Diseases Caused by Fungi and Fungus-Like Organisms

Alternaria tenuissima Causing Leaf Spot Disease on *Amygdalus triloba* in Xinjiang of China

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Amygdalus triloba (Rosaceae; previously *Prunus triloba*) is a deciduous, flowering shrub that is widely used in the greening and beautification of lawns, parks, and courtyards in China. In late May 2019, a leaf spot disease of *A. triloba* was observed on approximately 35% of plants in the Xinjiang Alaer city (40°33'20"N, 81°17'19"E). The disease symptoms began as small, suborbicular, brown spots on the leaves. As the disease progressed, the spots enlarged and coalesced into large necrotic areas and resulted in premature defoliation. Leaf sections (5 × 5 mm) from infected leaves were surface sterilized with 75% ethanol for 30 s and 0.1% HgCl₂ for 1 min, rinsed three times in sterile distilled water, and then incubated on potato dextrose agar (PDA). Fifteen fungal isolates showing similar morphological characteristics were obtained by single-spore isolation. On the PDA plates, all fungal colonies had a dark olive color with loose, cottony mycelium. On potato carrot agar, the fungus formed unbranched spore chains but occasionally formed one or two lateral branches. Conidiophores were short, hazel-colored, septae, arising singly, and measuring 15.1 to 61.8 × 1.8 to 4.2 μm (35.2 ± 1.4 × 2.3 ± 0.1 μm, *n* = 50). Mature conidia were ellipsoidal to ovoid with a short conical beak at the tip, light brown, with zero to three longitudinal septa and one to five transverse septa, and measuring 19.3 to 30.8 × 7.2 to 12.5 μm (21.8 ± 0.3 × 9.5 ± 0.2 μm, *n* = 50). Based on the cultural and morphological traits, the pathogen was preliminarily identified as *Alternaria tenuissima* (Simmons 2007). Genomic DNA was extracted from the representative isolate YALAR-1, and the internal transcribed spacer (ITS) region, the partial coding sequence of endopolygalacturonase (endoPG), the glyceraldehyde-3-phosphate dehydrogenase (GAPDA), and

the partial region of the histone 3 (H3) genes were amplified using primers ITS1/ITS4 (White et al. 1990), PG2b/PG3a (Andrew et al. 2009), GDF1/GDR1 (Berbee et al. 1999), and H3-1a/H3-1b (Glass and Donaldson 1995), respectively. The amplicons were sequenced and deposited in GenBank (MT459807 [ITS], MT459808 [endoPG], MT459805 [GAPDA], MT459806 [H3]). MegaBLAST analyses revealed that our ITS, endoPG, GAPDA, and H3 sequences were 99 to 100% identical to those of *A. tenuissima* isolates in GenBank (AF347032 [ITS], KP124026 [endoPG], AY278809 [GAPDA], KF997086 [H3]), confirming the identity of the pathogen as *A. tenuissima*. Pathogenicity tests were performed by inoculating the fungus onto healthy, mature leaves of *A. triloba* in the field. Twenty-five leaves (five leaves per plant) were sprayed with spore suspensions (1 × 10⁶ spores/ml) of each fungal pathogen, and the same number of leaves were sprayed with distilled water as controls. Inoculated and control leaves were covered with clear plastic bags for 3 days. The experiment was repeated three times. Twelve days after inoculation, the observed symptoms were similar to the original symptoms, and the same fungal pathogen was reisolated from the inoculated leaves and identified as *A. tenuissima* based on morphological features and sequence analysis. The control leaves remained asymptomatic and no fungus was isolated from these leaves. Previously, a leaf spot of *A. triloba* caused by *Alternaria brassicae* was reported in Dalian, China (Xie et al. 2017). To control this disease effectively, further studies are needed on the biology and ecology of *A. tenuissima* and *A. brassicae*, respectively. To our knowledge, this is the first report of *A. tenuissima* associated with leaf spot disease on *A. triloba* in China. In late September 2020, the diseased plant rate increased to 38% in Alaer city. If the disease control and prevention is neglected, the landscape of Alaer city will be affected seriously. So, to effectively control the spread of the disease, it is urgent now to study the sensitivity of the pathogen to fungicide and carry out field efficacy trials.

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