Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot on *Akebia trifoliata* Caused by *Phytophthora nicotianae* in China

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Akebia trifoliata (Thunb.) Koidz. is a species in the family Lardizabalaceae, which belongs to deciduous woody lianas. It is an important species of plant used in Chinese medicine. In July 2019, a leaf spot disease was observed on *A. trifoliata* in a nursery garden in Jingzhou (N 30° 21', E 112° 19'), Hubei Province, China. Symptoms initially appeared as small brown spots and subsequently developed into subcircular or irregular-shaped brown necrotic lesions. In severe cases, the leaves became completely necrotic and abscised. The incidence of leaf symptoms on affected plants ranged was between 30 and 40%. To isolate the pathogen, pieces of symptomatic leaves were collected and excised at the margins of lesions, surface disinfected with 70% ethanol and 0.1% HgCl₂, rinsed three times with sterile water, placed on potato dextrose agar (PDA) amended with 50 µg/ml of kanamycin, and incubated at 28°C in the dark for 3 days. Isolated colonies were subcultured by transferring hyphal tips. Six fungal isolates were isolated from the collected tissues. All six isolates had similar colony morphologies on PDA and were composed of white flocculent aerial hyphae. The average radial growth rate of colonies after 7 days was 11.2 mm/day. Isolates were later cultured on 20% V8 juice agar for 20 days

to encourage sporulation. Sporangia were produced on V8 medium and were colorless, inverted, pear-shaped, and terminal, with obvious mastoid, 22 to 34 × 28 to 46 µm (*n* = 50); oospores were light brown and suborbicular, with a thick wall, 18 to 26 µm (*n* = 20); globose chlamydospores were light brown and suborbicular, 12 to 32 µm (*n* = 50). Antheridia were not observed, suggesting homothallism. These morphological characteristics were identical to those reported for *Phytophthora nicotianae* (Erwin and Ribeiro 1996). We selected a single isolate, B2, for molecular identification because it was the most aggressive in leaf pathogenicity assays. The internal transcribed spacer (ITS) region of rDNA was amplified and sequenced using primers ITS1/ITS4 (White et al. 1990). BLAST analysis revealed that the ITS sequence (GenBank accession no. MT472132) was 100% identical to other *P. nicotianae* strains (GenBank accession no. KJ754387). To fulfill Koch's postulates, a 50-ml zoospore suspension (10⁶ spores/ml) of B2 was sprayed on the foliage of three 1-year-old healthy seedlings. Sterile distilled water was used to inoculate control plants. After 10 days, typical symptoms of dark brown spots were observed on all the inoculated leaves, whereas the control leaves remained asymptomatic. *P. nicotianae* was reisolated from the inoculated, symptomatic leaves, thus confirming Koch's postulates. The experiment was repeated three times. To the best of our knowledge, this is the first report of *P. nicotianae* causing leaf spot on *A. trifoliata* in China. *P. nicotianae* is a common stramenopile pathogen that infects many plant hosts. The presence of this pathogen in an *A. trifoliata* nursery should be carefully considered to mitigate possible outbreaks of this disease in other fields in this growing region.

References:

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