Diseases Caused by Oomycetes

First Report of Damping-Off of Ovate-Leaf Atractylodes Caused by *Pythium aphanidermatum* in South Korea

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Plant Dis. 105:711, 2021; published online as <https://doi.org/10.1094/PDIS-09-20-1975-PDN>. Accepted for publication 7 October 2020.

Ovate-leaf atractylodes (*Atractylodes ovata*) is a well-known medicinal plant in Korea, where the dried rhizome and the root extract are used in herbal medicines. In 2019, severe damping-off of ovate-leaf atractylodes at the early seedling stage was observed in a commercial planting in Sangju, South Korea. Approximately 35% of young seedlings suddenly wilted and then died despite adequate soil moisture. Putative causal agents were isolated from roots of diseased plants. Roots were washed thoroughly with tap water, cut into 1-cm-long pieces, and then split in half longitudinally. Root pieces were surface disinfected in 0.1% NaOCl solution for 1 min, washed three times with sterilized distilled water, and finally blotted dry. Root tissue was placed on potato dextrose agar (PDA, Difco and Becton Dickinson) amended with tetracycline (0.05 g/liter) and incubated at 25°C in the dark. Twelve pure cultures of the potential causal agent were obtained by hyphal tipping twice. Mycelia of the 7-day-cultures were white, and the colony produced numerous oogonia. The oogonia were smooth, globose, terminal and rarely intercalary, and 17.3 to 27.1 µm (mean ± SD, 24.3 ± 2.25 µm) in diameter. Antheridia were declinuous or monoclinalous (rare) with clavate cells that were 17.9 to 31.6 × 7.1 to 8.8 µm (mean, 26.8 × 7.5 µm). The stalks of the antheridia were unbranched. Oospores were plerotic or nearly plerotic and 16.3 to 25.4 µm (mean ± SD, 20.1 ± 3.2 µm) in diameter. The morphological characteristics of the isolates were comparable to those of *Pythium* spp. including *P. aphanidermatum* (Van der Plaats-Niterink 1981). The internal transcribed spacers (ITS), cytochrome oxidase 1 (cox1), and cytochrome oxidase 2 (cox2) regions of genomic DNA from SRRS1, SRRS2, and SRRS4 were amplified using primer sets ITS5 + ITS4, FM52R + FM55, and FM58 + FM66, respectively

(Ueta and Tojo 2016) and sequenced. Resulting sequences were deposited in GenBank under accession numbers LC569777 to LC569779 and LC569785 to LC569790. The concatenated sequence data contained 46 taxa for the ITS-cox2 phylogenetic analysis and 17 taxa for cox1. The maximum likelihood estimation and Bayesian inference consensus tree showed that the present isolates formed a clade with *P. aphanidermatum* (strains NBRC 100101, P36-3, and 1987-61). The pathogenicity of three isolates was tested using the procedure described by Herrero et al. (2003) with some modification. Five surface-disinfected seeds were sown in a plastic pot containing autoclaved peat soil. There was a total of 12 pots. After emergence, seedlings were thinned to three similar-sized seedlings per pot and grown for 25 days at 25°C in 16-h light. Seedlings were then inoculated with mycelial plugs (10 mm diameter) from 4-day-old cultures on PDA. Three plugs per pot were placed 1 cm apart from the seedlings (one plug per seedling). Three pots per isolate were inoculated, and three pots with non-inoculated seedlings were used as a control. All plants were grown in a growth chamber at 25°C with 16-h light and 80% relative humidity and irrigated twice per week with sterile water. All inoculated seedlings (100%) suddenly collapsed 10 days after inoculation, whereas the control plants remained healthy and vigorous. *P. aphanidermatum* was reisolated from the inoculated seedlings and identified following the procedures previously described, thus completing Koch's postulates. This pathogen has also reported as the causal agent of damping-off of common bean, cucumber, wheat, tomato, and tobacco (Al-Saadi et al. 2007; Gilardi et al. 2018; Herrero et al. 2003). To our knowledge, this is the first report of *P. aphanidermatum* causing damping-off disease on ovate-leaf atractylodes in South Korea, and this pathogen could threaten production. Outcomes of the present study will help manage this disease with effective control measures.

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The author(s) declare no conflict of interest.

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Keywords: damping-off, *Pythium aphanidermatum*, pathogenicity

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