Diseases Caused by Fungi and Fungus-Like Organisms

Fusarium culmorum and *Fusarium equiseti* Causing Root Rot Disease on *Lycium barbarum* (Goji Berry) in China

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Lycium barbarum L. is a woody shrub and widely cultivated as an important economic crop in Qinghai province, China. The berry is used in traditional Chinese medicine (Inbaraj et al. 2008). In recent years, root rot of *L. barbarum* has reduced yields by approximately 35% at Nuomuhong Farm (36°28'29.766" N, 96°33'43.657"E, altitude 2,730 m) in Qinghai province. The plantations of *L. barbarum* at Nuomuhong Farm covered about 91.3 km² area. Large-scale outbreaks of root rot were observed after the rainy season and irrigation during the months of June and July, resulting in nearly 30% mortality of *L. barbarum* trees. Diseased plants exhibited symptoms including root rot, leaf etiolation, and wilting, resulting in reduced transport of water and nutrients. Severe disease stunted growth and sometimes resulted in death of the plant. To determine the causal agent of the disease, Nuomuhong Farm was divided evenly into 10 parts, and 10 symptomatic plants were chosen randomly from a 1,000 m² area in each part. One hundred samples were collected per year; a total of 400 samples were collected from 2016 to 2019. Diseased roots were surface sterilized with 0.1% HgCl₂ for 40 s, dipped in 75% ethanol for 30 s, rinsed three times with sterile distilled water, blotted dry with sterile paper towels, plated on Spezieller Nährstoffarmer agar (SNA) amended with antibiotics,

and incubated at 25°C under continuous fluorescent lights (Nirenberg and O'Donnell 1998). After 1 week, both red and white colonies were obtained, which produced abundant multiseptate macroconidia. The large conidia were slightly curved, fusiform to falcate. The macroconidia of red colonies were 21.3 to 37.8 × 3.8 to 7.5 μm (10 macroconidia measured per isolate) with blunt apical cells, notched foot cells, one to five septations, usually three septations, producing chlamydospores, with absent microconidia. The macroconidia of white colonies were 21.3 to 40.5 × 2.8 to 5.0 μm (10 macroconidia measured per isolate) with blunt apical cells, notched foot cells, one to five septations, usually three septations, producing chlamydospores, with microconidia absent. A portion of the second largest subunit of RNA polymerase (RPB2) locus was amplified and sequenced with PCR primers 5f2 and 7cr (O'Donnell et al. 2010). A BLASTn search of GenBank using the 896-bp RPB2 sequence of the red colony revealed that it shared 100% sequence identity with *Fusarium culmorum* (GenBank accession nos. MH582079 and GQ915490, respectively). A BLASTn search using the 820-bp RPB2 sequence of the white colony revealed that it shared 99.88% sequence identity with *F. equiseti* (GenBank accession no. MH582110). Nucleotide sequences of the *F. culmorum* and *F. equiseti* isolates were deposited in GenBank (accession nos. MK848688 and MK848689). Phylogenetic analysis further supported two cultures belonging to *F. culmorum* and *F. equiseti*. To assess their pathogenicity to *L. barbarum*, inocula were prepared from 7-day-old pure cultures of 10 *F. culmorum* and *F. equiseti* isolates grown on SNA, respectively. On the roots of four 4-year-old *L. barbarum* plants, a 40-mm² wound was created and inoculated with a 5-mm-diameter mycelium plug. Sterile SNA was used as the negative control. Then, all plants were maintained at 22 to 25°C in a greenhouse. After 4 weeks, inoculated plants exhibited the same symptoms observed on diseased plants in the field, whereas the negative control plants remained healthy. The pathogenicity tests were conducted more than 100 times from 2016 to 2019, and all results were similar. The identity of the isolates used in the pathogenicity experiment were confirmed morphologically after they were reisolated from the experimental plants, thereby fulfilling Koch's postulates. To our knowledge, this is the first report of root rot caused by *F. culmorum* and *F. equiseti* on *L. barbarum* in China.

References:

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