Disease Notes

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum karstii* Causing Fruit Anthracnose of *Carissa grandiflora* in Spain

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The species *Carissa grandiflora* A. DC., commonly called Natal plum, is a shrub native to the coastal region of Natal, South Africa. In southern Spain, Natal plum is used as an ornamental plant due to its beautiful flowers and red ripened fruits. In March 2019 and 2020, we surveyed nine public gardens in the cities of Cadiz and Sanlúcar de Barrameda (Andalusia, Spain), and Natal plum fruit showing anthracnose symptoms were observed in six (67% prevalence) of them. Affected fruits showed necrotic and circular lesions with acervuli in the center causing the complete mummification of the fruit. Affected fruits were collected from four gardens and disinfested according to the method of Moral et al. (2010). Six fungal isolates were recovered from small (3 to 4 × 1 to 2 mm) pieces of the affected fruits in potato dextrose agar (PDA), and hyphal tips from them were transferred to fresh PDA to obtain pure cultures. The six isolates were initially identified as *Colletotrichum karstii* according to their morphology and the sequences of the ITS1-5.8S-ITS2 (ITS) region (Damm et al. 2012). The six *Colletotrichum* isolates showed similar colony morphology, and their ITS sequences were identical. Overall, *C. karstii* isolates showed cylindrical and straight conidia that were 12.1 to 14.2 µm long and 4.9 to 5.6 µm wide ($n = 50$). The aerial mycelia of the fungus varied from grayish-white to dark gray. A multilocus approach was conducted for more precise identification of the *Colletotrichum* species. For that, ITS, β -tubulin (TUB2), actin (ACT), partial sequences of the chitin synthase 1 (CHS-1), histone 3 (HIS3), and a 200-bp intron fragment of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

of a representative isolate (FITP19001) were amplified and sequenced according to the method of Damm et al. (2012). GenBank accession numbers for ITS, TUB2, ACT, CHS-1, HIS3, and GAPDH were MT757643, MT759805, MT759806, MT759807, MT759808, and MT759809, respectively. Sequences showed 100% identity with homologous sequences belonging to *C. karstii* (GenBank taxid: 1095194). To test Koch's postulates, 10 unripened and 10 ripened *C. grandiflora* fruits, harvested from asymptomatic plants, were inoculated. For each group, five fruits were inoculated using a drop of 10 µl of 5×10^4 conidia/ml suspension of *C. karstii* (FITP19001), and another five fruits were inoculated using a mycelial plug of the same isolate. Inoculated fruits were incubated in a humid chamber at room temperature (19 to 24°C) under light for 2 weeks. Noninoculated control fruits were treated with sterile water or a PDA plug and incubated under the same conditions. The pathogenicity test was conducted twice. After 10 days, typical anthracnose symptoms developed on both unripened and ripened inoculated fruits but not on noninoculated controls. Overall, the severity of anthracnose lesions was higher on ripened fruits than unripened fruits. Likewise, the severity of symptoms was higher on the fruits inoculated using a mycelial plug than on those fruits inoculated with a spore suspension. The species *C. karstii* was reisolated from lesions of all inoculated fruits as described above but not from noninoculated fruits. The species *C. karstii* has been described affecting numerous species worldwide (Damm et al. 2012). Previously, *C. gloeosporioides* was reported causing fruit anthracnose of Natal plum in Florida (Alfieri et al. 1984). To our knowledge, this is the first report of *C. karstii* causing anthracnose on the fruit of Natal plum in Spain and worldwide.

References:

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