Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Blight Caused by *Nigrospora oryzae* on Poplar in China

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Populus alba L. × *P. berolinensis* Dipp. (a hybrid poplar, ‘PaPb poplar’) exhibits fast growth and beautiful tree shape with high drought and cold tolerance, and is widely planted in the cities of Northeast China because of its ornamental and ecological value (Wang et al. 2008). In October 2020, an unknown leaf blight symptom was observed on PaPb poplar seedlings at Shenyang Agricultural University (41°49’N, 123°34’E) in Shenyang, Liaoning Province, China. Disease incidence was 50% in a survey of 200 seedlings on the campus. Typical symptoms were brown-to-black, irregular lesions. Five symptomatic leaves were collected, and pieces were cut at the margin of diseased and healthy tissue. These pieces were surface sterilized with 2% sodium hypochlorite for 2 min, rinsed three times with sterile distilled water, air dried, placed on potato dextrose agar (PDA), and incubated at 28°C. After 5 days of incubation, three isolates with similar morphological characteristics were observed. Isolate N03 was chosen for pathogen identification. The fungal colonies were initially white, and later turned gray to black. Conidia were single-celled, black, spherical or oblate, and 9.19 to 12.78 × 12.61 to 14.81 μm in diameter (*n* = 40). These were borne on hyaline vesicles at the tip of a conidiophore. Morphologically, N03 was identified as *Nigrospora oryzae* (Berk. and Broome) Petch (Wang et al. 2017). The genomic DNA was extracted with an SP Fungal DNA Kit (D5542-01, OMEGA). The internal transcribed spacer (ITS) region, translation elongation factor 1-α (*TEF1-α*), and partial beta-tubulin (*TUB*) genes were amplified using the ITS5/ITS4 (White et al. 1990), EF1-728F/EF-2R (Carbone and Kohn 1999; O’Donnell et al. 1998), and Bt-2a/Bt-2b (Glass and Donaldson 1995)

primer sets, respectively. PCR products of ITS, *TEF1-α*, and *TUB* were amplified, sequenced, and deposited in GenBank with accession nos. MZ148528, MZ182080, and MZ182079, respectively. BLASTn analysis of the ITS, *TEF1-α*, and *TUB* sequences showed 99.3, 99.8, and 99.27% nucleotide identities to MK131325, KY019328, and KY019559, respectively. A phylogenetic tree based on combined ITS, *TEF1-α*, and *TUB* sequences was constructed using Maximum Likelihood with 1,000 bootstraps showing that N03 was grouped with other *N. oryzae* isolates. The fungus was identified as *N. oryzae* based on morphological characteristics and molecular analyses. Koch’s postulates were completed to confirm the pathogenicity of *N. oryzae* on PaPb poplar. The N03 spore suspension (10⁵ spores/ml) was used to inoculate detached leaves and field leaves in two experiments. The two experiments were repeated three times. In the detached leaf test, 10 healthy leaves collected from 1-year-old PaPb poplar seedlings were inoculated with N03 by spraying with the spore suspension followed by incubation at 28°C on wet filter papers in a Petri dish for 7 days. Ten leaves were sprayed with sterile water as controls. For the field leaf test, leaves of five plants were spray-inoculated with the spore suspension at the 4-week-old growth stage, and an additional five plants were sprayed with sterile water. Seven days after inoculation, brown-to-black, irregular lesions on the margins were observed on inoculated leaves but not on controls. All detached leaves inoculated with N03 were symptomatic. In field tests, symptoms appeared on 20 of 30 inoculated leaves. *N. oryzae* was reisolated from all inoculated detached leaves and inoculated plants, but not from the controls. *N. oryzae* is a known pathogen of several hosts, such as *Costus speciosus* (Koen.) Sm. and *Mentha spicata* L., but has not been reported on any species of *Populus*. To our knowledge, this is the first report of leaf blight of PaPb poplar caused by *N. oryzae* in China and the world. This disease could affect growth and development of PaPb poplar seedlings and may cause economic losses in the future. Appropriate strategies should be developed to manage this disease.

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