

Disease Note

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum chrysophilum* Causing Apple Bitter Rot in Spain

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Bitter rot of apple (*Malus domestica* Borkh.) is a cosmopolitan disease affecting fruit, and it causes considerable losses worldwide. In September 2020, symptoms of bitter rot were observed on ‘Pink Lady’ apples in two orchards (~2.5 ha each) in Gualta, Catalonia, Spain (42.03803 N, 3.09831 E and 42.03942 N, 3.10931 E). Early symptoms consisted of light-brown and sunken circular lesions (1 to 4 mm) that enlarged over time, which later became dark brown and water soaked and extended cone-shaped toward the core. Sporulation was mostly noticed in larger lesions. Estimated incidence was 2 and 20% of 150 trees surveyed in each orchard, respectively. Twenty-one fungal isolates were obtained from diseased fruit by culturing small pieces of necrotic tissue on potato dextrose agar (PDA) amended with rifampicin at 50 mg/liter. Colonies on PDA looked identical. They were cottony, initially light gray colored on top, and darkened with age; the colony reverse was initially cream colored and darkened with age. Conidia were produced in orange acervular masses on Spezieller Nährstoffarmer agar and were aseptate, hyaline, cylindrical with obtuse ends, and measured 10.1 to 14.7 × 4.5 to 7.1 μm (average 13.1 ± 1.04 × 5.3 ± 0.67 μm [mean ± SD], n = 50) with a mean length/width ratio of 2.6 ± 0.39 (n = 16 isolates). Perithecia were not observed. Based on the conidial morphology, the isolates were tentatively identified as belonging to the *Colletotrichum gloeosporioides* species complex (Weir et al. 2012). Total genomic DNA was extracted from all isolates, and six nuclear regions were amplified and partially sequenced: the internal transcribed spacer region of rDNA (ITS), the mating type protein 1-2-1 gene and the Mat1-2-1-Apn2 intergenic spacer region (ApMAT), actin (*ACT*),

calmodulin (*CAL*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), and tubulin (*TUB2*). The sequences for each region were 100% identical across all isolates. BLAST searches in GenBank showed 99 to 100% identity with sequences of various *Colletotrichum chrysophilum* W.A.S. Vieira, W.G. Lima, M.P.S. Câmara & V.P. Doyle strains, including the ex-type CMM4268 (Vieira et al. 2017). Sequences of the representative isolate CJL1080 were deposited in GenBank (*ACT*, MZ488944; ApMAT, MZ442299; *CAL*, MZ488945; *GAPDH*, MZ488946; ITS, MZ443972; *TUB2*, MZ442300). A multilocus phylogenetic analysis through Bayesian inference conducted with the obtained sequences and the reference ones (Khodadadi et al. 2020) revealed that our isolates clustered well within *C. chrysophilum*, as suggested by the BLAST results. To confirm Koch’s postulates, isolates CJL1080 and CJL1095 were inoculated on Pink Lady apples. Six surface-sterilized fruits per isolate were wound inoculated four times each with either 20 μl of a conidial suspension (10⁵ conidia/ml) or sterile distilled water (control). After 7 days of incubation in a moist chamber at 22°C, symptoms compatible with *Colletotrichum* infection were observed around the wounds, whereas control inoculations remained symptomless. The fungus was reisolated from all the lesions and identified through its morphological traits and DNA sequencing (ApMAT, *CAL*, and *GAPDH*). No fungus was isolated from the controls. Taxa of the *C. gloeosporioides* species complex causing bitter rot have been recently reported in Europe (Grammen et al. 2019; Nodet et al. 2019). This is the first report of *C. chrysophilum* causing apple bitter rot in Spain, which expands the knowledge on the geographic distribution of this important pathogen of apples in Europe.

References:

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