The Effect of Different Temperatures and Moisture Levels on Survival of *Calonectria pseudonaviculata* in Boxwood Leaves and Twigs and as Microsclerotia Produced in Culture

Nina Shishkoff, Foreign Disease Weed Science Research Unit, United States Department of Agriculture–Agricultural Research Service (USDA-ARS), Frederick MD 21771; and Mary J. Camp, Northeast Area Statistics Group, USDA-ARS, Beltsville, MD 20705-2350

Abstract

Shishkoff, N., and Camp, M. J. 2016. The effect of different temperatures and moisture levels on survival of *Calonectria pseudonaviculata* in boxwood leaves and twigs and as microsclerotia produced in culture. Plant Dis. 100:2018-2024.

Leaves and twig sections of boxwood infected with *Calonectria pseudonaviculata* were incubated in sand at two moisture levels (36% [carrying capacity] and 5% water [vol/vol]) and at five temperatures (–10, 0, 10, 20, and 30°C). Percent sporulation from monthly tissue samples plated on glucose yeast-extract tyrosine media declined to zero after 5 months at 30°C and after 7 months at –10°C. At 0, 10, and 20°C, sporulation was observed through 30 months. Statistical analysis of data collected over 16 months of sampling showed a significant effect for temperature in all sample types, with maximum survival at 10°C. For discrete

microsclerotia grown on cellophane sheets, sporulation was not observed after 2 months at 30°C or after 7 months at –10°C. At all other temperatures, they continued to germinate over the 30 months of sampling. Statistical analysis showed significant effects for temperature and moisture level, with maximum survival at 0°C in moist soil over 16 months. These results suggest that extremes of heat and cold will kill the pathogen in plant debris but, at moderate temperatures, it will remain in soil for long periods, making replanting in affected sites in well-moderated climates difficult.

In North America, boxwood blight is caused by *Calonectria pseudonaviculata* (Crous, J. Z. Groenew. & C. F. Hill) L. Lombard, M. J. Wingf. & Crous, although a second species, *C. henricotiae* Gehesquière, Heungens & J. A. Crouch, is also present in Europe (Gehesquière et al. 2016). The disease was first observed in 2011 in the states of Connecticut and North Carolina (Douglas 2012; Ivors et al. 2012) and in the province of British Columbia, Canada (Elmhirst and Auxier 2013); it has been reported from 16 U.S. states (Malapi-Wight et al. 2014). Boxwood blight causes lesions on leaves and stems of boxwood and other hosts but appears to also have a persistent presence in soil. Henricot reported survival in litter for at least 5 years (Henricot et al. 2008) but specifically mentioned a lack of visible microsclerotia. Microsclerotia were first reported in boxwood leaf and stem tissue by Weeda and Dart (2012). Dart et al. (2015) found that conidia and microsclerotia could remain viable in soil for up to 3 and at least 40 weeks, respectively. Ganci et al. (2014) conducted field experiments over a period of 2 years in commercial boxwood production fields in western North Carolina and concluded that *C. pseudonaviculata* could survive from season to season in diseased leaves on the surface or subsurface (5 cm) of the soil and soilless potting mix. Results from these field experiments indicated that the fungus survived better in diseased leaves that were buried rather than on the surface of the soil or soilless potting mix. In the field experiments, there was also a strong correlation between the percentage of leaves with microsclerotia and their ability to form spores.

Microsclerotia are resting structures known to persist for long periods of time. In order to manipulate soil conditions to be less favorable to their survival, it is necessary to know what their survival is under natural conditions. Viable microsclerotia of *Cylindrocladium scoparium* (= *Calonectria morganii*) were recovered from soil which had been in clean fallow for 7 years (Thies and Patton 1970). *Macrophomina phaseolina* microsclerotia sampled from the field survived 157 days in a crop rotational system and 163 days in a monoculture system (Reis et al. 2014). Pataky and Beute (1983) looked at field survival of microsclerotia of *Cylindrocladium crotalariae* (= *Calonectria ilicicola*) and found that survival in debris on the soil surface during the winter was less than that of samples buried 25 cm below the surface. Graham and Griffin (1988) placed soil naturally infested with microsclerotia of *C. ilicicola* at depths of 5, 13, or 25 cm in late fall and sampled for 1 year: germination of microsclerotia declined rapidly at all soil depths during the winter; leveled off during April, May, and June; and declined gradually from July to November. Shishkoff and Campbell (1990) found survival of microsclerotia of *Pyrenochaeta lycopersici* in tomato root debris left in fields after harvesting for up to 33 months. Buchwaldt et al. (1996) conducted a 4-year survival study of *Colletotrichum truncatum* in lentil fields and showed that infectivity of infested debris placed on the soil surface declined during the first 12 months of exposure, whereas infectivity of buried debris remained high, until a rapid decline occurred at 48 months.

In field studies, it is difficult to separate the effects of moisture, temperature, and soil depth. Pataky and Beute (1983) noted that moisture content and weight of buried samples were less variable than for samples placed on the soil surface and felt that temperature and moisture appeared to interact in their effect on microsclerotial viability. Graham and Griffin (1988) noted that soil at their 5-cm depth was frozen in the winter and exposed to 0°C or lower for numerous days, whereas soil at the 25-cm depth was saturated and exposed extensively to cold but not freezing temperatures; they felt that chilling plus saturation of soil with water was highly detrimental to microsclerotium germinability, and similar in its effect to soil freezing.

Some scientists performed laboratory and greenhouse studies to better understand the factors influencing survival of microsclerotia. Nadakavukaran and Horner (1961) artificially infested screened, air-dried, nonsterile sandy loam with *Verticillium albo-atrum* microsclerotia; soil moisture was adjusted to air dryness, 25, 50, 75, and 100% of

Corresponding author: N. Shishkoff; E-mail: nina.shishkoff@ars.usda.gov

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Accepted for publication 8 May 2016.

<http://dx.doi.org/10.1094/PDIS-09-15-1098-RE>
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moisture-holding capacity and flooding level, at temperatures from 5 to 40°C. Generally, microsclerotia survived poorly in soils held above 25°C. Flooding rapidly reduced the microsclerotial population at all temperature levels. The surviving population decreased rapidly as temperature increased from 25 to 40°C. Survival was best in soils held between 5 and 15°C and at 50 to 75% moisture-holding capacity. Germination of *Cylindrocladium crotalariae* (= *Calonectria ilicicola*) microsclerotia in natural soil was studied in the laboratory by Roth et al. (1979), who saw a decrease over 4 weeks when naturally infested soils were incubated at 6°C and was lower still at -3°C, while no germination was seen at -10°C. However, when soils incubated at -10 or 6°C for 4 weeks were transferred to 26°C for 4 weeks, the low-temperature effect was partially reversed. Conductivity measurements of solutions bathing microsclerotia incubated at 6°C were higher than those incubated at 26°C for 4 weeks and indicated that chilling injury might have accounted, in part, for decreased germinability of microsclerotia. Graham and Griffin (1988) conducted laboratory tests in which high soil temperatures (30, 35, and 40°C) greatly decreased microsclerotium germinability compared with a more moderate soil temperature (25°C), and the effects of high temperatures were much greater at low water potentials than at high water potentials. Almeida and Bolkan (1981) studied the effects of soil humidity and incubation temperature on survival of *Cylindrocladium clavatum* (= *Calonectria brassicae*) under controlled conditions and found that 25°C was the optimum temperature for microsclerotium survival, and found no effect for soil moisture for the two levels tested (-0.25 and -0.8 bars). Kuruppu et al. (2004) used field soil artificially infested with microsclerotia of *C. ilicicola* incubated for 1, 2, 3, or 6 weeks at 20, 25, 30, 35, and 40°C (temperatures approximating soil temperatures for soybean fields in south Louisiana). Germinable microsclerotia were enumerated after incubation at different temperatures, and soybean seed were planted in these soils. Results showed that soil temperature is a critical factor in survival of microsclerotia. The optimal soil temperature range for survival of microsclerotia was 20 to 30°C, and the maximum soil temperature limit was 35°C, above which microsclerotia did not survive. Yang et al. (2004) examined the effect of soil factors on survival of microsclerotia of *V. dahliae* and found optimum survival at 10°C and poor survival at 40, 30, and 0°C. They found lower survival in moist soil, with best survival at a soil moisture of 5%.

In this study, we assessed the survival of *C. pseudonaviculata* in leaves and twigs and as discrete microsclerotia on the surface of sand moistened to holding capacity (36% water [vol/vol]) or kept relatively dry (5% [vol/vol]) and incubated at -10, 0, 10, 20, or 30°C in plastic containers in incubators. This experimental design provided separation of moisture and temperature effects and allowed each variable to be assessed for effect on pathogen survival.

Materials and Methods

Preparing infected plant material. In each of three trials, 40 boxwood plants (*Buxus sempervirens* 'Suffruticosa') were inoculated with *C. pseudonaviculata* (isolate CBS 114417) by dipping the foliage of plants in a spore suspension (approximately 2,000 spores/ml of water) and incubating plants in a dew chamber at 20°C for 4 days. Plants were then placed in a greenhouse at 20 ± 3°C with twice-daily overhead irrigation. On a daily basis, plants were shaken to detach diseased leaves into an adjacent plastic mesh tray lined with cheesecloth and filled with a 3-cm layer of autoclaved river sand. Plants were incubated for 1 month in the greenhouse, as symptomatic leaves were collected on the moist sand surface. At the end of the month's incubation, the defoliated plants were examined for stem symptoms and 1-cm symptomatic twig segments were excised. Control plants (6 to 10) were treated with water and kept in an adjacent greenhouse under similar temperature and irrigation; leaves were intended as a check, because it was unclear whether *Pseudonectria rouselliana* (*Volutella buxi*) and other incidental fungi would affect the ability to rate survival of *C. pseudonaviculata* over time at different temperatures. Control plants were defoliated and their leaves and twig segments incubated for a month in a tray of moist sand in another greenhouse under the same conditions.

Production of microsclerotia on cellophane. Microsclerotia were produced using a modification of Nakajima et al. (1988) by placing culture plugs (1 cm in diameter) of the pathogen (isolate CBS114417) onto the surface of autoclaved cellophane sheets (Bio-rad GelAir cellophane support; Bio-Rad Laboratories, Inc.) covering the surface of glucose-yeast extract-tyrosine agar (GYET; Hunter 1992) in 9-cm-diameter plates (20 to 25 plates per trial). GYET media is semiselective for *Calonectria* spp., which produce a characteristic chestnut-colored pigment on it. After 1 month of incubation at 20°C, the surface of the cellophane was covered with microsclerotia. To prepare squares of microsclerotia-infested cellophane for sampling over time, the cellulose film on cultures was peeled from the surface of the agar and cut into approximately 1-by-1-cm squares, each appearing by eye to contain 50 to 100 microsclerotia.

Survival experiment. For each trial, samples of infected leaves and twigs were incubated in 20 plastic food storage containers, 10 containing autoclaved river sand moistened to water-holding capacity (moist; in this case, water at 360 ml/liter of sand) and 10 containing sand that was slightly moist and crumbling to the touch (dryish; water at 50 ml/liter of sand). Diseased twig segments (approximately 100) and leaves (approximately 200) were placed in each box on the surface of the moist or dryish sand spread at a depth of 3 cm in the plastic containers. The containers were weighed after the samples were added. The average weight of 10 leaves and five twig segments was also recorded. The squares of cellophane bearing microsclerotia were placed in 20 plastic boxes (60 cellophane squares per box) containing either the moist or dryish sand face up on the surface of the 3-cm layer of sand. Those containers were also weighed. The first two trials used Lock & Lock plastic boxes (14 by 14 by 4 cm) with a 0.74-liter capacity (Lock & Lock USA Distributor, Inc.); the third trial used Sterilite plastic boxes (16 by 11 by 4.5 cm) with a 0.6-liter capacity (Sterilite Corp.). The third replicate also used a Watchdog 1000 series microstation 1650 Datalogger with an SMEC 300 soil moisture sensor (Spectrum Technologies) to confirm moisture levels of the samples. Four duplicate containers of each treatment were placed in each of five Sanyo MIR-554 incubators (set at -10, 0, 10, 20, and 30°C). In addition to these samples, a single container per moisture level holding similarly prepared noninoculated boxwood leaves and twig segments was placed in each incubator.

At time zero, 10 leaves and five twig segments from *C. pseudonaviculata*-infected plants and 6 leaves and five twigs from control plants were plated on GYET media, with the leaves face down and the twigs in firm contact with the agar surface. Tissue was considered positive for *C. pseudonaviculata* if sporulation was observed within 1 week or colonies of the pathogen grew from plant tissue into the media. Four pieces of cellulose-bearing microsclerotia were placed face-up on the surface of media and the number of germinated microsclerotia per total was recorded (a microsclerotium was considered to have germinated if conidiophores were seen growing from it).

In subsequent samplings, boxes kept at -10°C were moved to 0°C 2 to 3 days before sampling to gradually thaw them before sampling, and were put back at 0°C for 1 day afterward to cool down gradually before being replaced at -10°C. Every month, duplicate leaf and twig segment samples (10 to 13 leaves and five twig segments per box) at each sand moisture level and each temperature were taken for 18 months. For the first two trials, boxes stored at 0, 10, and 20°C were also sampled at 25 and 30 months. Microsclerotium samples (two cellophane squares per box) were taken for 30 months (trial 3 to month 18). One control was taken at the same time for each treatment. Boxes were weighed and water added if necessary according to a formula (weight of box at last sampling minus weight of sample) at early samplings (through 12 months). In later samplings, due to the advanced decomposition of samples at some temperatures, the final moisture level was assessed by touching the sand. Water was added to moist sand until a wet sheen formed around a pressure point and, in dryish sand, the texture had to be barely moist but crumbly. Boxes containing microsclerotia were weighed and water was added to keep the weight the same throughout the experiment (the cellophane squares being of negligible weight). After this survival study had commenced, other research in the lab demonstrated that exposure

to 10% bleach (0.08% sodium hypochlorite) did not significantly affect survival of microsclerotia of *C. pseudonaviculata* but killed most other organisms that might be present on the leaves and twigs (Shishkoff 2016). This allowed a check to make sure that using sporulation as an indication of viability was reasonable. The procedure was tested at the 10- and 14-month sample dates for trial one and at the 6- and 10-month sample dates for trial 2, then used on all samples at 18 and 25 months. Additional leaves and twig segments were taken from each box and placed in 10% bleach for 5 min, rinsed in water for 30 min, then plated. One cellophane square from each box was also taken, treated with bleach, and rinsed before being plated on GYET. The bleached samples were examined for sporulation on the sample and presence of characteristic chestnut colonies in the media. In addition, conidia from sporulating 18-month samples were collected in a 10-ml volume of sterile water and 1-ml aliquots were plated on GYET and examined 24 h later for germination.

Statistics. A nonparametric χ^2 approach was used to determine whether the distribution of pathogen presence differed between the moisture levels across temperature. The Kruskal-Wallis exact χ^2 goodness-of-fit test (StatXact; Cytel Software Corp; Mehta and Patel 2013) for a singly ordered frequency table was used to determine

whether the distribution of the pathogen was the same for moisture level across the temperatures for all months and for just months 0 to 16. For microsclerotia, percent germination (values from 0 to 1) was modeled from a β distribution and analyzed as a three-factor repeated-measures generalized linear model using PROC GLIMMIX (SAS Institute) for moisture and temperature, with month as the repeated factor. The first-order autoregressive [ar(1)] variance-covariance structure was used to model the correlation. Means and means comparisons were done with Sidak-adjusted *P* values so that the experiment-wise error was 0.05.

Results

The pathogen was still able to sporulate in some samples after 30 months of incubation at 0, 10, or 20°C (Figs. 1 and 2). By 30 months, leaf and twig samples were quite decayed at 20°C but sporulation of the pathogen was still readily apparent on the skeletonized remnants.

Pathogen sporulation in different treatments was inconsistent from month to month from microsclerotia, leaves, and twigs; therefore, a nonparametric χ^2 approach was used to determine whether the distribution of pathogen presence differed between the moisture levels across temperature. The Kruskal-Wallis exact χ^2 goodness-of-fit test for a singly ordered frequency table was used to determine whether the distribution of the pathogen was the same for moisture levels across the temperatures for all months and for months 0 to 16. The presence of the pathogen for the leaf (pL) and twig (pT) substrates in dryish and wet soil was not statistically different (all months: pL = 0.0806 and pT = 0.1798; months 0 to 16: pL = 0.1116 and pT = 0.1991). For microsclerotia, the results were statistically significant for both cases (*P* = 0.00002 and 0.00009, respectively). This was due to the exceptionally low pathogen population in the dryish soil at a temperature of 20°C (Tables 1 and 2; Fig. 3). Treating microsclerotia cultured on cellophane was quickly found to be the best method for quantifying relative survival of the pathogen and was used for further analysis of the moisture-temperature treatments over the following months. Month 0 was excluded from the analysis because the focus of this study was the pathogen's survival after the initial conditions. Given the large number of treatments with all zero values, only two sets of treatments were analyzed: (i) temperatures of 0 and 10°C for both moistures and (ii) temperatures of 0, 10, and 20°C for the high moisture treatment. Percent germination (values from 0 to 1) was modeled from a β distribution and analyzed as a three-factor repeated-measures generalized linear model using PROC GLIMMIX (SAS Institute) for moisture and temperature, with month as the repeated factor. The first-order autoregressive [ar(1)] variance-covariance structure was used to model the correlation. The results are shown in Table 3. Means (of average percent germination) and means comparisons are given in Tables 4 and 5. The comparisons were done with Sidak-adjusted *P* values so that the experiment-wise error was 0.05. Although temperature-month was statistically significant for model 1, the main effects for moisture, temperature, and month were the largest explanations of variability. In model 2, although temperature and month were statistically significant, the difference in the average percent germination by temperature was the largest contributor. Model 2 shows that the mean percent germination for microsclerotia was statistically greatest at 0°C, lowest at 20°C, and intermediate for 10°C.

Control leaves occasionally had sporodochia of *P. rouselliana* (*V. buxi*) or fruiting bodies of *Dothiorella candollei* on them, but no *Calonectria* sp. was ever detected. On the sample dates on which additional leaves, twigs, and microsclerotia were treated with bleach, samples gave approximately the same percent sporulation as unbleached samples, with no colonies of the pathogen appearing in the media from nonsporulating plant tissue or microsclerotia. No colonies were seen in any bleached samples incubated at -10°C or 30°C, suggesting that presence of sporulation was a good indicator of viability of the organism. Conidia collected from 18-month samples germinated if plated on GYET.

Discussion

These current laboratory studies with *C. pseudonaviculata* show a strong effect of temperature survival of microsclerotia in plant tissue

Fig. 1. Sporulation from tissue and microsclerotia during the survival study. **A**, Sporulation from twig incubated under moist conditions at 10°C for 14 months; **B**, sporulation from a leaf incubated under dryish conditions at 10°C for 14 months; and **C**, microsclerotia grown on cellophane and incubated under dry conditions at 0°C for 8 months.

or as discrete microsclerotia on cellophane and an effect of sand moisture for discrete microsclerotia that was not significant for microsclerotial survival in leaves or twigs. These experiments did not reflect natural conditions but shed light on them. The use of microsclerotia from culture was artificial because other work has

shown that microsclerotia from culture were, on average, larger than those formed in leaf tissue (N. Shishkoff, unpublished). Their use, however, allowed a finer quantification of survival than was possible using leaves or twigs. Whereas leaves and twigs were scored as either sporulating or not sporulating, the use of microsclerotia on

Fig. 2. Survival of *Calonectria pseudonaviculata* over 30 months at two moisture levels (sand at approximately 36% water and 5% water [vol/vol]) and five temperatures (–10, 0, 10, 20, and 30°C), the average of three trials, with the third trial only to month 18. **A**, Sporulation from infected leaves, results for 0, 5, 10, 15, 18, 25, and 30 months shown, with samples incubated at –10 and 30°C not sampled at 25 and 30 months; **B**, sporulation from infected twigs, results for 0, 5, 10, 15, 18, 25, and 30 months shown, with samples incubated at –10 and 30°C not sampled at 25 and 30 months; and **C**, sporulation on microsclerotia grown on squares of cellophane; monthly data to 30 months.

Table 1. Frequency distributions of germination of *Calonectria pseudonaviculata* from infected tissue (leaves and twigs) and from microsclerotia cultured on cellophane when incubated at five temperatures and two moisture levels over 16 months^z

Sample	Total nonzero values									
	Over all months					Over 0 to 16 months				
	–10°C	0°C	10°C	20°C	30°C	–10°C	0°C	10°C	20°C	30°C
Leaves										
Dry	15	37	75	72	10	15	37	71	68	10
Wet	20	103	110	109	14	20	97	102	101	14
Twigs										
Dry	8	28	75	59	9	8	28	73	57	9
Wet	10	79	110	108	6	10	75	102	101	6
Microsclerotia										
Dry	25	142	102	46	10	25	102	73	33	10
Wet	19	152	131	124	12	18	102	96	86	12

^z Nonzero values represent samples where sporulation was observed.

cellophane squares allowed a high percentage of germination of microsclerotia to be distinguished from weak germination or no germination.

The use of autoclaved river sand was for convenience, because survival would likely be different in any soil type tested, but it was meant to act as a baseline. It is hoped that these results might allow other researchers to test different soil types already having some idea what temperature and moisture levels would be conducive to survival. The moisture levels tested were also chosen as reasonable approximations of moist and dryish and different results could have been reached if different levels had been chosen.

Only one isolate of the pathogen was tested here but a study currently underway compares the survival of three isolates of *C. pseudonaviculata* and one of *C. henricotiae* (N. Shishkoff, unpublished).

Despite the artificiality, this work allows broad generalizations to be made for a new pathogen whose basic epidemiology is still imperfectly understood. At the highest and lowest temperatures, the pathogen did not

Table 3. Analysis of variance table for microsclerotium survival study over 16 months

Source	df	F value	P value
Model 1			
Moisture	1	26.17	<0.0001
Temperature	1	28.86	<0.0001
Month	15	3.21	<0.0001
Moist × temperature	1	0.11	0.7398
Moist × month	15	1.65	0.6200
Temperature × month	15	1.77	0.0397
Moist × temperature × month	15	0.81	0.6612
Model 2			
Temperature	2	45.31	<0.0001
Month	15	2.62	0.0013
Temperature × month	30	1.00	0.4795

Table 2. Months and temperatures with all zero values (no sporulation observed) for microsclerotium survival study

Month	Dry					Wet				
	−10°C	0°C	10°C	20°C	30°C	−10°C	0°C	10°C	20°C	30°C
0	...	...	...	...	...	...	...	...	...	...
1	...	...	...	...	...	...	...	...	...	...
2	...	...	...	...	X	...	...	...	...	...
3	...	...	...	...	X	...	...	...	...	X
4	...	...	...	X	X	...	...	...	...	X
5	...	...	...	...	X	X	...	...	...	X
6	...	...	...	...	X	X	...	...	...	X
7	...	...	...	X	X	X	...	...	...	X
8	X	...	...	...	X	X	...	...	...	X
9	X	...	...	X	X	X	...	...	...	X
10	X	...	...	X	X	X	...	...	...	X
11	...	...	...	X	X	X	...	...	...	X
12	X	...	...	...	X	X	...	...	...	X
13	X	...	...	...	X	X	...	...	...	X
14	X	...	...	X	X	X	...	...	...	X
15	X	...	...	...	X	X	...	...	...	X
16	X	...	...	...	X	X	...	...	...	X

Fig. 3. Loess plot of survival of microsclerotia of *Calonectria pseudonaviculata* over 16 months at two moisture levels (sand at approximately 36% water [A] and 5% water [B] [vol/vol]) and five temperatures (−10, 0, 10, 20, and 30°C); average of three trials.

survive long (7 months at -10°C and 2 to 5 months at 30°C). These are not extreme temperatures, and give some hope that the pathogen would not persist for long periods of time in the far northern or southern United States. In moderate climates, microsclerotia might be at risk for survival and nurseries in such regions would want to select from a menu of Best Management Practices: remove fallen leaves; sanitize tools, boots, and clothing; and quarantine new stock before placing it in the field.

Litter may be important in the survival and spread of the boxwood blight pathogen. Buchwaldt et al. (1996) investigated windborne dispersal of *Colletotrichum truncatum* and survival in infested lentil

debris. Different windborne materials were inoculated onto lentil assay plants. Dust generated during harvest of anthracnose-infested lentil crops was shown to be a source of inoculum that could be dispersed by wind at least 240 m from a combine. Plant debris and soil dispersed by high winds in the fall from fields with infested lentil stubble also were sources of inoculum. Samples that had overwintered outside also caused anthracnose on inoculated assay plants. The authors concluded that dispersal of *C. truncatum* by wind has likely contributed to the spread of lentil anthracnose in western Canada. They also found that infectivity of infested debris placed on the soil surface declined during the first 12 months of exposure, whereas infectivity of buried debris remained high, leading them to conclude that long-term survival of *C. truncatum* in buried lentil debris might explain the high level of infestation found in many fields, although windborne inoculum could also be a source of infection. There is further research to be done in mitigation of infested soil and composting of infected litter in such a way as to kill the pathogen.

Virginia Cooperative Extension has published a series of Best Management Practices (<http://pubs.ext.vt.edu/category/plant-diseases.html>) that deal, in part, with the problem of the boxwood blight pathogen surviving in litter and soil (Bordas et al. 2014; Bush et al. 2015; Dart et al. 2014a,b; Hansen et al. 2015). Recommendations include bagging and removing debris, heating soil with an agricultural flamer, and burning or burying litter, all of which recognize the risk the survival of microsclerotia poses to boxwood health.

Acknowledgments

We thank F. Eskandari and K. Johnson for technical assistance. Funding support was provided from the 2012-2013 Farm Bill project through the Animal and Plant Health Inspection Service.

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Table 4. Model 1: Means (average percent germination) and means comparisons for survival study for microsclerotia over 16 months

Factor	Temperature ^y		Mean ^z
	0°C	10°C	
Month			
1	0.6590 abA	0.6596 aA	...
2	0.6080 abA	0.4257 abA	...
3	0.6164 abA	0.4404 abA	...
4	0.7951 aA	0.5698 abA	...
5	0.7272 aA	0.5003 abA	...
6	0.6801 abA	0.5009 abA	...
7	0.5953 abA	0.4580 abA	...
8	0.7035 abA	0.6087 abA	...
9	0.6838 abA	0.3505 abA	...
10	0.4453 abA	0.2675 abA	...
11	0.7443 aA	0.3657 abA	...
12	0.6007 abA	0.4759 abA	...
13	0.4656 abA	0.1835 abA	...
14	0.4819 abA	0.3350 abA	...
15	0.7447 aA	0.2143 bB	...
16	0.3235 bA	0.3959 abA	...
Moisture			
Dry	...	...	0.4200 b
Wet	...	...	0.6186 a

^y Month means within temperature with different lowercase letters are different at the 0.05 significance level. Temperature means within month with different uppercase letters are different at the 0.05 significance level.

^z Moisture means with different lowercase letters are different at <0.00001 significance level.

Table 5. Model 2: Means (average percent germination) and means comparisons for survival study of microsclerotia over 16 months

Factor	Mean ^z
Month	
1	0.5250ab
2	0.3955ab
3	0.3738ab
4	0.6374a
5	0.5916ab
6	0.5874ab
7	0.5997ab
8	0.6572a
9	0.5674ab
10	0.2892b
11	0.5043ab
12	0.4755ab
13	0.3767ab
15	0.4531ab
15	0.5614ab
16	0.4860ab
Temperature	
0°C	0.7178 a
10°C	0.5101 b
20°C	0.2851 c

^z Month means and temperature means with different letters are different at the 0.05 significance level.

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