Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Cercospora apii* Sensu Lato 'sp. H' Causing Leaf Spot on Geranium (*Pelargonium × hortorum*) in Brazil

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Pelargonium × hortorum (Geraniaceae), geranium, is a highly popular ornamental worldwide, including in Brazil (Lorenzi 2013). Currently only three fungi are reported to occur on geraniums in Brazil (Mendes and Urban 2019): *Alternaria* sp., *Leptosphaeria pelargonii*, and *Puccinia pelargonii-zonalis*. In 2018, a leaf spot disease was found on geranium plants grown in a disease demonstration garden on the campus of the Universidade Federal de Viçosa (UFV), state of Minas Gerais (MG), Brazil (20°45'14" S, 42°52'54" W). The disease, in combination with an attack by the rust fungus *P. pelargonii-zonalis*, caused severe defoliation of plants. The same situation was observed independently in garden areas of the campus of UFV and also in private gardens in MG and also in the state of Rio de Janeiro. Leaf spots were numerous and initially small whitish dots (1 to 2 mm). Spots grew and became circular, surrounded with a dark brown border. Older spots became dark and coalesced, leading to extensive leaf necrosis (3 to 5 cm). Upon observation under a dissecting microscope, a dematiaceous fungus was found growing on the majority of the necrotic spots. A sample of diseased leaves was selected, dried in a plant press, and deposited in UFV's herbarium (VIC 47336). Pure cultures of the fungus were obtained by transfer of conidia from individual separate leaf spots onto PDA plates with the help of a sterile fine-pointed needle. Colonies had identical morphology, and one was selected and deposited in the local culture collection (COAD 2916). Fungal structures were scraped from the surface of necrotic tissues with a scalpel, and slides were mounted in lactoglycerol and examined with a light microscope (Olympus BX 51). Measurements were taken from at least 30 structures. The fungus had the following morphology: conidiophores fasciculate, cylindrical, geniculate, proliferating sympodially, 85 to 130 × 4 to 6 µm, three to six septate; conidia acicular to filiform, 92 to 210 × 2.5 to 4 µm, nine to 23 septate, hyaline, smooth. This morphology is typical of *Cercospora apii* sensu lato (Crous and Braun 2003). Genomic DNA was extracted from COAD 2916 grown on PDA using a Wizard Genomic DNA Purification Kit (Promega) per the manufacturer's instructions. Three loci were PCR amplified, namely:

calmodulin, actin, and histone h3 using primers CAL228F/CAL2Rd, ACT512F/ACT783R, and CYLH3FQ/CYLH3R, respectively. The resulting sequences were deposited in GenBank (MT199213, MT199214, and MT199215, respectively). A multilocus Bayesian phylogenetic analysis was performed using sequences from other closely related taxa obtained from GenBank. COAD 2916 clustered with *Cercospora* sp. H (Groenewald et al. 2013). This is a yet unresolved clade of the *C. apii* sensu lato assemblage. Inoculation of five 3-month-old healthy geranium plants was performed. COAD 2916 was grown on PDA for 12 days, and plugs were taken from the margin of growing colonies and placed on young leaves. Five other healthy plants receiving uncultured sterile PDA plugs on leaves served as controls. All plants were placed in a dew chamber for 24 h, transferred to a bench in a greenhouse, and observed daily for the emergence of symptoms. Symptoms appeared on all inoculated leaves by 7 days. Controls remained healthy. COAD 2916 sporulated on the diseased tissues, and the fungus was reisolated in pure culture. Colonies had the same morphology of those originally used for inoculation. Three species of *Cercospora* have been reported on *Pelargonium* spp.: *C. apii*, *C. brunkii*, and *C. cf. flagellaris* (Bakhshi et al. 2018; Farr and Rossman 2020). COAD 2916 is phylogenetically distant from *C. apii* sensu stricto, *C. brunkii*, and *C. cf. flagellaris* and is morphologically different from *C. brunkii*. This is the first record of *C. apii* sensu lato 'sp. H' causing leaf spot on *P. × hortorum* worldwide. The other record of *C. apii* on *Pelargonium* is on *P. zonale* from Iran (Pimia et al. 2012), but no molecular information was included in that publication.

References:

- Bakhshi, M., et al. 2018. IMA Fungus 9:299.
- Crous, P. W., and Braun, U. 2003. *Mycosphaerella* and Its Anamorphs. CBS-KNAW, Utrecht, The Netherlands.
- Farr, D. F., and Rossman, A. Y. 2020. Fungal Databases, Syst. Mycol. Microbiol. Lab., ARS, USDA. Retrieved March 26, 2020, from <https://nt.ars-grin.gov/fungaldatabases/>.
- Groenewald, J. Z., et al. 2013. Stud. Mycol. 75:115.
- Lorenzi, H. 2013. Plantas para jardim no Brasil: Herbáceas, arbustivas e trepadeiras. Editora Plantarum, São Paulo, Brazil.
- Mendes, M. A. S., and Urban, A. F. 2019. Fungos relatados em plantas no Brasil, Laboratório de Quarentena Vegetal. Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF. <http://pragawall.cenargen.embrapa.br/aiqweb/michtml/fgbanco01.asp>. Accessed November 19, 2019.
- Pimia, M., et al. 2012. Mycotaxon 120:157.

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