Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Phyllosticta capitalensis* Causing Black Spot Disease on *Psidium guajava* in Mainland China

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Guava (*Psidium guajava*) cv. Watermelon Bar was introduced into Guangxi province of China in 2015 and subsequently planted on >5,000 ha in Yulin city alone. Black spot disease on fruit was observed between June and October 2015. Once a few diseased fruits were found on a tree, >30% of fruits were found to be infected several days later. In the field, the fruits from >90% of guava trees were infected by the disease. Spots containing pycnidia were dark brown to black, sunken, and 3 to 22 mm in diameter. Nine diseased guava fruits were collected from three trees in West Bank village (22.695387N, 110.112299E), Yuzhou District, Yulin City, Guangxi, China. Tissues from the margin between healthy and diseased portions of the fruit were excised and surface sterilized by immersing in 75% ethanol for 3 min, washed with sterile water three times, cut into 0.2- to 0.3-cm² pieces, and placed on PDA. After 3 days of incubation at 27°C, greyish-green fungal colonies developed. Hyphal tips were subcultured on PDA and oat agar, incubated at 27°C in a 12 h light/12 h dark cycle for 9 to 10 days until small black pycnidia appeared under the mycelia, after which conidia were observed from pycnidia under the optical microscope. Pycnidia were black, granular, and in clusters. Conidia were hyaline, unicellular, obovate, 7.47 to 14.49 × 5.01 to 9.63 µm, with mucoid sheath and apical mucilaginous appendage. After incubating for 20 days, black, granular, and agglomerate perithecia under the mycelia were observed. When the perithecia were pressed slightly on a slide, asci were squeezed out, which were eight-spored, subcylindrical to clavate, 56.74 to 88.27 × 7.88 to 12.16 µm. Ascospores were hyaline, unicellular, multiguttulate, fusiform, wider in the middle, and 10.50 to 16.19 × 3.64 to 5.86 µm. Spermatia were hyaline, bacilliform with swollen ends, 3.15 to 8.99 × 1.02 to 1.88 µm. The single-spore isolate YLWB01 was selected as one of 24 representative

isolates for molecular identification. DNA of YLWB01 was extracted, and the internal transcribed spacer (ITS) region and actin (ACT) and translation elongation factor 1 alpha (TEF) gene fragments were amplified using primers ITS1/ITS4 (White et al. 1990), ACT512F/ACT783R, and ER728F/EF986R (Carbone and Kohn 1999), respectively. BLAST analysis in GenBank showed that the obtained sequences of ITS (MN958712), ACT (MN958710), and TEF (MN958711) had 100% identity to *Phyllosticta capitalensis* (AM403717, JN791558, and FJ538377, respectively). Phylogenetic analysis by neighbor joining using MEGA 6.0 (Guarnaccia et al. 2017; Tamura et al. 2013) results showed that ITS, ACT, and TEF sequences clustered together with *P. capitalensis* (strain CPC18848) in the phylogenetic tree. Based on these morphological characteristics and sequence analysis (Wikee et al. 2013), the fungus was identified as *P. capitalensis*, cause of guava black spot disease (Arafat 2018). Pathogenicity of YLWB01 was demonstrated on fruits surface sterilized by 75% ethanol in the laboratory. Six symptomless mature guava fruits were stab wounded, three stabs per fruit. Three fruits were inoculated with a 5-mm-diameter colonized disk of PDA incubated for 10 days, with three inoculated with a blank PDA disk as a control. After inoculation, fruits were incubated in glass covers at room temperature. Symptoms began to appear after 2 days and were similar to those of naturally infected guava fruits after 5 days. Control fruits remained healthy. The same fungus was reisolated from the inoculated fruits but not control fruits, using the methods described above. Guava black spot disease caused by *P. capitalensis* has been previously reported in Egypt (Arafat 2018). Zhang et al. (2011) also reported *P. capitalensis* in China on imported guava fruit with black spots inspected by the Ningbo Entry-Exit Inspection and Quarantine Bureau in Zhejiang province but not collected from the field. This is the first report that *P. capitalensis* is the pathogen of guava black spot disease in mainland China.

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