Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Coinfection of *Sarcococca hookeriana* (Sweetbox) by *Coccinectria pachysandricola* and *Calonectria pseudonaviculata* Causing a Foliar Disease of Sweetbox in Pennsylvania

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Funding: Funding was provided by the USDA-ARS projects 8042-22000-298-00-D and 2072-22000-043-00-D, USDA-ARS Floriculture Nursery Research Initiative project 0500-00059-001-00-D, and by the appointment of V. Castroagudín to the ARS Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an inter-agency agreement between the U.S. Department of Energy (DOE) and the USDA. ORISE is managed by ORAU under DOE contract number DE579AC05-06OR23100. Plant Dis. 105:1568, 2021; published online as <https://doi.org/10.1094/PDIS-06-20-1198-PDN>. Accepted for publication 27 November 2020.

Sweetbox (*Sarcococca hookeriana*) is a high-value ornamental shrub susceptible to disease caused by *Calonectria pseudonaviculata* (*Cps*) and *Coccinectria pachysandricola* (*Cpa*) (Malapi-Wight et al. 2016; Salgado-Salazar et al. 2019). In July 2018, 18-month-old sweetbox plants with leaf spots and defoliation were observed in a residential landscape in Lancaster County, Pennsylvania. Small tan leaf spots grew to cover half of the leaf, developing a concentric banding with dark brown rings and a yellow halo. The symptoms agreed with those of *Cpa* disease of sweetbox reported from Washington D.C. (Salgado-Salazar et al. 2019). Diseased plants were located ~1.5 m from *Buxus sempervirens* with boxwood blight. Morphological and genetic characterization of isolated fungi and pathogenicity tests followed Salgado-Salazar et al. (2019). White to salmon pink spore masses developed on the

abaxial leaf surface after humid chamber incubation. Two distinct fungal cultures were recovered (JAC 18-61, JAC 18-79) on potato dextrose agar (Fisher Scientific, Pittsburgh, PA). JAC 18-61 presented cultural and morphological characteristics as described for *Cps* (Crous et al. 2002). JAC 18-79 produced flat, filamentous, light salmon colonies with tan centers and white filiform borders containing pale pink sporodochia, verticillate and simple conidiophores ($\bar{x}$: $61.8 \pm 20.12 \mu\text{m}$, $n = 20$) with lateral, cylindrical phialides ($\bar{x}$ = $18.1 \pm 5.83 \times 2.4 \pm 0.7 \mu\text{m}$, $n = 20$), and ellipsoid, hyaline conidia without septa ($\bar{x}$ = $15.2 \pm 1.9 \times 3.3 \pm 0.7 \mu\text{m}$, $n = 20$). Sexual structures and chlamydospores were not observed. The characteristics of JAC 18-79 agree with those reported for *Cpa* (Salgado-Salazar et al. 2019). Bidirectional sequencing of the ITS, beta-TUB, and RPB1 and RPB2 regions was performed as described (Salgado-Salazar et al. 2019). BLASTn comparisons against NCBI GenBank revealed JAC 18-61 sequences (MT318150 and MT328399) shared 100% identity with *Cps* sequences (JX535321 and JX535307 from isolate CB002). Sequences from JAC 18-79 (MT318151, MT341237 to MT341239) were 100% identical to *Cpa* sequences (MH892596, MH936775, MH936703 from isolate JAC 16-20 and JF832909, isolate CBS 128674). The genome of JAC 18-79 was sequenced and yielded an assembly of 26.3 Mb (204 contigs >1,000 bases, N_{50} = 264.3 kb, 92× coverage, JABAHV0000000000) that contained the MAT1-2 mating-type idiomorph and shared 98.9% similarity with *Cpa* BPI910731. Isolate JAC 18-61 (*Cps*) caused lesions on wounded and unwounded sweetbox and boxwood leaves. In general, JAC 18-79 (*Cpa*) infected only wounded leaves of both hosts; however, in one trial, one unwounded sweetbox and two unwounded boxwood plants developed lesions, possibly due to the presence of natural wounds. Control plants did not develop symptoms. These results diverge to some degree from previous reports of *Cpa* infecting unwounded sweetbox and not infecting wounded boxwood (Salgado-Salazar et al. 2019). These results indicate that virulence variation among *Cpa* isolates might occur. Plating of symptomatic tissue and examination of spores fulfilled Koch's postulates for both pathogens. To our knowledge, this is the first report of *Cpa* blight on sweetbox in Pennsylvania, and the second U.S. report of the disease. This is also the first report of coinfection of *Cpa* and *Cps* on diseased sweetbox foliage. Given the capacity of *Cpa* to infect both sweetbox and boxwood, inspection for *Cpa* on both hosts is advisable.

References:

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The author(s) declare no conflict of interest.

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Keywords: boxwood blight, *Buxus*, emergent diseases, *Sarcococca*, *Volutella* blight, woody ornamentals

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