

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum grossum* Causing Apple Bitter Rot Worldwide (Italy)

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Apple bitter rot is a globally widespread disease that is observed on both preharvest and postharvest fruits, contributing to considerable economic losses. While the *Colletotrichum acutatum* species complex is predominant in Europe (Baroncelli et al. 2014; Amaral Carneiro and Baric 2021), in recent years, the *Colletotrichum gloeosporioides* species complex is emerging, raising many concerns (Amaral Carneiro et al. 2023). Circular, slightly sunken, brown lesions with acervuli produced in concentric spots were observed on the cultivar Story Inored harvested in September 2022 from an organic orchard in Masi (Padova Province, Italy), with a disease incidence close to 30%. Tissue samples were excised under aseptic conditions from 10 surface-cleaned diseased fruits at the margin between healthy and diseased pulp tissues, transferred to potato dextrose agar medium, and incubated in the dark at 25°C for 7 days, whereafter five single-spore cultures were obtained. Pure colonies grown at 25°C for 7 days appeared light gray-white on the upper side with floccose aerial mycelium, whereas the reverse side was dark gray with a distinct margin. Conidia were hyaline, cylindrical in shape with both ends rounded or one end acute, and measured $16.6 \pm 1.4 \times 6.1 \pm 0.5 \mu\text{m}$ (mean $\pm$ SD) ($n = 50$) as described by Diao et al. (2017). To identify the species, genomic DNA of a representative isolate (C38) was extracted, and beta-tubulin (TUB2), calmodulin (CAL), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glutamine synthetase (GS), and Apn2-Mat1-2 intergenic spacer (ApnMat) genes and

the internal transcribed spacer (ITS) region were amplified by PCR and Sanger sequenced (Rojas et al. 2010; Weir et al. 2012). The obtained DNA sequences of TUB2, CAL, GAPDH, GS, ApnMat, and ITS were submitted to GenBank under the accession numbers OR025589, OR025586, OR025587, OR025588, OR025585, and OR004800, respectively. A MegaBLAST analysis resulted in 100% identity to the epitype CAUG7 of *Colletotrichum grossum* (Diao et al. 2017) for GAPDH (KP890159) and TUB2 (KP890171), 99.85% for CAL (KP890147), and 99.5% for ITS (KP890165). The phylogenetic tree constructed by concatenation with the obtained sequences, as well as references, revealed that the isolate C38 clustered within *C. grossum*, confirming the BLAST approach. Pathogenicity tests were performed on 40 ‘Story Inored’ apples. The apples were cleaned, wounded with a sterilized needle, and exposed to two different conditions: 20 apples (10 inoculated with 20 μl of a spore suspension [10^4 spores/ml] and 10 inoculated with sterile water as controls) were incubated at 20°C with a 12-h photoperiod for 14 days, whereas the remaining 20 apples, prepared with the same approach, were placed at 1°C for 3 months and then at room temperature for 14 days. Symptoms appeared after 6 days on apples incubated at 20°C, whereas those stored at 1°C displayed symptoms at 11 days after being placed at room temperature. In both conditions, lesions were similar to those observed on the original fruits, whereas the controls remained asymptomatic. Identity of reisolated fungal colonies was confirmed by CAL, GAPDH, and GS region sequence analysis. *C. grossum* has been reported rarely: in 2017 on *Capsicum annuum* var. *grossum* in China, in 2018 on *Mangifera indica* leaves in Cuba, and in 2021 on *Rhyncospermum jasminoides* in Italy (Diao et al. 2017; Guarnaccia et al. 2021; Manzano León et al. 2018). To the best of our knowledge, this is the first report of apple bitter rot caused by *C. grossum* worldwide.

References:

- Amaral Carneiro, G., and Baric, S. 2021. Plant Dis. 105:3118.
- Amaral Carneiro, G., et al. 2023. J. Plant Pathol. 105:1141.
- Baroncelli, R., et al. 2014. Plant Dis. 98:1000.
- Diao, Y.-Z., et al. 2017. Persoonia 38:20.
- Guarnaccia, V., et al. 2021. J. Plant Pathol. 103:127.
- Manzano León, A. M., et al. 2018. J. Plant Pathol. 100:329.
- Rojas, E. I., et al. 2010. Mycologia 102:1318.
- Weir, B. S., et al. 2012. Stud. Mycol. 73:115.

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