Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Moldy Core of Minneiska Apples from New Zealand Caused by *Alternaria arborescens*

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Moldy core is a fungal disease of apple fruits characterized by mycelial growth in the seed locules, sometimes accompanied by penetration of the immediate surrounding flesh. The disease can go undetected until the fruit is cut open, as no external symptoms appear. *Alternaria*, *Aspergillus*, *Cladosporium*, *Coniothyrium*, *Epicoccum*, *Phoma* and *Stemphylium* spp. are common pathogens associated with moldy core (Gao et al. 2013; McLeod 2014; Serdani et al. 2002). It is more common in cultivars with an open calyx, where spores may initiate infections during the growing season or at the post-harvest storage stage (Spotts et al. 1988). Moderate to severe moldy core symptoms were observed when 10 apples of Minneiska cultivars imported from New Zealand were cut open. In comparison, Minneiska apples grown in Nova Scotia showed no moldy core symptoms when 10 random fruits were cut open. Small pieces of the diseased fruit tissue from the core region were surface disinfected for 1 min in 1% NaOCl, rinsed three times with sterilized water, and placed onto PDA dishes. The PDA dishes were incubated in the dark at 22°C, and single-spore isolation was carried out to fresh PDA dishes. The isolates produced colonies of regular shape, tan-black, with prominent white gray margin and gray conidia. Colonies turned dark black after 3 weeks of growth on PDA. Mycelia were septate, and conidia were oval or obclavate or club-shaped with a tapering end with four to six longitudinal and transverse septa. Size of conidia was 12.5 to 20 × 8.7 to 12.5 µm on 20-day-old PDA dishes. Based on the size and shape of conidia and other morphological characteristics, the fungi were identical to *Alternaria* spp. (Simmons 2007). To assess the identity by multilocus sequence analysis, genomic DNA was extracted from the pure cultures of two isolates (5.8 and 8) using the

E.Z.N.A. SP Fungal DNA Kit (Omega Bio-Tek). The glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), major allergen (*Alt a 1*), *OPA10-2*, internal transcribed spacer (*ITS*) region of ribosomal DNA, and translation elongation factor 1-α (*TEF1-α*) region from two isolates (5.8 and 8) were amplified and sequenced using primers *gpd1/2* (Berbee et al. 1999), *A21F/A21R* (Gabriel 2015), *OPA10-2/OPA10-2L* (Andrew et al. 2009), *ITS1/ITS4* (White et al. 1990), and *EF1-up/EF1-low* (O'Donnell et al. 1998), respectively. The resulting sequences of both isolates were deposited in NCBI GenBank (*GAPDH*, MW411052, MW411053; *Alt a 1*, MW411050, MW411051; *OPA10-2*, MW415762, MW415763; *ITS*, MK140445, MT225559; *TEF1-α*, MT305773, MT305774). Sequences of *GAPDH*, *Alt a 1*, *OPA10-2*, *ITS*, and *TEF1-α* genes of both isolates were identical to each other and showed 100, 100, 99.21, 100, and 100% identity to *Alternaria arborescens* (AY278810.1, AY563303.1, KP124712.1, KY965831.1, KY965831.1), respectively. Identity with reference strain CBS 102605 confirms that both strains 5.8 and 8 are *A. arborescens*. The pathogenicity of the two isolates was confirmed by artificially inoculating healthy Minneiska fruit by dispensing the conidial suspension directly on the seed locule. Briefly, surface-disinfected fruits were air dried for 5 min and then peeled using a sterilized knife and cut transversally. Each half of the fruit was inoculated with 100 µl of conidial suspension (~1 × 10⁴ conidia/ml) in potato dextrose broth (PDB) and incubated at 22°C in a humid chamber for 7 to 10 days, or until symptoms with visible mycelial growth were observed. Control fruits were treated with 100 µl of sterilized PDB. Both *A. arborescens* isolates produced visible moldy core symptoms on the inoculated fruits, whereas no symptoms were observed on control fruits. The experiment was repeated three times with at least three replicates with similar results. *A. arborescens* was successfully reisolated from the artificially inoculated fruits to complete Koch's postulates. This is the first report of *A. arborescens* causing moldy core disease in Minneiska apples from New Zealand.

References

- Andrew, M., et al. 2009. Mycologia 101:95.
- Berbee, M. L., et al. 1999. Mycologia 91:964.
- Gabriel, M. F., et al. 2015. Med. Mycol. 53:636.
- Gao, L. L., et al. 2013. Plant Dis. 97:510.
- O'Donnell, K., et al. 1998. Proc. Natl. Acad. Sci. U.S.A. 95:2044.
- McLeod, A. 2014. Page 40 in: Compendium of Apple and Pear Diseases and Pests, 2nd Ed. American Phytopathological Society, St Paul, MN.
- Serdani, M., et al. 2002. Mycol. Res. 106:561.
- Simmons, E. G. 2007. *Alternaria: An Identification Manual*. CBS Biodiversity Centre, Utrecht, the Netherlands.
- Spotts, R. A., et al. 1988. Australas. Plant Pathol. 17:53.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

The author(s) declare no conflict of interest.

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