Diseases Caused by Oomycetes

Occurrence of Root Rot on Alfalfa (*Medicago sativa*) Caused by *Pythium coloratum* in China

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Alfalfa (*Medicago sativa*) is an important perennial pasture legume in China (>0.7 million hectares in Gansu Province) with high yield, nutrition, and adaptability. Root rot diseases threaten alfalfa production. In summer 2017 to 2019, 30 to 80% of plants in major alfalfa-producing regions in Gansu Province had root rot. To identify associated oomycetes, diseased plants were collected from three fields (2 to 5 ha) in Zhangye, Wuwei, and Jiuquan under flood irrigation every 40 to 50 days during the growing season. Diseased plants (3 to 4 years old) were poorly growing, stunted, and wilted, and some died. Irregular brown necrotic areas were observed on tap roots, and lateral roots were brown discolored, poorly developed, and rotted. Roots were washed under running tap water, cut into 3 × 4-mm pieces, rinsed in deionized (DI) water, and surface sterilized in 75% ethanol for 2 min. Pieces were washed with autoclaved DI water at least three times, dried on autoclaved filter paper, and placed onto cornmeal-based 3P selective medium (Ali-Shtayeh and Saleh 1999). Forty single-hyphal-tip cultures of Wuwei samples were subcultured on cornmeal agar (CMA) and PDA and incubated at 25°C. These isolates had similar morphology; five were selected for morphological and molecular identification. Colonies on CMA were submerged without a regular pattern, and they produced cottony aerial hyphae on PDA. Hyphae were colorless, without septa, and 5 to 9 µm in diameter. Sporangia were filamentous, terminal and intercalary, occasionally slightly inflated, and formed dendroid structures. Sporangia formed long discharge tubes, and vesicles were up to 50 µm in diameter. Oogonia were globose or pyriform, terminal or occasionally intercalary, and 20 to 27 µm in diameter. Antheridia, one to four per oogonium, were monoclinal and diclinal with mostly straight necks. Oospores were

aplerotic and globose, 15 to 25 µm in diameter, with 2- to 4-µm-thick walls. Morphological characters suggested *Pythium coloratum* (Van der Plaats-Niterink 1981). ITS regions (ITS1, ITS2, and 5.8S rRNA) were amplified and sequenced with primers ITS1/ITS4 (White et al. 1990) and CoxI genes with OomCoxI-Levup/OomCoxI-Levlo (Robideau et al. 2011). Sequences were compared with GenBank reference sequences by BLAST. ITS showed identity with *Pythium* spp. and the best matches (99.5%; 865/869 base pairs) to *P. coloratum* (e.g., MK813919, MK434167). CoxI also had the best match (100%; 680/680 base pairs) to *P. coloratum* (HQ708547). ITS and CoxI sequences of a representative isolate were deposited in GenBank (MT256101 and MT571499, respectively). Five representative isolates were submitted to the Grassland Culture Collection Center, Lanzhou University (LZU-MSR-P.c1 to P.c5). Pathogenicity of two isolates (P.c1, P.c3) was determined on alfalfa at 22°C, 12-h photoperiod, and 65% RH. Inoculum was prepared by subculturing agar pieces from a PDA culture in a 250-ml flask containing autoclaved millet seeds incubated at 22°C in darkness for 2 weeks and shaken every 2 days to ensure uniform colonization. Inoculum was mixed with pasteurized potting mix at 0.5% (w/w) and placed into pots (12 × 12 cm). Two-week-old seedlings were transplanted into the infested mix. There were 12 pots per isolate and five seedlings per pot, and pots were watered every 2 days. Three weeks postinoculation, 87% of the inoculated plants were stunted, and the roots were brown to dark-brown discolored; 58% of plants died. No disease was observed on noninoculated plants. Reisolation of *P. coloratum* from diseased roots was confirmed morphologically and by sequencing. Root rot on alfalfa caused by *Pythium* spp. has been reported in many counties (Ao et al. 2018; Basu 1983; Berg et al. 2017), and root rot caused by *P. coloratum* on cauliflower was reported in China (Song et al. 2019). This is the first report of root rot on alfalfa caused by *P. coloratum* in China. Since alfalfa is a perennial pasture and populations of *P. coloratum* can accumulate in the soil and roots, thus this pathogen should be of concern, particularly in commercial fields with excess soil moisture associated with flood irrigation.

References:

- Ali-Shtayeh, M., and Saleh, A. F. 1999. Mycopathologia 145:143.
- Ao, S., et al. 2018. Plant Dis. 102:1043.
- Basu, K. 1983. Can. Plant Dis. Surv. 63:51.
- Berg, E. L., et al. 2017. Plant Dis. 101:1860.
- Robideau, G. P., et al. 2011. Mol. Ecol. Resour. 11:1002.
- Song, W. W., et al. 2019. Plant Dis. 103:2697.
- Van der Plaats-Niterink, A. J. 1981. Stud. Mycol. 21:50.
- White, T. J., et al. 1990. Page 315 in: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, CA.

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