Diseases Caused by Oomycetes

First Report of Root and Crown Rot of American Ginseng Caused by *Pythium spinosum* in China

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American ginseng (*Panax quinquefolius*) is a perennial herb whose dried roots are used for health care products, medicine, and food in China (Yuan et al. 2010). Shandong Province is the main area growing it and contributes >50% of the production in China. Wendeng city, in the east of Shandong Peninsula, is the primary production area in Shandong Province. It has four seasons, sufficient light, loose soil (pH 5.5 to 7.0), and thus similar conditions to the production areas in North America. In March 2016, 2-year-old American ginseng plants that were planted directly into the ground in the greenhouses in Wendeng city contained up to 6 to 10% stunted plants. Water-soaked lesions were observed on the crowns and the tips of fine roots. The leaves of infected plants became scalded, with dark green starting at the top of the plants and gradually moving downward. Leaves and petioles gradually curled, withered, and drooped, and the whole plant collapsed. Tissue samples (10 mm) were excised from the water-soaked roots and crowns of diseased plants, rinsed under running water for 24 h, dipped in a 0.2% calcium hypochlorite solution for 10 min, placed on sterile filter paper to dry, and then placed on V8 medium (200 ml of V8 juice, 15 g of agar, 0.2 g of CaCO₃, and 1 liter of distilled water) and incubated in the dark at 28°C for 5 days. Five *Pythium*-like isolates that were arachnoid-cottony on cornmeal agar were isolated, and they all produced hyphal swellings, oogonia, antheridia, and oospores. Oospores were globose, smooth and plerotic, with some being aplerotic. The dimensions of hyphal swellings, oogonia, and oospores respectively ranged from 9.0 to 21.3 (avg. 14.1) µm, 12.9 to 22.5 (avg. 18.2) µm, and 12.5 to 20.5

(avg. 16.7) µm. Finger-like projections were uniformly distributed on the walls of the oogonia, and the antheridia were curved rods. The five isolates were identified as *Pythium spinosum* based on morphological characteristics (van der Plaats-Niterink 1981). Genomic DNA was extracted from the isolates using a DNA extraction kit (Omega). The cytochrome c oxidase subunit I (COI) gene and internal transcribed spacer (ITS) region rDNA were amplified and sequenced using primers FM55/FM52R (Long et al. 2012) and ITS1/ITS4, respectively (White et al. 1990). The five COI sequences were aligned and were identical for all five isolates, as well as the five ITS sequences. BLASTn analysis of the 538-bp COI sequence (MT822775) resulted in 99% identity with *P. spinosum* strain CBS122663 (HQ708832.1), and the 916-bp ITS sequence (MN847595) showed 100% identity with GenBank AB217665 belonging to *P. spinosum*. Koch's postulates were confirmed. Corn kernels that had been soaked for 24 h in water, autoclaved for 2 h at 121°C, and allowed to cool were inoculated with agar plugs of *P. spinosum* grown on corn meal agar for 10 days. Inoculated corn kernels were incubated at 28°C for 13 to 15 days, until the corn kernels were covered with white hyphae of *P. spinosum*. Ten healthy ~2-year-old American ginseng plants growing in Wendeng greenhouses were transplanted into a sterilized potting soil artificially infested with the corn inoculum (3 g of inoculum per 100 g of loam mixture). Inoculated and noninoculated control plants were maintained in a greenhouse with a roof covered with sunshade net at 28°C and 100% RH. The experiment was repeated once. Four days after inoculation (DAI), the crown of inoculated plants developed water-soaked symptoms similar to those observed in field. By 7 DAI, the inoculated fine roots and crowns showed water-soaked lesions identical to those observed in field, whereas control plants remained symptomless. The reisolated isolate of *P. spinosum* was identical morphologically and by DNA sequence analysis to the original isolate. This is the first report of root rot on American ginseng caused by *P. spinosum* in China and worldwide. Identification of the pathogen will assist in devising strategies to protect this important medicinal plant from the pathogen and to prevent yield losses.

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