Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Tuber Soft Rot of Jerusalem Artichoke (*Helianthus tuberosus*) Caused by *Rhizopus arrhizus* in Qinghai Province of China

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Jerusalem artichoke (*Helianthus tuberosus* L.) is a tuber-producing plant in the Asteraceae that has been widely planted throughout the Qinghai Plateau as an erosion control measure, due to its sand-binding root system. Its tubers can safely survive through the winter (−25°C) on the plateau, and its root system possesses a strong sand fixation ability. In recent years, it has been used for desertification control in the area surrounding Qinghai Lake and Tengri. However, tubers stored in winter are susceptible to fungal infection, resulting in soft rot symptoms. During cold storage of harvested Jerusalem artichoke tubers, the fungus *Rhizopus stolonifer* may cause disease (Ghoneem et al. 2016; Kays and Nottingham 2007; Kosaric et al. 1984). At the experimental site belonging to the Academy of Agricultural and Forestry Sciences of Qinghai University (N 36°43′, E 101°44′, 2,265 m elevation), tubers stored during 2016 to 2018 suffered annual losses to storage decay of 30 to 50%. The initial symptom was white mycelium on affected tubers. The fructan in the tuber decomposed and fermented continuously; thus, the tuber gradually rotted and softened. Moldy tubers were sterilized in 1% NaOCl for 2 min and 70% ethanol for 15 s and then washed with sterile distilled water four times. To isolate the pathogen, symptomatic tubers were cut into ~1-cm pieces from the treated tuber epidermis, inoculated onto PDA, and placed at 25°C for 7 days. When fungal colonies had grown out of the pieces of diseased tissue into the culture medium, a sterile cork borer was used to take plugs from the margins of selected colonies. Plugs from each colony were transferred to

PDA plates and incubated. After three to five rounds of subculturing, 10 morphologically identical fungal isolates were recovered from tuber tissue. The colony was initially circular with distinct margins, and the mycelium was cottony with hyaline stolons. With age, mycelium turned gray, and black sporangia on long sporangiophores were found scattered throughout the colony. Colonies grew rapidly, filling the 40-mm-diameter culture dish in 2 days. Microscopical observation showed that the sporangiophores were slightly curved, fascicular, each with an apical sporangium. Sporangia were spherical or nearly spherical, yellowish-white when immature, and black at maturity, when they measured 158.4 (132.1 to 225.3) μm ($n = 50$). The sporangium was yellow white at birth and then turned black at maturity. The sporangiospore produced in the sporangium was 6.0 (3.2 to 8.3) × 5.1 (3.6 to 6.9) μm ($n = 50$). The internal transcribed spacer (ITS) (Jung et al. 2012) was amplified by ITS1/ITS4. The sequence obtained by ITS sequencing was uploaded to GenBank as MN242807. BLASTn analysis showed that the similarity to *Rhizopus arrhizus* was >99%. To confirm to Koch's postulates, healthy tubers were inoculated with one of the isolates recovered from diseased tissue. A 1.0-ml sterile syringe was used to inject 50 μl of a 10⁶ conidia/ml suspension into each of five healthy tubers, and five more tubers were injected with sterile water as a control. This test was repeated three times. These were cultured in an incubator at 18°C for 7 days. *R. arrhizus* showed symptoms of water leaching on the tuber 3 days later, and black-gray mold was found rapidly spreading throughout the tuber at about 7 days. Results of the three trials were the same. This showed that the fungus isolated from diseased tissue still exhibited strong pathogenicity upon reinoculation and that symptoms from artificial inoculation resembled the original symptoms. The pathogen was reisolated from the inoculated tubers and was confirmed to be *R. arrhizus* through ITS sequencing. It has been reported that *R. arrhizus* infects cassava tubers and potato tubers (Amadioha and Markson 2007; Cui et al. 2019). To our knowledge, this is the first report of *R. arrhizus* causing storage root soft rot of Jerusalem artichoke in China.

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