Diseases Caused by Oomycetes

First Report of *Aphanomyces euteiches* Race 1 and Race 2 Causing Aphanomyces Root Rot on Alfalfa (*Medicago sativa*) in South Dakota

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Aphanomyces euteiches causes Aphanomyces root rot (ARR) in alfalfa (*Medicago sativa*), along with root rot on many other legumes, including pea, clover, and lentil (Malvick et al. 2009). In 2020, South Dakota (SD) planted the most acres of alfalfa in the United States, which demonstrates the importance of alfalfa to the state. Several SD growers reported alfalfa establishment problems likely to be associated with ARR. Soil samples were collected from 16 fields under commercial alfalfa production in Lake County, SD, in June 2020. Composite soil samples based on 24 subsamples were collected in a W-shaped pattern at a depth of 15 cm. Collected soil was sieved, and 80 cm³ was placed in plastic pots (6 × 6 cm). Each pot was planted with 25 seeds, covered with an additional 15 cm³ of soil, and placed in a growth chamber with a 16-h photoperiod at temperatures of 24 and 19°C (day and night). Alfalfa seedlings, including Saranac (susceptible to races R1 and R2), WAPH-1 (resistant only to R1), WAPH-5 (resistant to both R1 and R2), and Mustang 625 (resistant to both R1 and R2 and coated with mefenoxam) grew in collected soil for 7 days, followed by 4 days under flooded conditions. Trays were drained, and at 21 days after planting (DAP), roots were removed from soil, washed in distilled water, and rated to measure severity of disease symptoms (Samac et al. 2015). The average severity index (ASI) used a 1 to 5 disease severity scale, 5 being a dead plant and 1 being no symptoms present (<http://www.naaic.org/stdtests/Aphano.html>). Race was based on ASI, where R1 included an ASI of ≥3 for Saranac and <3 for WAPH-1, and R2

included an ASI of >3.0 for Saranac and WAPH-1 and <3.0 for WAPH-5 (Malvick and Grau 2001). Race-typing experiments were repeated twice with six replicate pots per alfalfa cultivar per experiment and determined the presence of both R1 and R2 in Lake County, SD. ASI values for Mustang 625 and WAPH-5 were similar across all fields evaluated, which indicates limited confounding effects of other root-rotting pathogens. DNA was extracted from three symptomatic roots from each field and was PCR amplified using *A. euteiches*-specific primers (Vandemark et al. 2002). A PCR product was observed in all 16 fields evaluated, and the absence of a product was observed when DNA was extracted from alfalfa roots grown in vermiculite. Following race typing, infected alfalfa roots were surfaced sterilized and placed on *Aphanomyces* selective medium consisting of mefenoxam and benomyl in cornmeal agar (CMA) (Pfender et al. 1984). Isolates were identified as *A. euteiches* based on hyphal morphology (Malvick and Grau 2001). Alfalfa seedlings (Saranac) were grown in vermiculite under growth conditions used for the race-typing assay and inoculated at 6 DAP with two isolates of *A. euteiches*. Inoculation was completed using half plates of 1-week-old *A. euteiches* mycelium on CMA blended with 1 liter of water (Samac et al. 2015). At 35 DAP, control alfalfa seedlings inoculated with blended CMA and water remained asymptomatic, and alfalfa infected with *A. euteiches* displayed symptoms including honey-brown colored lesions. For confirmation of Koch's postulates, DNA from three reinfected seedlings was again PCR amplified using *A. euteiches*-specific primers and confirmed our previous work. This is the first report of either R1 or R2 of *A. euteiches* causing ARR on alfalfa in SD. To avoid future yield loss, SD growers should consider planting available alfalfa cultivars that have resistance to both races of *A. euteiches*.

References:

- Malvick, D. K., and Grau, C. R. 2001. Plant Dis. 85:740.
- Malvick, D. K., et al. 2009. Eur. J. Plant Pathol. 123:171.
- Pfender, W. F., et al. 1984. Plant Dis. 68:845.
- Samac, D. A., et al. 2015. Plant Dis. 99:614.
- Vandemark, G. J., et al. 2002. Phytopathology 92:265.

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