Diseases Caused by Fungi and Fungus-Like Organisms

Occurrence of Leaf Spot Caused by *Mycocentrospora acerina* on Japanese Ginseng (*Panax japonicus*) in China

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Panax japonicus C. A. Mey., known as Japanese ginseng or “Zhujiashen” in China, is a perennial medicinal herb (family Araliaceae) native to China and is widely grown in many provinces including Hubei, Hunan, and Sichuan. In recent years, cultivation of Japanese ginseng has increased tremendously in China because of its high value. Its root is widely used in traditional Chinese medicine for the treatment of inflammation. In early May 2020, severe necrotic lesions on leaves with 40 to 50% disease incidences were observed on 3-year-old Japanese ginseng plants in a cultivated field in Xuanen County (30.05°N, 109.83°E), Hubei Province, China. The total area affected by the disease was approximately 30 ha. Initial symptoms showed small, circular, brown, necrotic spots uniformly distributed on leaves. The center of the spots was light tan, surrounded by a dark brown ring and a chlorotic halo. As the disease progressed, multiple lesions merged into large disease spots with visible white fungal hyphae, causing leaf wilting. Ten small pieces (0.1 × 0.1 cm in size) of leaf tissue were removed from the lesion margins, surface sterilized with 0.5% sodium hypochlorite for 1 min and 75% alcohol for 20 s, washed with sterile distilled water three times, dried, placed on Petri plates with potato dextrose agar (PDA) medium containing 10 µg/ml of ampicillin, and incubated at 20°C for 5 days. Colonies with dense mycelia were initially white and gradually becoming black. The hyphae were septate, branched, and 3 to 7 µm in width. Conidiophores were flexuous, not branched, and produced a single spore. Spores (ranged from 95.4 to 255.5 × 6.2 to 13.5 µm)

were elongate, multiseptate, with a long, strongly curved beak (ranged from 25.5 to 95.4 µm). The number of septae ranged from 4 to 13. Chlamydospores with a smooth or slightly rough wall were spherical to ovoid and averaged 8.5 to 25.4 × 7.2 to 16.5 µm. The six isolates were preliminarily identified as *Mycocentrospora acerina* (R. Hartig) Deighton based on the morphological characteristics (Gilchrist et al. 2015). To confirm the identification, isolates ZJS1, ZJS3, and ZJS5 were chosen for DNA sequencing. The internal transcribed spacer (ITS) region of rDNA was amplified using the ITS1 and ITS4 primers (White et al. 1990) and sequenced. The identical sequences of the 491-bp amplicons were deposited in GenBank (accession nos. ZJS1, MZ277314; ZJS3, OL333859; and ZJS5, OL333860). BLAST analysis of the sequences showed 100% identity to *M. acerina* (MH856114). Moreover, the three isolates were further confirmed as *M. acerina* by amplifying the large subunit (LSU) of the ribosome gene (accession nos. ZJS1, MZ277321; ZJS3, OL333861; and ZJS5, OL333862), as their identical sequences exhibited 99.83% similarities with *M. acerina* (MH868490). Isolate ZJS1 was chosen to fulfill Koch's postulates with 30 healthy 2-year-old *P. japonicus* grown in plastic pots filled with a sterilized mixture of peat moss and vermiculite (3:1). One leaf of each plant was inoculated with one 5-mm-diameter mycelium-agar disc and placed in a greenhouse at 20 ± 1°C, with ambient lighting and relative humidity of 85%. By 5 days after inoculation, all inoculated plants showed symptoms identical to those observed in the field, and no symptoms were observed on control plants. The fungus was reisolated from the inoculated plants and identified as *M. acerina* using the above method. The experiment was conducted thrice with similar results. To our knowledge, this is the first report of leaf spot caused by *M. acerina* on Japanese ginseng in China and the world. There is a need to develop effective management measures to reduce the occurrence of this disease.

References:

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