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DISEASE NOTES



First Report of *Colletotrichum fructicola* Causing Apple Bitter Rot in Europe

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Bitter rot is one of the prevalent diseases of apple (*Malus pumila* Mill.) worldwide. The disease affects the fruit preharvest in orchards and/or postharvest in storage, resulting in considerable economic losses. Until recently the reported causal agents in Europe belong to the *Colletotrichum acutatum* species complex (Baroncelli et al. 2014; Nodet et al. 2016); however, species belonging to *C. gloeosporioides* species complex were reported in the United States (Munir et al. 2016), South America (Velho et al. 2018), Korea (Park et al. 2018), and recently in Belgium (Grammen et al. 2019). In September 2017, bitter rot symptoms were observed on apple fruit (cultivars Joya Cripps Red, Granny Smith, and Pink Lady) in four orchards in the region of Occitanie in France. The rot began as circular brown spots, 1 to 2 mm in diameter, which enlarged rapidly. Sixteen isolates were obtained from symptomatic apples by culturing pieces of necrotic tissue on potato dextrose agar. Cultures showed light-gray, cottony mycelium that became darker with age, with the reverse color being brownish and becoming black with age. Conidia were produced in small orange masses and were

mainly cylindrical, with rounded ends. For all isolates, the production of perithecia was observed in culture, and asci and ascospores were observed under the microscope. The width and length of 50 conidia were examined and ranged from 3.1 to 4 μm (average 3.5 μm) and from 8 to 13 μm (average 10.5 μm), respectively. Based on these morphological characteristics, those isolates correspond to teleomorph of species belonging to *C. gloeosporioides* species complex (Weir et al. 2012). Total genomic DNA was extracted from the 16 isolates, and the internal transcribed spacer region of rDNA was amplified using the universal primers ITS4 and ITS5 and then sequenced. For all isolates, the resulting sequences were 100% identical to *C. fructicola* sequences obtained by a BLAST search in GenBank. Three other loci (partial GAPDH, TUB2, and ApMat genes) were amplified and sequenced to further characterize two isolates (UBOCC-A-118064 and UBOCC-A-118065; GenBank accession nos. MK114103 to MK114110, respectively). Multilocus phylogenetic analysis carried out with the obtained and reference sequences (Da Lio et al. 2018) revealed that the isolates clustered within *C. fructicola*, as suggested by the BLAST results; this is also consistent with their initial identification as *C. gloeosporioides*. To confirm Koch's postulates, for the two characterized isolates, 10 'Golden Delicious' apples were surface sterilized and then wound inoculated with 20 μl of a conidial suspension (10^5 conidia/ml). After 10 days of incubation at 20°C, symptoms identical to those initially observed developed around the inoculation point, whereas controls inoculated with water remained symptomless. Fungal thalli reisolated from the lesions were morphologically similar to the original isolate. To our knowledge, this is the first report in Europe of *C. fructicola* causing bitter rot on apple. According to Munir et al. (2016), *C. fructicola* was reported to be more aggressive than species belonging to the *C. acutatum* species complex such as *C. fioriniae* previously identified in France (Nodet et al. 2016); moreover, this species is associated with Glomerella leaf spot, an emerging leaf disease never described in Europe (Velho et al. 2018). All those points encourage the development of species-specific management strategies for this pathogen in European countries.

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