Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by a Provisionally Novel Species of *Pseudocercospora* on *Ligustrum japonicum* in South Korea

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Ligustrum japonicum Thunb. (Oleaceae) is a hedge plant along streets in South Korea. In August 2019, leaf spots on trees were observed in Naju in Jeonnam Province (35°00'32.4"N, 126°49'17.6"E). Diseased leaves on each affected tree within a 0.3-ha area were ~70%. The initial symptoms were orbicular or irregular, pale-brown to dark-brown spots on leaves that were typically 0.5 to 1.3 cm in diameter. When leaves were severely affected, larger blotches were formed, leading to bleaching symptoms and eventually defoliation. Thin narrow or bunched hyphae emerged through the stomata, which were smooth, septate, branched, hyaline or pale brown, and 2 to 3 µm in diameter. The stromata emerging through the cuticle were large, well-developed, globular, olivaceous brown, 35 to 55 µm diameter, and partly superficial or immersed. The conidiophores were aggregated in dense fascicles emerging through stomatal openings and were cylindrical, straight to slightly geniculate, 17 to 53 × 5.5 to 7.5 µm in diameter, and smooth with usually a single septum. Conidia were guttulate cylindrical to narrowly obclavate, straight to mildly curved, obtuse to rounded at the apex, truncate at the base, variable in length, and 17 to 57 × 3 to 4.5 µm in diameter, with one to three septa. Diseased leaves obtained from 15 locations in the area were randomly selected, and fungal isolations were performed by transferring spores from leaf spots on MEA. Among 15 cultures, 10 isolates were retained based on culture characteristics, and two randomly selected monoconidial cultures were deposited in the culture collection (CDH) of the National Institute of Forest Science, Korea (CDH2019-4 and 5). To confirm the identity of the fungus isolated in this study, two isolates, CDH2019-4 and 5, were selected for molecular study, and multiple sequence comparisons were performed based on partial sequences of the

ITS, translation elongation factor-1 alpha (*TEF*-1α), actin, and the second largest subunit of RNA polymerase II (*rpb2*) using the respective primer pairs ITS1/ITS4, EF1-728F/EF1-986R, ACT-512F/ACT-783R, and RPB2-5f2/rRPB2-7cR (Nakashima et al. 2016). The sequence data were deposited in GenBank: MN453602 and MT484236 (ITS), MN497240 and MT491200 (*TEF*-1α), MN497241 and MT491201 (actin), and MN497242 and MT491202 (*rpb2*). The resulting sequences were concatenated as a single dataset and subjected to phylogenetic analysis with the maximum likelihood method. The results showed that the isolated cercosporoid fungus formed a clearly distinct lineage that provisionally represents an undetermined species of *Pseudocercospora*, which is most closely related to *P. cornicola* (Tracy & Earle) Y.L. Guo & X.J. Liu, MUCC 909 (GU269283, ITS; GU384400, *TEF*-1α; GU320389, actin; MG594035, *rpb2*) (Crous et al. 2013). Sequence comparisons revealed that the sequences of this Korean cercosporoid fungus differed from *P. cornicola* at 10 of 499 characters (~2.0%) for ITS, four of 300 (~1.3%) for *TEF*-1α, and 42 of 741 (~5.7%) for *rpb2* but were identical for actin. This isolate is morphologically distinct from *P. cornicola* because it has longer conidiophores and smaller conidia. Pathogenicity was confirmed by performing inoculation trials on five seedlings of *L. japonicum*. Leaves of each plant were sprayed with 5 ml of conidial suspension (10⁵ conidia/ml, CDH2019-5). Three control plants were sprayed with sterile water. After 3 weeks in the greenhouse at 25°C and in 80% RH, leaf spots appeared, identical to those observed in the field. No symptoms were observed on control plants. *Pseudocercospora* sp. was reisolated from 90% of lesions, fulfilling Koch's postulates. Leaf spots associated with a *Pseudocercospora* sp. on *L. japonicum* have not been recorded elsewhere (Farr and Rossman 2020). This disease poses a serious threat by reducing plant vigor and quality. To control the disease, more attention should be paid on the host range of the pathogen and other regions where the disease caused by the pathogen might occur.

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