Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Preharvest Fruit Rot of ‘Pink Lady’ Apples Caused by *Colletotrichum fruticola* in Italy

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In late summer 2019, a severe outbreak of fruit rot was observed in commercial ‘Pink Lady’ apple orchards (>20 ha in total) in the region Emilia-Romagna (Northern Italy). The symptoms on the fruit appeared as small circular red to brown lesions. Disease incidences of over 50% of the fruits were observed. To isolate the causal agent, 15 affected apples were collected, and small portions of fruit flesh were excised from the lesion margin and placed on potato dextrose agar (PDA). The plates were incubated at 20°C in the dark, and pure cultures were obtained by transferring hyphal tips on PDA. The cultures showed light to dark gray, cottony mycelium, with the underside of the culture being brownish and becoming black with age. Conidia ($n = 20$) were cylindrical, aseptate, hyaline, rounded at both ends, and 12.5 to 20.0×5.0 to $7.5 \mu\text{m}$. The morphological characteristics were consistent with descriptions of *Colletotrichum* species of the *C. gloeosporioides* species complex, including *C. fruticola* (Weir et al. 2012). The identity of two representative isolates (PinkL2 and PinkL3) from different apples was confirmed by means of multilocus gene sequencing. Genomic DNA was extracted using the LGC Mag Plant Kit (Berlin, Germany) in combination with the Kingfisher method (Waltham, MA). Molecular identification was conducted by sequencing the ITS1/ITS4 region and partial sequences of four other gene regions: chitin synthase (*CHS*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), actin (*ACT*), and beta-tubulin (*TUB*). The sequences have been deposited in GenBank under accession numbers MT421924 and MT424894 (*ITS*), MT424612 and MT424613 (*CHS*), MT424616 and MT424617 (*GAPDH*), MT424614 and MT424615 (*ACT*), and MT424620 and MT424621 (*TUB*). MegaBLAST analysis revealed that our ITS

sequences matched with 100% identity to *C. fruticola* (GenBank JX010177). The *CHS*, *GAPDH*, *ACT*, and *TUB* sequences of both isolates were 100% identical with *C. fruticola* culture collection sequences in GenBank (JX009807, JX009923, JX009436, and JX010400, respectively), confirming the identity of these isolates as *C. fruticola*. Koch’s postulates were performed with 10 mature Pink Lady apples. Surface sterilized fruit were inoculated with $20 \mu\text{l}$ of a suspension of 10^5 conidia/ml after wounding with a needle. The fruits were incubated at 20°C at high relative humidity. Typical symptoms appeared within 4 days on all fruit. Controls mock-inoculated with sterile water remained symptomless. The fungus was reisolated and confirmed as *C. fruticola* by morphology and sequencing of all previously used genes. Until recently the reported causal agents of bitter rot of apple in Europe belonged to the *Colletotrichum acutatum* species complex (Grammen et al. 2019). *C. fruticola*, belonging to the *C. gloeosporioides* species complex, is known to cause bitter rot of apple in the United States, Korea, Brazil, and Uruguay (Kim et al. 2018; Velho et al. 2015). There is only one report of bitter rot associated with *C. fruticola* on apple in Europe (France) (Nodet et al. 2019). However, *C. fruticola* is also the potential agent of Glomerella leaf spot of apple (Velho et al. 2015, 2019). To the best of our knowledge this is the first report of *C. fruticola* on apples in Italy. It is important to stress that the *C. gloeosporioides* species complex is still being resolved and new species on apple continue to be identified (e.g., *C. chrysophilum*, which is very closely related to *C. fruticola*) (Khodadadi et al. 2020). Given the risks of this pathogen, the presence of *C. fruticola* in European apple orchards should be assessed and management strategies developed.

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