First report of *Pseudocercospora punicae* causing black spot of pomegranate fruit in Mexico

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During 2015 and 2016, symptoms of black spot were observed on pomegranate fruit in two orchards in Oaxaca, Mexico. Diseased fruits exhibited small irregular black spots, which later coalesced into large spots. The pathogen was isolated on V8 juice agar medium and colonies exhibited moderate aerial mycelia with irregular margins, and dark grey in the upper side and dark green on the underside. Microscopic examination showed that stromata appeared to be oval to ellipsoidal, erumpent to semi-immersed, and brown to dark brown. Conidiophores were fasciculate or solitary, arising from stromata, 0–1-septate, slightly constricted at the septa, subcylindrical–ampulliform, and smooth. Conidia were solitary, subcylindrical–obclavate, straight to slightly curved, pale brown, 2 to 7-septate, $48.2\text{--}75.9 \times 2.7\text{--}3.8 \mu\text{m}$. The fungus was morphologically identified as *Pseudocercospora punicae* (Phengsintham et al. 2011; Bakhshi et al. 2014). To confirm the identification, genomic DNA was extracted, and the internal transcribed spacer (ITS) region, and part of translation elongation factor 1 α (EF-1 α) and actin (ACT) genes were amplified and sequenced. The sequences were deposited in GenBank (Accession numbers ITS:MG255299; EF-1 α :MG923953; ACT:MG923952). A phylogenetic analysis under the Maximum Parsimony criterion and including published ITS, EF1- α , and ACT sequence data for *Pseudocercospora* spp.,

showed that the fungus belongs to the *P. punicae* clade. Pathogenicity tests were carried out on 20 ripe pomegranate fruits. Fruits were wounded with a sterile toothpick and a mycelial plug was placed on each wound. Ten pomegranate fruits as control received only non-colonized V8 plugs. All inoculated fruits developed small black lesions nine days after inoculation, whereas no symptoms were observed on the control fruits. *Pseudocercospora punicae* was consistently re-isolated from inoculated fruit, fulfilling Koch's postulates. Previously, García-Alvarez (1976) reported the occurrence of *P. punicae* (as *Mycosphaerella lythracearum*) in Mexico. However, there is no evidence of morphological characterization or pathogenicity tests. To our knowledge, this is the first confirmed report of black spot of pomegranate fruit caused by *P. punicae* in Mexico.

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