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Flaming to Reduce Inocula of the Boxwood Blight Pathogen, *Cylindrocladium pseudonaviculatum*, in Field Soil

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In September 2011, a new disease of *Buxus* spp. called boxwood blight, caused by *Cylindrocladium pseudonaviculatum* (= *C. buxicola*), was reported for the first time in North America (7). To date, the boxwood blight pathogen has been reported with isolated finds in ten states (Connecticut, Massachusetts, Maryland, North Carolina, New York, Ohio, Oregon, Pennsylvania, Rhode Island, and Virginia) and two Canadian providences (British Columbia and Ontario). In October 2011, the boxwood blight pathogen was confirmed in field-planted American boxwood (*Buxus sempervirens* 'Arborescens') in Carroll County, VA (7,11). Defoliation among infected plants ranged from 10 to 80% with over 40% of the plants showing symptoms of boxwood blight by the end of October 2011. A histological study conducted on infected boxwood at this site determined symptomatic leaves were often heavily colonized with darkened mycelia and microsclerotia of *C. pseudonaviculatum* in Carroll Co., VA (11). The significant role microsclerotia and infested plant tissue can play in the epidemiology of *Cylindrocladium* spp. in ornamental nursery systems has been documented (8,9). Microsclerotia have been shown to remain viable in soil for as many as 15 years, making them especially troublesome (1). In an effort to reduce inoculum levels in the infested Carroll Co. field, symptomatic plants were removed, collected into a pile, and destroyed by burning in late February 2012. Before the symptomatic boxwood was destroyed, infected leaves had fallen to the ground which represented an additional potential inoculum source. Propane flammers have been used to manage *Verticillium* spp. in soils by flaming potato (*Solanum tuberosum*) vines (5) and pepper-mint (*Mentha × piperita*) stubble after harvest (4). Additionally, flammers have been used to reduce primary inoculum of apple scab (caused by *Venturia inaequalis*) by flaming apple (*Malus domestica*) leaves on orchard grounds (3). Therefore, we sought to reduce inocula at the infested site using a propane push flamer and investigated the hypotheses that soil flaming would be effective to reduce viable inocula of *C. pseudonaviculatum* in the upper layer of soil.

Soil flaming treatment. In an attempt to reduce inocula at the site, a propane push flamer (Red Dragon, La Crosse, KS) was used to scorch leaf material colonized with *C. pseudonaviculatum* left behind on the soil surface. The propane flamer was pushed by hand over the soil (clay-loam) which was mostly free of weeds and was low in organic matter except for visible boxwood leaf litter at the time of treatment. The operator treated the soil with flame until visible leaf and plant debris were burned and no longer visible on the soil surface. This was at a speed of approximately 1 m/45 sec but varied depending on factors such as leaf litter levels and roughness of the soil surface.

Soil sample collection, processing, and microsclerotia quantification. A previously developed technique (10) for quantifying *C. crotalariae* from peanut fields was adapted for this study. Ten (1-m radius) plots were randomly selected in an area where boxwood plants infected with *C. pseudonaviculatum* had been located. Twelve subsamples of 100 g of soil were collected from each plot prior to and after flaming from the top 5 cm of soil using a trowel. The trowel was sterilized using Steramide (Edwards-Councilor, Norfolk, VA) quaternary ammonia solution (200 ppm) between uses. Subsamples were mixed together in a composite sample.

Ten grams of each composite sample was separated into particle sizes larger than 850 μm (to capture abscised leaf material), 250 to 850 μm , and 38 to 250 μm using 850- μm , 250- μm , and 38- μm sieves. Each fraction was suspended in 800 ml distilled water and blended in an Ice Breaker blender (Hamilton-Beech, Richmond, VA). Each suspension was filtered through a 3- μm Isopore (Millipore, Billerica, MA) membrane filter. The filtrate was scraped off each filter using a razor blade into 25-ml of distilled water, and vortexed for 30 s. One-ml aliquots of each soil suspension fraction were transferred to ten Petri plates containing 1/4-strength potato dextrose agar amended with 100 mg/liter streptomycin sulfate and 100 mg/liter chloramphenicol.

All plates were incubated for 7 days at room temperature (25 to 28°C) and viewed with a compound microscope (Nikon Eclipse E400, Melville, NY) at magnification levels ranging between 4 and 40 \times . Colonies of *C. pseudonaviculatum* were identified based on conidial and vesicle morphology (2,6).

Comparison of viable microsclerotia levels in non-flamed versus flamed soil. An overall reduction in CFUs of *C. pseudonaviculatum* was observed in the flamed soil samples. Non-flamed samples had an average of 25.2 CFUs/10 g soil whereas the flamed soil had an average of 4 CFUs/10 g soil. The difference in CFUs between the flamed and non-flamed samples was significant ($P = 0.00019$) using a two tailed paired Student's t-test.

While separating the fractions of soil, more leaf litter was visibly evident in the non-flamed samples (Fig. 1). The leaf litter was captured in the 850- μm sieve which is the soil fraction a majority of the *C. pseudonaviculatum* CFUs were present (Fig. 2). Implications for pathogen mitigation.

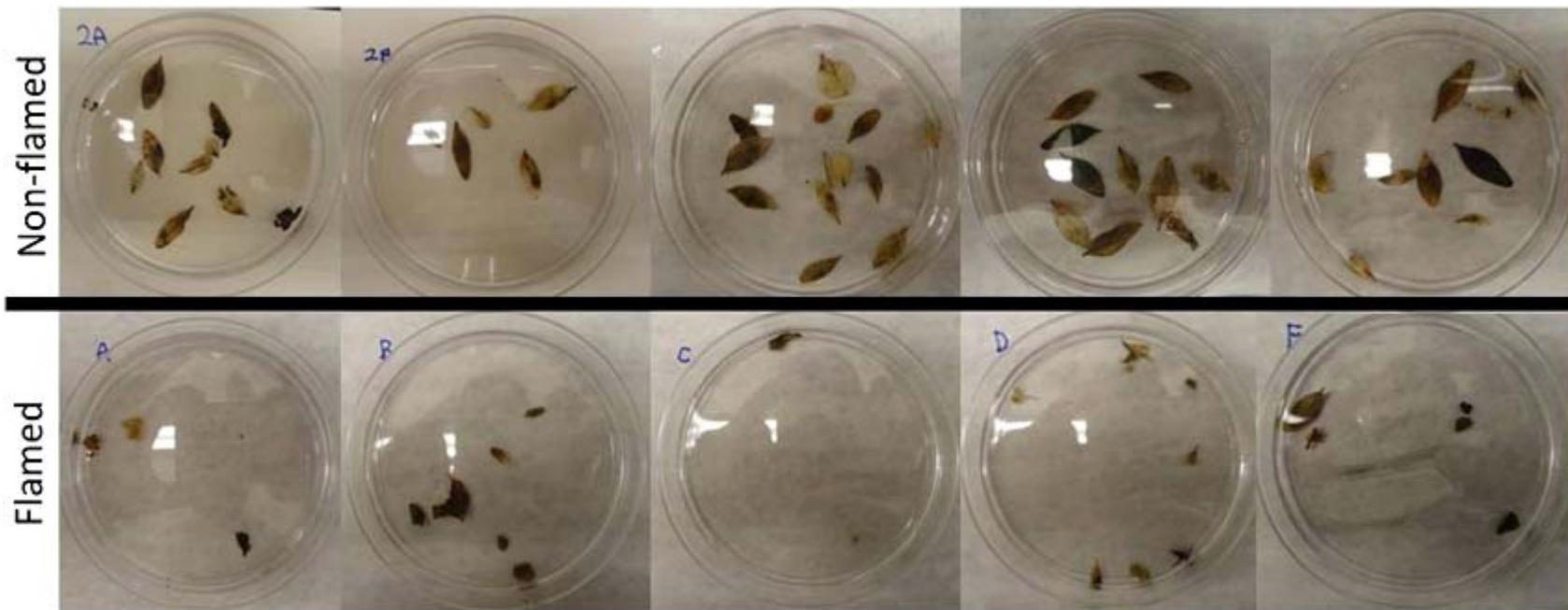


Fig. 1. Examples of boxwood leaf debris collected from the 850- μ m sieve in non-flamed (top row) versus flamed (bottom row) soil samples.

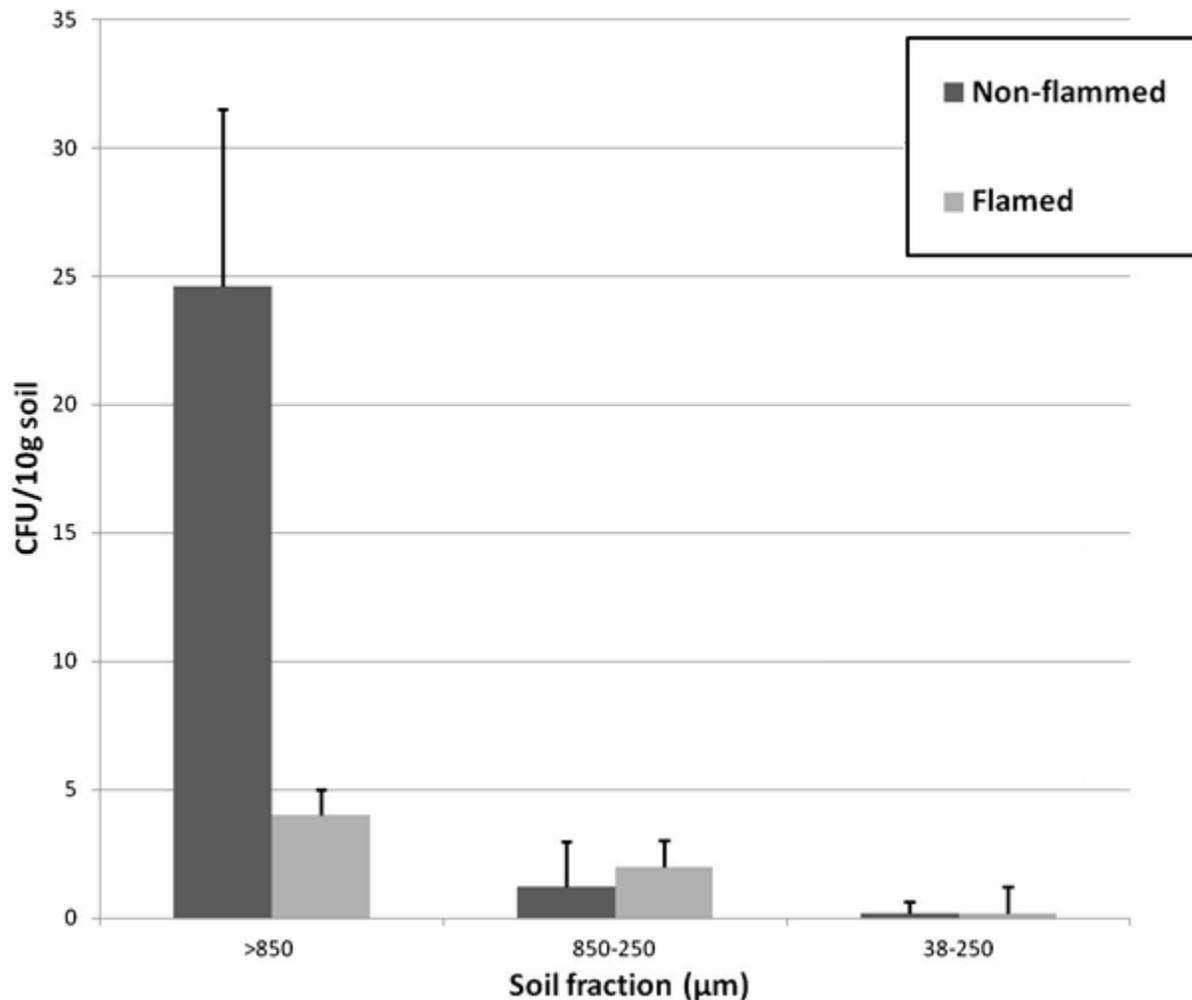


Fig. 2. Average number of *Cylindrocladium pseudonaviculatum* colony-forming units in non-flamed versus flamed soil by particle fraction size.

These results support the hypothesis that flaming soil surfaces can significantly reduce levels of inocula of *C. pseudonaviculatum* in the upper layer of soil. They also support the hypothesis that at our study site a majority of *C. pseudonaviculatum* inocula in the upper 5 cm of soil is present as inocula associated with abscised leaf material. It is likely that as infected leaf material decomposes, inocula will be more abundant at smaller fraction sizes potentially making it more difficult to eradicate by burning. Great care should be taken to destroy or remove infected leaf debris as soon as *C. pseudonaviculatum* is detected on a site before leaf debris are blown by wind, buried by erosion or begin to decompose.

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