Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Diaporthe tulliensis* and *D. pseudomangiferae* Causing Cacao Pod Rot in Puerto Rico

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Worldwide, cacao pod rot is a devastating disease of *Theobroma cacao*; infected cacao pods turn necrotic reducing yield up to 30%. From July 2020 to August 2021, a survey was conducted at the USDA-ARS cacao germplasm collection in Mayaguez, Puerto Rico. Incidence of cacao pod rot was 73.9%, observed in 142 of the 196 accessions sampled. The disease was observed at different stages of pod development (small, green, mature pods and dry, mummified, large pods). Diseased tissue (1 mm²) from three pods per cacao accession was surface disinfested by immersion in 70% ethanol for 1 min, rinsed with sterile distilled water, and plated on potato dextrose agar (PDA) amended with 250 mg/liter of ampicillin and 60 mg/liter of streptomycin. After 30 days of incubation at 25°C, seven isolates developing white, fast-growing colonies with black, globose pycnidia were observed. All isolates produced hyaline, one-celled, biguttulate, and cylindrical and rounded at the apex α conidia 5.1 to 7.3 $\times$ 2.5 to 3.0 μ m in size and were identified as *Diaporthe* spp. (Crous et al. 2015; Gomes et al. 2013). To determine the species, seven isolates were sequenced for the internal transcribed spacer (ITS), sections of β -tubulin (BT), and translation elongation factor 1 alpha (EF1- α) and compared using BLASTn with *Diaporthe* spp. type specimens deposited in NCBI GenBank. ITS, BT, and EF1- α sequences of Phomocac16, Phomcac17, Phomcac18, and Phomcac21 isolates (GenBank accession nos. OL353698 to OL353701, OL412430 to OL412433, and OL412437 to OL412440 for ITS, BT, and EF1- α , respectively) were grouped to the holotype BRIP 62248a (Bootstrap BS = 100) of *Diaporthe tulliensis* R.G. Shivas, Vawdrey & Y.P. Tan. The other three isolates (Phomcac8P1, Phomcac8P3, and Phomcac8P4) were grouped to the ex-type (CBS 101339) of *Diaporthe pseudomangiferae* R.R. Gomes, Glienke & Crous, ITS, BT, and EF1- α

sequences (GenBank accessions nos. OL353702 to OL353704, OL412434 to OL412436, and OL412441 to OL412443, for ITS, BT, and EF1- α , respectively). Pathogenicity tests were conducted using isolates Phomcac16, Phomcac17, Phomcac18, and Phomcac21 of *D. tulliensis* and isolates Phomcac8P1, Phomcac8P3, and Phomcac8P4 of *D. pseudomangiferae* on five healthy, detached, green, yellow and red pods of the following cacao varieties: TARS27, ICS16, ICS1, GS29, UF601, SIAL56, Amelonado, SIAL98, EET94, ICS129, and GNV58. Cacao pods were wounded and inoculated with 5-mm mycelial disks from 8-day-old pure cultures grown in PDA of each isolate and wrapped with Parafilm. Untreated controls were inoculated with PDA disks only. Fruits were kept in a humid chamber for 8 days at 25°C. Tests were repeated twice. Eight days after inoculation with *D. tulliensis* and *D. pseudomangiferae*, all cacao pods turned dark brown; untreated controls showed no symptoms of pod rot, and no fungi were isolated from tissue. Both *D. tulliensis* and *D. pseudomangiferae* were reisolated from their respective diseased tissues, fulfilling Koch's postulates. *D. tulliensis* has been reported from rotted stem ends of cacao pods in Australia (Crous et al. 2015), and *D. pseudomangiferae* was reported in a shipment of cacao seed pods in California; however, pathogenicity tests were not conducted at either location. In California, *D. pseudomangiferae* is considered a quarantine pathogen with a temporary Q rating (Chitambar 2017). To our knowledge, this is the first report of *D. tulliensis* and *D. pseudomangiferae* causing cacao pod rot in Puerto Rico. Knowing the identity and incidence of these new cacao pathogens is the first step for developing specific control measures and potential sources for resistance to cacao pod rot caused by *Diaporthe* spp.

References:

- Chitambar, J. 2017. California Pest Rating for *Diaporthe pseudomangiferae* R. R. Gomes, C. Glienke & Crous. <https://blogs.cdfr.ca.gov/Section3162/?p=3285>
- Crous, P. W., et al. 2015. *Persoonia* 35:264.
- Gomes, R. R., et al. 2013. *Persoonia* 31:1.

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