Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Colletotrichum siamense* and *C. gloeosporioides* Causing Anthracnose of *Citrus* spp. in Mexico

J. L. Pérez-Mora,¹ G. A. Mora-Romero,¹ H. Beltrán-Peña,¹ E. García-León,² N. B. Lima,³ M. Camacho-Tapia,⁴ and J. M. Tovar-Pedraza^{5,†}

¹ Universidad Autónoma de Occidente, Unidad Regional Los Mochis, Departamento de Ciencias Naturales y Exactas, Los Mochis, 81223, Sinaloa, Mexico

² Instituto Nacional de Investigaciones Agrícolas, Forestales y Pecuarias, Campo Experimental Valle del Fuerte, Guasave, 81110, Sinaloa, Mexico

³ Instituto de Patología Vegetal (CIAP-INTA), Unidad de Fitopatología y Modelización Agrícola (UFYMA), CONICET, Córdoba, X5020, Argentina

⁴ Universidad Autónoma Chapingo, Laboratorio Nacional de Investigación y Servicio Agroalimentario y Forestal, Texcoco, 56230, Estado de México, Mexico

⁵ Centro de Investigación en Alimentación y Desarrollo, Coordinación Regional Culiacán, Laboratorio de Fitopatología, Culiacán, 80110, Sinaloa, Mexico

Funding: This research was supported by the National Council of Science and Technology of Mexico (CONACYT), project INFRA-2019-302117. Plant Dis. 105:496, 2021; published online as <https://doi.org/10.1094/PDIS-08-20-1743-PDN>. Accepted for publication 9 September 2020.

Citrus anthracnose, caused by *Colletotrichum* spp., is a major disease in many citrus-growing regions of the world. During the spring of 2019, symptoms of petal necrosis and necrotic lesions on fruits were detected on Mexican lime (*Citrus aurantifolia*), sweet orange (*Citrus sinensis*), and grapefruit (*Citrus paradisi*) trees in three commercial orchards distributed in northern Sinaloa (El Fuerte and Ahome municipalities), Mexico. *Colletotrichum*-like colonies were consistently isolated on potato dextrose agar (PDA) medium from symptomatic petals and fruits, and 30 monoconidial isolates (10 per orchard) were obtained. Five isolates were selected as representative for morphological characterization, multilocus phylogenetic analysis, and pathogenicity tests. The isolates were designated as FAVF355 to FAVF359 and were deposited in the Culture Collection of Phytopathogenic Fungi of the Faculty of Agronomy of El Fuerte Valley at the Autonomous University of Sinaloa (Mexico). Colonies grown on PDA at 25°C were cottony, dense, with grayish white aerial mycelium and with pink conidial masses. Conidia ($n = 100$) were cylindrical, hyaline, aseptate, 13.7 to 18.8×4.3 to $5.8 \mu\text{m}$, with both ends rounded. Based on morphological features, the five isolates were tentatively identified in the

Colletotrichum gloeosporioides species complex (Weir et al. 2012). For molecular identification, total DNA was extracted, and the internal transcribed spacer (ITS) region (White et al. 1990) and partial sequences of actin (ACT), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and β -tubulin (TUB2) genes were amplified by polymerase chain reaction (Weir et al. 2012) and sequenced. A phylogenetic tree based on Bayesian inference for species belonging to the *C. gloeosporioides* species complex was constructed. The multilocus phylogenetic analysis distinguished the isolates FAVF355 to FAVF357 as *C. gloeosporioides* sensu stricto and the isolates FAVF358 to FAVF359 as *C. siamense*. The sequences were deposited in GenBank (ITS, MT850050 to MT850054; ACT, MT834528 to MT834532; GAPDH, MT855979 to MT855982; TUB2, MT834533 to MT834536). Pathogenicity of the five isolates was verified on healthy fruits of their original host species. Five fruits per isolate were inoculated using the colonized agar plug method. Fruits were wounded with a sterile toothpick, and mycelial plugs (5 mm in diameter) removed from the margin of a 6-day-old culture were placed onto three wound sites in each fruit. Noncolonized agar plugs were placed on the wounds of 10 fruits used as the control. The fruits were kept in a moist chamber at 25°C for 8 days. The experiment was repeated twice. All inoculated fruits developed circular and necrotic lesions 6 days after inoculation, whereas the control fruits remained symptomless. The fungi were consistently reisolated from the diseased fruits and were morphologically identical to those originally inoculated, fulfilling Koch's postulates. To date, only *C. gloeosporioides* sensu lato and *C. acutatum* sensu lato have been associated with sweet orange and Mexican lime in Mexico (Farr and Rossman 2020), whereas *C. gloeosporioides* sensu stricto has been recently recorded in a different area (Iguala, Guerrero) of Mexico (Cruz-Lagunas et al. 2020). To our knowledge, this is the first report of *C. gloeosporioides* sensu stricto causing anthracnose on sweet orange, and of *C. siamense* on Mexican lime in Mexico, as well as *C. gloeosporioides* sensu stricto causing disease on grapefruit in Sinaloa, Mexico.

References:

- Cruz-Lagunas, B., et al. 2020. Australas. Plant Dis. Notes 15:31.
- Farr, D. F., and Rossman, A. Y. 2020. Fungal Databases, Syst. Mycol. Microbiol. Lab., ARS, USDA. Retrieved July 15, 2020, from <https://nt.ars-grin.gov/fungaldatabases/>.
- Weir, B. S., et al. 2012. Stud. Mycol. 73:115.
- 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.

e-Xtra

Keywords: fungi, phylogeny, morphology, pathogenicity

[†]Indicates the corresponding author.

E-mail: J. M. Tovar-Pedraza; juan.tovar@ciad.mx