Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Sclerotinia sclerotiorum* on Watercress (*Nasturtium officinale*) in an Aquaponic System in Hungary

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Watercress (*Nasturtium officinale*) is an aquatic dicotyledonous vegetable belonging to Brassicaceae (Aiton 1812). Watercress was grown in an aquaponic system on fired clay ball medium at the Aquaponic Research Station of the University of Debrecen, in the city of Debrecen (Hungary). During January 2020, 3-month-old plants showed symptoms in aquaponic cultivation. A visual survey showed 30% of plants with symptoms. Leaves and stems withered and showed white cotton-like mycelium. Mycelia from infected plants were placed on potato dextrose agar (PDA) and incubated at 25°C for 7 days. Single hyphal tips were transferred to produce a pure culture. All 10 fungal isolates showed similar morphological characteristics on PDA. Colonies consisted of white mycelia after 3 days, and globose to irregular and black 2.5 to 7 (average, 3) mm ($n = 100$ from 10 plates) sclerotia formed 10 days later, which are the typical morphological features of *Sclerotinia sclerotiorum* (Mordue et al. 1976). Molecular identification was performed with one of the 10 isolates (Scl_B). Mycelia were grown in 250 ml of potato dextrose broth in a rotary shaker at 175 rpm at 24°C for 6 days. DNA was extracted from mycelium using a NucleoSpin plant II (Macherey-Nagel, Germany) according to the manufacturer's protocol. Polymerase chain reaction (PCR) amplification (Kim et al. 2014) was performed with primers ITS1/ITS4 for the internal transcribed spacer (ITS) region (White et al. 1990) on a Primus 96 thermal cycler (MWG Biotech, Germany). Specific PCR was

performed with primers SSasprF/SSasprR (Abd-Elmagid et al. 2013). PCR products were sequenced by Microsynth Austria GmbH. NCBI BLAST analysis of the 440-bp ITS sequence (GenBank MW012403.1) showed 100% identity with the sequence of *S. sclerotiorum* (MT177267.1, etc.). The 170-bp specific gene sequence (GenBank MW959042.1) had a 100% similarity to hypothetical proteins (GenBank MK028159.1), with a 99.4% similarity to a portion of the *S. sclerotiorum* aspartyl protease gene (AF271387.1). Pathogenicity tests were carried out by inoculating surface-disinfested, 30-day-old watercress plants in plastic pots (15 × 15 × 12 cm). In three repeated experiments 90 watercress plants were measured. Fifteen plants (one plant per pot) were planted into the five-times-autoclaved substrate (Biorgmix: pH 6.1 ± 0.5%; N, 1.5%; P₂O₅, 0.7%; K₂O, 0.5%; organic matter content, 50%) and inoculated by 10 wheat kernels that were colonized by *S. sclerotiorum* (Scl_B) (Garibaldi et al. 2019). Fifteen plants were planted into the substrate with 10 noninoculated kernels as a control. Plants were kept in an MLR-352 climatic test chamber (PHCbi, Japan) at 21 ± 1°C with a 12-h light/dark cycle. On the first day of the experiment, complex nutrient solution (Tek-Land: N, 5%; P₂O₅, 5%; K₂O, 5%; B, 0.01%; Cu, 0.01%; Mn, 0.02%; Mo, 0.002%; Zn, 0.016%) was used, followed by autoclaved water daily. Eight days later white mycelium appeared on every inoculated plant, and 5 days later dark sclerotia formed on the stems. Based on the morphological characteristics the reisolated pathogen was *S. sclerotiorum*. Similar results were detected in three repeated experiments with white mold fungus being reisolated from all 45 infected watercress plants. The 45 noninoculated plants did not show any symptoms and any diseases. This pathogen has already been reported on watercress in the field (Boland and Hall 1994; Farr et al. 1989; Garibaldi et al. 2019). This is the first reported case of white mold on watercress in an aquaponic system in Hungary.

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