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

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Cylindrocladium pseudonaviculatum (= Cylindrocladium buxicola), the causal agent of boxwood blight, was first reported in the United States in October 2011 (5). This pathogen was reported in the United Kingdom in the mid 1990s and by 2002 had spread to several other European countries and New Zealand (2,3,4,5). The pathogen causes severe defoliation and dieback to species in the genus Buxus (3). One challenge of managing soil-borne plant pathogens is their ability to survive for extended periods in soil. One evolutionary adaptation enabling many soil-borne plant pathogens, including many Cylindrocladium species, to survive in soil is the formation of specialized structures called microsclerotia. Indeed, microsclerotia formation in host tissue enables some Cylindrocladium species to survive as long as 15 years in soil (1). Cylindrocladium scoparium and C. floridanum have been shown to produce abundant microsclerotia after abscission of inoculated azalea leaves (Rhododendron obtusum) (6). Furthermore, azalea leaves colonized with Cylindrocladium microsclerotia can produce conidia that disperse and subsequently cause new infections (7). These findings demonstrated the potential of Cylindrocladium to spread as microsclerotia in fallen leaf material that subsequently becomes incorporated in soil, stuck to equipment, or carried by wind to later infect susceptible hosts. One study demonstrated the ability of C. pseudonaviculatum to survive in infested leaf material for at least 5 years; however, the authors did not observe any microsclerotia and suggested the pathogen survived as mycelia (3). If the boxwood blight pathogen has indeed lost or never evolved the ability to produce microsclerotia in tissues, one could infer that the pathogen is either less equipped for long term survival in soil than other Cylindrocladium species or the pathogen has evolved another mechanism to enable it to persist in soil. Based on these assumptions, we conducted a histological study to determine the potential role, if any, of microsclerotia in the lifecycle of C. pseudonaviculatum.

To determine if C. pseudonaviculatum forms microsclerotia in field-infected host tissue, leaves (N = 27) and stems (N = 17) were sampled from 14 infected American boxwood (Buxus sempervirens ‘Arbvorescens’) plants collected from two fields in a Carroll Co., VA, nursery. C. pseudonaviculatum was sporulating heavily on collected leaf and stem material. Additionally, leaves sporulating heavily with Macrophoma candollei (N = 12) and Volutella buxi (N = 20) were collected for comparison. Following 48 h incubation in a moist chamber at 22° C, all plant material showed no evidence of other fungi. Plant tissue was stained by boiling in lactoglycerol (1:1:1, lactic acid/glycerol/distilled water) with 0.05% trypan blue for 30 s in a microwave. Tissue was cleared for 24 h in 1:1 glycerol/distilled water. Once cleared, infected tissue was viewed with a
compound microscope (Nikon Eclipse E400, Melville, NY). Microsclerotia were visible in all *C. pseudonaviculatum* infected leaf and stem tissue (Fig. 1, 2, and 3). In *M. candollei* and *V. buxi* infected leaves, intercellular mycelia were observed but no microsclerotia were evident (Fig. 4).

Pure cultures of *C. pseudonaviculatum* isolates were obtained from infected leaf material from Carroll Co., VA. Cultures were grown for 7 days on 2% potato dextrose agar (PDA) and were used to inoculate healthy detached leaves of American boxwoods. Boxwood leaves were surface sterilized in a 10% bleach solution for 5 min, then rinsed in distilled water. Leaves were then placed on colonized media for 7 days to allow infection and colonization. As a control, boxwood leaves (N = 24) were placed on 2% PDA in the absence of a fungus. All cultures were grown in ambient light at 22°C. All leaf material was stained, cleared, and viewed microscopically as described previously. Microsclerotia were visible in the leaves inoculated with *C. pseudonaviculatum*. No microsclerotia were observed in the controls.

Based on these observations, *C. pseudonaviculatum* was found to produce abundant microsclerotia in host tissue. These specialized structures are ecologically important for this species and may contribute to its ability to survive in soil for extended periods of time and allow the pathogen to spread in fallen leaf material incorporated in soil, stuck to equipment or blown by wind more effectively. To reduce inoculum levels, great care should be taken to remove and dispose of infected leaf and stem material in nurseries and landscape settings. Phytosanitary precautions should also be taken when
working with or around potentially infected plant material as mud or plant debris is able to harbor microsclerotia.

Literature Cited