+	1 week	-	+
6 days	-	+	2 weeks	-	+
9 days	-	+	3 weeks	-	+
12 days	-	-	4 weeks	-	-

*a Spore germination scored as positive (+) or negative (-).

alcohol or fungicide application may be necessary. Additional research will be required to determine survival of *C. pseudonaviculata* conidial clumps over time and the role of mucilage in survival, adhesion and infection of boxwood. This observational data may explain episodes of spread under landscape conditions managed under current BMPs. Additional research is being conducted under a range of temperature and humidity conditions as well as on plants.

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