Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Epicoccum sorghinum* Causing Leaf Spot on *Atractylodes lancea* in Hubei Province, China

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Atractylodes lancea is a perennial herb of the Compositae family, widely used in the practice of traditional Chinese medicine for the treatment of digestive disorders, rheumatic diseases, night blindness, and other conditions. Since June 2018, a leaf disease on *A. lancea* has been observed in almost all the fields in Yingshan county of Hubei province, China. The estimated incidence of leaf spot varied from 37 to 65% per field. Initially, affected leaves showed small, water-soaked, chlorotic spots at the edge and tip of the leaf, gradually expanded to form irregular light-brown necrotic spots cover one-third or half of the leaf, and eventually led to leaf necrosis and abscission. The yield of fresh leaves and leaf buds was reduced due to the disease, which seriously affected the growth and quality of *A. lancea*. Sixteen leaf samples were collected and surface disinfected in 0.5% bleach for isolation. Samples of tissue between the healthy and necrotic area (0.2 to 0.5 mm²) were cut with a sterile scalpel from each sample, rinsed in sterile distilled water three times, dried on sterilized filter paper, and then placed on potato dextrose agar (PDA) medium and incubated at 25°C in darkness. After 3 days, the hyphal tips from the edges of growing colonies were transferred to fresh PDA plates for further purification. Finally, six isolates were obtained. The colonies of these isolates were regular and villous at 28°C in darkness. In the initial 5 days colonies were white; after approximately 10 days they became gray-brown, while the reverse side became reddish-brown due to the accumulation of a diffusing pigment. After 15 days chlamydospores were observed, globoid or nearly spherical in shape and grayish brown. The brown unicellular or multicellular chlamydospores measured 5.8 (3.1 to 14.81, *n* = 20) × 23.1 (12.6 to 31.6, *n* = 20) μm. The cultural and morphological characteristics corresponded to

Epicoccum sorghinum (Fu et al. 2019). The isolate CZ01 was chosen for molecular identification. The internal transcribed spacer, actin (ACT), and β-tubulin genes of the CZ01 isolate were amplified using primers ITS1/ITS4, ACT512F/ACT783R, and T1/βt2b (Aveskamp et al. 2010; Carbone and Kohn 1999; White et al. 1990), respectively. The subsequent sequences were deposited in GenBank (accession nos. MN944541, MN956831, and MT254581). Results of BLASTn search showed that the sequences of the three genes have 100, 99.63, and 98.52% identity with existing sequences of *E. sorghinum* (syn. *Phoma sorghina*) in GenBank (MN215621, FJ426957, and MN917744), respectively. In the phylogenetic tree constructed based on the ACT sequences, CZ01 clustered with *P. sorghina* (FJ426957). Thus, CZ01 was identified as *E. sorghinum*, based on cultural, morphological, and molecular features. Pathogenicity tests were conducted on two-leaf-stage *A. lancea* plants by inoculating spore suspensions (10⁶ conidia/ml) or mycelial plugs (diameter = 5 mm) to needle wounds on leaves, whereas the control group was wounded and inoculated with sterile PDA discs or sterile water and then covered with wet cotton for 48 h. Six plants received each treatment, and two leaves were inoculated for each plant. All inoculated and control plants were enclosed in transparent plastic bags and incubated in a greenhouse at 26°C with a photoperiod of 12 h. The first lesions appeared 12 h after inoculation on leaves, and leaf spot symptoms similar to those seen in the field developed 2 days later from both types of inoculation. Uninoculated controls remained asymptomatic. The inoculations were repeated three times with identical results. To fulfill Koch's postulates, the fungus was isolated from the margins of leaf spots on inoculated leaves on PDA and confirmed as *E. sorghinum*. *E. sorghinum* is reported to infect multiple plants including *Paris polyphylla*, *Digitaria sanguinalis*, and *Bletilla striata* (Fu et al. 2019; Kang et al. 2019; Zhou et al. 2018). To our knowledge, this is the first report of *E. sorghinum* causing leaf spot disease on *A. lancea*. The leaf spot caused by *E. sorghinum* may lead to serious yield loss of *A. lancea* unless the proper control measures are applied. This report will facilitate the diagnosis and control of leaf spot of *A. lancea*.

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