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First Report of *Colletotrichum gloeosporioides* Causing Anthracnose of Tejocote (*Crataegus gracilior*) Fruits in Mexico

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Crataegus, is a genus classified in family Rosaceae and includes several tree species commonly called Tejocote that are widely cultivated for their pome fruits in Mexico. During fall of 2014, 2015, and 2016, severe symptoms of anthracnose were observed on approx. 60% of tejocote (*Crataegus gracilior*) fruits in an orchard located in Tulancingo, Oaxaca, Mexico. Affected fruits showed sunken, prominent, dark brown to black necrotic lesions, and were exuding salmon spore masses. To isolate the fungus, small pieces from tissue adjacent to the lesions of 10 symptomatic fruits were excised and surface disinfested by immersion in a 1% sodium hypochlorite solution for 2 min, rinsed three times in sterile distilled water, placed in Petri plates containing potato dextrose agar (PDA), and incubated

at 25ºC for 5 to 7 days in darkness. Mycelial plugs were excised from the edge of the actively growing fungal colony and aseptically transferred to fresh PDA medium and incubated at 25°C for 6 days. Five monoconidial cultures were obtained by transferring germinated spores to Petri plates with fresh PDA. One isolate was selected as representative for morphological and molecular identification. Colonies of pure cultures exhibited greyish-white aerial mycelium and abundant salmon-pink conidial masses. Conidia (n= 100) were subcylindrical, hyaline, straight, one-celled, with rounded ends, measuring 13.6 to 17.7 × 4.4 to 5.9 µm. Conidial appressoria were ovoid and brown to dark brown. Based on morphological characteristics, the fungus was identified within the *Colletotrichum gloeosporioides* species complex (Weir et al. 2012). The isolate was designated UACH-177 and deposited in the Culture Collection of Phytopathogenic Fungi at the Chapingo Autonomous University. For molecular identification, the ITS region (White et al. 1990), and fragments of (Apn2) (Rojas et al. 2010), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and β-tubulin 2 (TUB2) genes (Weir et al. 2012) were amplified by PCR, and sequenced. The sequences were deposited in GenBank (Accessions numbers ITS:MG821312; Apn2:MG821310; GAPDH:MG821311; and TUB2:MG821313). A phylogenetic analysis using Bayesian inference and including published ITS, Apn2, GAPDH, and TUB2 data for *C. gloeosporioides* and other *Colletotrichum* species was performed. The phylogenetic analysis showed the sequences were grouped into the clade of *C. gloeosporioides*. To confirm the pathogenicity of the fungus, 20 tejocote fruits were surface disinfested by immersion in a 1% sodium hypochlorite solution for 1 min, washed three times with sterile distilled water and dried on sterilized filter paper. Inoculations were performed by deposition of 10 µl of a conidial suspension (10^6 spores ml^{-1}) on the fruit surface. Ten fruit were mock inoculated with distilled water as a control. All fruits were kept in a moist chamber at 25°C for 10 days. Pathogenicity test was repeated twice. Disease symptoms were observed on all inoculated fruit after 7 days, whereas control fruit did not develop symptoms. Fungal colonies were re-isolated from all symptomatic fruits and were found to be morphologically identical to the original isolate inoculated on tejocote fruits, thus fulfilling Koch's postulates. In Mexico, García-Alvarez (1976) reported *Colletotrichum* sp. on fruits of *Crataegus mexicana*, however, that report was not supported by morphological characterization nor pathogenicity tests. To our knowledge, this is the first report of *C. gloeosporioides* causing anthracnose of *Crataegus gracilior* in Mexico and worldwide.