Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Cercospora* cf. *malloti* Causing Brown Eyespot on Strawberry in Brazil

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Strawberry (*Fragaria × ananassa* Duch.) is one of the most consumed fruits from the Rosaceae family in Brazil. It is cultivated on ~4,500 ha in all regions of the country, with a wide range of fungi causing leaf diseases. Strawberry leaves (cv. San Andreas) with symptoms of eyespot usually related to *Mycosphaerella fragariae* (*Ramularia tulasnei*) (Mazaro et al. 2006) were observed in April 2019 in a field in Santa Maria, Rio Grande do Sul state, Brazil (29°43'19.6"S/53°43'04.6"W). However, the symptoms slightly differed from *Mycosphaerella* blight as well as the fungi structures observed under an optical microscope. The symptomatic leaves had small circular spots, initially reddish-purple to dark brown, which later increased in size. The center of the lesion was pale gray where dark brown conidiophores were found over it. The symptoms occurred in 50% of the leaves of all plants during the crop season. To identify the causal agent, symptomatic leaves were collected and superficially disinfected with 70% ethanol for 30 s followed by 0.5% bleach for 30 s and then washed in sterile water. The leaves were placed in germination boxes with a moistened paper filter and incubated at 25°C and 12 h photoperiod for 4 days. After the formation of the fungal structures, the fungus was isolated in potato dextrose agar (PDA) medium and kept for 20 days at 25°C. Colonies were dark brown with pale pink aerial mycelium. The conidia were hyaline,

single- to multiseptate with a filiform shape, and straight or slightly curved. The fasciculate conidiophores on the stroma measured 20 to 70 × 2.5 to 3.5 µm. Conidia averaged 10.2 to 82.4 × 4 to 8.8 µm, similar to morphological characteristics of the genus *Cercospora*. The isolate M6 was deposited in the Brazilian National System of Genetic Registration (SISGEN) no. A57AE19. DNA was extracted from a monospore culture of M6 on PDA plates, which presented all typical aspects previously described. To confirm the identity, PCR amplification was carried out for internal transcribed spacer (ITS) region ITS 1-5.8S - ITS 2 and Calmodulin region (CAL) genes (Acqua et al. 2011). The ITS (No. MZ713249) and CAL (No. MZ713254) sequences were deposited in the GenBank database. BLASTn analysis revealed the isolate was 99% similar to *Cercospora* cf. *malloti* (GenBank acc. no. KT193689 and KT193738) (Nguanhom et al. 2015). A pathogenicity test was performed to fulfill Koch's postulates. Strawberry plants with healthy leaves (60 days old), were inoculated by spraying a conidial suspension (1 × 10⁵ conidia/ml). Control plants were sprayed with sterile water. The plants were incubated in a greenhouse at 28°C with a 12 h photoperiod and 80% relative humidity under drip irrigation keeping at least 60% of field capacity. Six plants were used as replicates. Twenty days after inoculation, small circular reddish-purple spots were found, which later increased in size. Ten days later, the spots presented dark brown conidiophores over the center of the lesion. The symptoms described above were observed in all inoculated leaves. The control plants remained symptom free. The pathogen was then reisolated from the lesions and identified as *C. cf. malloti* based on the morphological aspects described above on PDA. In Thailand *C. cf. malloti* is reported on crops like *Brassica alboglabra* (cabbage) (Nguanhom et al. 2015), but not on strawberries. To our knowledge, this is the first report of *C. cf. malloti* pathogenic to strawberries in Brazil.

References:

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