Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Caused by *Didymella americana* on *Cassia nomame* in China

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Cassia nomame (Sieb.) Kitagawa is an annual plant in the Leguminosae family. The aerial parts of *C. nomame* have been used as a tonic and diuretic in Korea and Japan (Syed et al. 2019). A leaf spot was observed on the leaves of a 1-year-old *C. nomame* landrace in Changli County (39.42°N, 119.10°E), Qinhuangdao City, Hebei Province, during August to October in 2018. In many fields ($n \geq 3$), the was a disease incidence over 80% in the middle and late stage of plant growth. Symptoms on leaves in one field began with many small, dark necrotic spot lesions. Later, the lesions became round-to-oval, slightly sunken in the center, and large necrotic patches with indefinite margins. Finally, lesions coalesced and resulted in defoliation. Lesions were occasionally observed on the pods. Symptoms on the pods were initially small, dark spots and then expanded to large necrotic patches with irregular edges. Symptomatic tissues ($n = 32$) from pods and leaves were cut into 3- to 8-mm² squares, surface disinfested with 75% ethanol for 10 s, rinsed with sterile distilled water, and placed on potato dextrose agar (PDA) at 28°C. After 3 days, 10 isolates with consistent characteristics were obtained with a frequency of 52.6%. The isolates on PDA were round, initially pale, and had little aerial mycelium, gradually turned olive green and had dense, wool-like aerial mycelia after 3 days. Conidia were hyaline, smooth, solitary, and elliptical. The conidia measured 5.4 to 8.2 × 2.5 to 3.8 μm ($n = 50$), and had two oil bodies positioned at opposite poles. Pigmented chlamydospores were spherical or nearly pear-shaped and solitary. Black fructifications (pycnidia) were produced profusely on PDA after subculture for 3 days. All the isolates were

similar to *Didymella* sp. in morphology (Aveskamp et al. 2009). Three isolates, YSGUO8, YSGGUO8-a, and YSGGUO8-b, were chosen to be further characterized by sequencing of the internal transcribed spacer (ITS), *actin* gene, and 28S large subunit of the nuclear rRNA gene (LSU) (Zhang et al. 2017). The sequences of three strains (MK836417, MZ484072, and MZ484073 for ITS; MK837604, MZ593675, and MZ593676 for *actin*; and MK843781, MZ836208, and MZ836207 for LSU, respectively) showed 99 to 100% similarity with *Didymella americana* K-004 (KY070279 for ITS, KY070285 for LSU), *Phoma americana* CBS 256.65 (FJ426973 for ITS, FJ426871 for *actin*, MH870196 for LSU), and *P. americana* CBS 185.85 (FJ426972 for ITS, FJ426870 for *actin*, GU237990 for LSU) in GenBank. The fungi were identified as *D. americana* (formerly *P. americana* or *Peyronellaea americana*) on the basis of morphological characteristics and sequence analysis. A pathogenicity test was conducted three times on a 1-year-old *C. nomame* strain at the four to six compound leaf stage. Conidia were obtained from 7-day-old PDA cultures grown at 28°C with a 12-h photoperiod. Koch's postulates were fulfilled by spray inoculating 10 healthy young plants with 10⁶ conidia per milliliter of *D. americana* strain YSGUO8, and sterile water as the control. After inoculation, the plants were managed at 28°C, 60% relative humidity, and a 12-h photoperiod. After 5 to 8 days, the inoculated leaves developed small dark spot lesions similar to those observed on the leaves with initial symptoms in the field. The control leaves remained symptomless. The same fungi were reisolated from infected leaves by morphological observation and sequence analysis, confirming Koch's postulates. *D. americana* has caused leaf spot on table beets in New York (Vaghefi et al. 2016). To our knowledge, this is the first report of *D. americana* causing leaf spot on *C. nomame* in China.

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