Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria tenuissima* Causing Brown Spot Disease on *Angelica dahurica* in China

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Angelica dahurica (Fisch. ex Hoffm.) is an abundantly cultivated Chinese herbal medicine plant in China with about 4,000 ha grown, and the annual production is up to 24,000 t. The medicinal part of *A. dahurica* is its root, which mainly functions to treat cold, headache, toothache, rhinitis, diabetes, and so on. Besides, *A. dahurica* is also used as a spice in Asia. In September 2018, brown spot was observed on the leaves of *A. dahurica* in fields of Anguo City, Hebei Province, China. In the field investigated, the incidence of brown spot disease reached 15%. The infected leaves showed brown spots surrounded with a pale yellow edge, resulting in withering of the whole leaf. It seriously endangers the growth of *A. dahurica*, reducing the yield and quality of medicinal materials and even leading to the death of plants. We isolated the pathogen from 10 leaves with the same lesions; small square leaf pieces of approximately 3 to 5 mm were obtained with sterile scissors from the junction of infected and healthy tissues, sterilized with sodium hypochlorite (10%) for 1 min followed by washing in sterile water for three times, and then incubated on potato dextrose agar (PDA) plates at 25°C for 4 days. The culture was transferred to new PDA plates and was cultivated in the dark at 25°C for 10 days. A total of three species of fungi were isolated, and

only one fungus species has been found to be able to cause the original pathological characteristics of *A. dahurica* leaves through the back-grafting experiment. The mycelium was black and began to sporulate after 8 days on PDA medium by single-spore separation. Multiple spores joined together to form a spore chain. The spores were spindle-shaped, yellow to yellow brown, and size ranged from 45 to 55 × 15 to 20 μm ($n = 50$), with zero to three longitudinal septa and one to five transverse septa. For pathogenicity tests, the spore suspension (3.5×10^5 spores/ml) was inoculated to healthy plants grown in an experimental field, the test was repeated four times, and 10 leaves were inoculated in each repetition; sterile water was inoculated as the blank control. Inoculated leaves were covered with transparent plastic bags for 24 h to keep humidity. Nine days later, it was found that there were lesions on the leaves inoculated with the pathogen, and the traits were the same as those in the field, whereas the controls were healthy. The fungus was consistently isolated from the inoculated leaves. Similar isolates were reisolated from the inoculated and infected leaves and identified as *Alternaria tenuissima* by DNA sequencing, fulfilling Koch's postulates. Fungal genomic DNA was extracted from 7-day-old culture. PCR amplifications were performed using primers ITS1/ITS4 and TEF/TEFR, respectively (Du 2008; Takahashi et al. 2006). The nucleotide sequence of PCR products, which were deposited in GenBank under the accession numbers MN153514 and MN735428, showed 99.8 to 100% identity with the corresponding sequences of *A. tenuissima* (MW194297 and MK415954). In order to further identify the pathogen species, we constructed a phylogenetic tree by combining TEF sequence and ITS sequence to distinguish the relationship between the pathogen and other minor species in the genus *Alternaria*; the isolate clustered in the *Alternaria* clade. Therefore, the pathogen was identified as *A. tenuissima* based on the morphological characteristics and molecular identification. To our knowledge, this is the first report of *A. tenuissima* causing leaf spot on *A. dahurica* in China.

References:

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