Diseases Caused by Fungi and Fungus-Like Organisms

Occurrence of Volutella Blight Caused by *Pseudonectria foliicola* on Boxwood in Tennessee

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Boxwood (*Buxus* sp. L.) is a popular evergreen shrub in the United States widely used as a landscape plant and fresh greenery. Boxwood 'Green Velvet' (*B. sinica* var. *insularis* × *B. sempervirens*) plants grown in field conditions exhibiting Volutella blight (VB) symptoms were found in a commercial nursery in Warren Co., Tennessee, in May 2019. Leaves appeared red, brown, or tan on affected plants. Waxy, salmon pink fruiting bodies (sporodochia) were observed underneath affected leaves using a hand lens. Leaf drop was also observed. Black lesions under the bark were observed in some plants. Disease severity (percentage leaf area diseased) was nearly 40%, and disease incidence was nearly 30% of 1,000 plants. Infected leaf and stem tissues collected from four symptomatic plants were surface sterilized with 70% ethanol and washed with sterile distilled water. Culturing the infected leaf and stem pieces (5 mm) on PDA consistently yielded white fluffy aerial mycelium growth with scattered salmon-color slimy masses of conidia forming from sporodochia after 10 days of incubation at 25°C in a 12-h fluorescent light/dark cycle. A total of two isolates (FBG2020_396, FBG2020_405) were hyphal-tip purified on PDA. Conidia ($n = 50$) were hyaline, aseptate, fusiform to ellipsoidal, measuring an average of $7.8 \times 3.3 \mu\text{m}$ (range: 4.84 to 13.2×2.2 to $4.64 \mu\text{m}$). To confirm pathogen identity, total DNA was extracted using an UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories) directly from a 5-day-old culture of isolates (FBG2020_396, FBG2020_405) on PDA. The ribosomal DNA internal transcribed spacer region (ITS), β -tubulin (tub2), and part of 28S large ribosomal subunit (LSU) regions were amplified by PCR using the primer pairs ITS 5/ITS 4, T1/BTb2, and LR0R/LR5, respectively (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997; Rehner and Samuels 1994; Vilgalys and Hester 1990; White et al. 1990). Newly generated sequences—GenBank/NCBI numbers MW459251, MW465902 (ITS), MW464656, MW464657 (tub2), and

MW459255, MW465903 (LSU)—were 100% identical to *Pseudonectria foliicola* L. Lombard & Crous ex-type (CBS 123190) sequences KM231776, KM232035, and NG_058095, respectively. To complete Koch's postulates, six Green Velvet plants grown in 10-cm square pots (containing 40% coarse sand and 60% ground pine bark) were inoculated by spraying conidial suspension (FBG2020_396 [1×10^5 conidia/ml]) obtained from 2-week-old PDA cultures. Plants were covered with clear plastic humidity domes for 3 days, and then they were maintained in a growth chamber at 25°C and 60% RH in a 12-h fluorescent light/dark cycle. Six control plants were maintained in the same environment without pathogen introduction. The pathogenicity test was conducted twice. After 10 days, typical symptoms of VB developed on inoculated plants, and microscopic examination revealed the same pathogen morphology as the original isolate. *P. foliicola* was consistently reisolated from leaves and stems. All control plants remained symptom-free, and *P. foliicola* was not reisolated. *P. foliicola* causing VB has been reported on *B. sempervirens* in Czech Republic (Spetik et al. 2020) and New Zealand (Lombard et al. 2015) and on *Buxus* sp. in Illinois, Maryland, Massachusetts, North Carolina, and Washington (Salgado-Salazar et al. 2019). This is the first report of VB of boxwood caused by *P. foliicola* in Tennessee. *P. foliicola* is an opportunistic pathogen and infects weak, stressed, and injured boxwood plants/cuttings (Rivera et al. 2018). This pathogen could cause a serious economic loss to boxwood nursery growers, because it can significantly affect the ornamental value of boxwood plants and fresh greenery. Integration of sanitation practices with other disease management strategies such as biorational products and reduced-risk fungicides will be necessary for limiting the spread of pathogen and successful management of *P. foliicola* on boxwood in both field and postharvest conditions.

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