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DISEASE NOTES

First Report of *Colletotrichum siamense* Causing Apple Bitter Rot in Central Argentina

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The introduction of low-chill apple cultivars in Argentina allowed the expansion of production into warmer northern and central areas. Bitter rot, caused by *Colletotrichum* species, is one of the prevalent apple diseases worldwide (Baroncelli et al. 2014; Munir et al. 2016; Velho et al. 2015). In December 2014, bitter rot symptoms were observed on apple fruit cultivars Eva, Caricia, and Princesa in four orchards of Santa Fe Province. The rot began as brown, 1 to 2 mm circular spots that enlarged rapidly and became sunken and extended toward the fruit core in a V-shaped pattern. Under high humidity conditions concentric rings of pinhead-size salmon acervuli formed in the lesions. The causal agent was isolated by touching acervuli with a sterile needle and monosporic cultures were obtained on PDA after 7 days at 25°C, with a 12-h light period. Colonies were white to gray on the top and pink on the underside, where concentric rings of salmon acervuli were clearly distinguished. The width and length of one hundred conidia were examined in three isolates (E3, E8, and E9), ranging from 3.37 to 5.54 µm (avg. 4.46), and from 11 to 17.85 µm (avg. 14.58), respectively. Conidia were mainly cylindrical, with rounded ends. After germination, conidia formed oval appressoria ranging from 9.17 to 10.31 µm (avg. 9.65), and from 6.88 to 7.81 µm (avg. 7.39). These morphological characteristics correspond to species belonging to *C. gloesporioides* complex (Weir et al. 2012). To accurately identify the species, DNA was extracted from isolates and genes corresponding to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), β-tubulin (TUB2), and calmodulin (CAL) were partially amplified and sequenced. CAL and GAPDH sequences presented a 100% of identity with species of *C. siamense*, whereas TUB2 sequences showed between 99 and 100% identity with the same species. The nucleotides sequences were deposited in GenBank (KY656675–77, GAPDH; KY656678–80, TUB2; and MF476801–03, CAL). Multilocus

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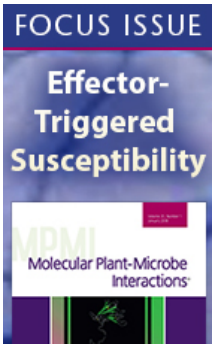

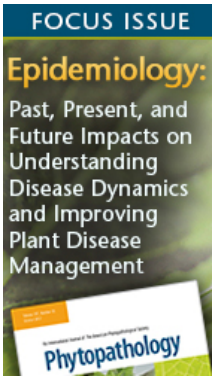

phylogenetic analyses performed with references sequences (Weir et al. 2012) showed that the three isolates clustered with *C. siamense*, in accordance with BLAST results. To confirm pathogenicity, each isolate was inoculated in eight fruits of the cultivar from which it was originally obtained. Two 10-µl drops of conidial suspension (1×10^5 conidia per ml) were deposited in wounded and nonwounded areas on fruits previously disinfested with 1% sodium hypochlorite solution for 1 min and rinsed twice with sterile distilled water. Drops of sterile water were deposited in eight fruits as control. Pathogenicity tests were repeated twice. Fruits were kept under high humidity conditions at 25°C for 10 days. First symptoms appeared 3 days after inoculation (DAI) in wounded areas and 5 DAI in nonwounded areas. After that, all of the isolates produced symptoms identical to those previously described, whereas the controls remain symptomless. The pathogen was reisolated from lesions, and identified as *C. siamense* by morphological characteristics and based on the CAL sequences, as previously described. To our knowledge, this is the first report of *C. siamense* in Argentina causing bitter rot on apple. *C. siamense* was previously reported to be more aggressive than other *Colletotrichum* species, but it is also more sensitive to fungicides (Munir et al. 2016), which encourages the development of species-specific management strategies for this pathogen in central Argentina.

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