APHANOMYCES ROOT ROT RESISTANCE (RACES 1 & 2)

Test accepted: August 1998 **Test updated:** June 2024
Pathogen: *Aphanomyces euteiches* Drechs. **Test authors:** Sharie Fitzpatrick, Jessica Brummer, Brian Hudelson, Dean Malvick and Craig Grau

PLANT CULTURE

Greenhouse/Growth Chamber

Container..... Seedling flats subdivided into compartments, with bottom drainage holes; flats are placed in a water reservoir (a flat without holes). Watertight tubs with drain holes that can be plugged/ unplugged to allow drainage may also be used.
Media..... Autoclaved sand, porous soil mix, or vermiculite
Temp/Light..... 20 to 24°C; 12-16 hour daylength
No. of Plants ..... 50 to 70 per replication
No. of Reps ..... 4 minimum

INOCULUM SOURCE

Standard Isolates..... MF-1 (Race 1)
NC-1 (Race 2)
Storage ..... Oatmeal or corn meal agar (see Note)
Temperature..... 4 to 12°C. For 4°C storage, use constant temperature incubator only, not self-defrosting refrigerator.

INOCULATION PROCEDURE

Age of Plant ..... 5 to 6 days (when cotyledons are fully expanded)
Type of Inoc. ..... Zoospore suspension or comminuted mycelium
Production ..... Zoospores produced by the method of Mitchell and Yang;[3] or one, 1 week old corn meal agar cultures are blended in 1 L distilled water (see Note on zoospore production)
Concentration..... 100 to 1000 zoospores or 1 mL comminuted mycelium per seedling
Method..... Add water to the surrounding reservoir to saturate the entire root zone, then pipette inoculum into the upper root zone

INCUBATION

Location ..... Environmentally controlled chamber or greenhouse
Counts..... Count at full emergence (7 to 8 days after seeding)
Culture ..... Maintain flooded conditions for 5 days; application of a complete nutrient solution to the water reservoir 7 days after inoculation aids in separation of plant reactions.
Age at Rating ..... 10 to 14 days after zoospore inoculation; 5 weeks after inoculation with mycelium

RATING

Percent resistant plants is the total of classes of 1 and 2.

1 Resistant..... No necrosis of roots and hypocotyls
2 Resistant..... Slight necrosis of roots and hypocotyls
3 Susceptible..... Necrosis of roots and lower hypocotyl, slight chlorosis of cotyledons, and moderate stunting of stem(s)
4 Susceptible..... Extensive necrosis of roots, hypocotyls and cotyledons, and severe stunting of stem(s)
5 Susceptible..... Dead seedling

CHECK CULTIVARS

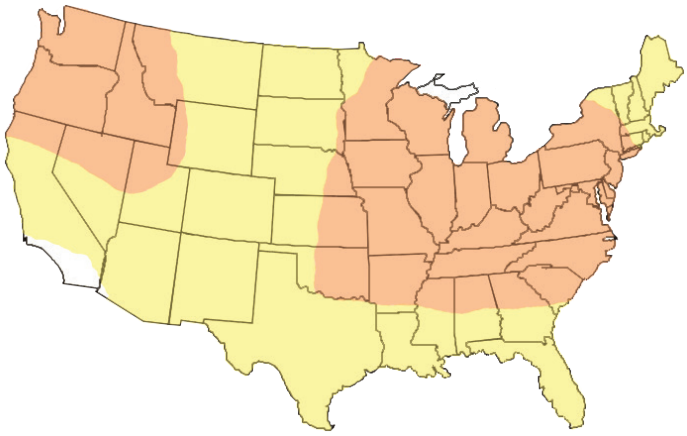
	Approximate Expected Resistance (%)	Acceptable Range of Reaction (%)
Race 1		
Resistant		
WAPH-1[7]	50	35-60
Susceptible		
Saranac	2	0-5
Race 2		
Resistant		
WAPH-5	50	35-60
Susceptible		
Saranac	2	0-5
WAPH-1	2	0-5

Values for resistant standards are percent of total plants in classes 1 and 2. Both Saranac and WAPH-1 must be used as race 2 susceptible checks.

(Click to see larger photo.)
Apahnomyces root rot ratings.

Thanks to John Edmonds, Jay Sandman, Sam Stratton, and Mike Velde for supplying additional data used in the development of this test.

DISTRIBUTION AND SEVERITY OF APHANOMYCES ROOT ROT



Not known to occur.
Occurs but is not considered a problem.
Occasionally causes significant losses on susceptible cultivars.
Frequently causes significant losses on susceptible cultivars.

Aphanomyces Root Rot, *Aphanomyces euteiches* Drechs
(Click map to the left for a larger version.)

A. euteiches, has been reported throughout North America, Europe, Australia, and New Zealand. The distribution of alfalfa strains *per se* has not been exhaustively studied. However, race 1 alfalfa strains have been detected in Idaho, Illinois, Indiana, Iowa, Kentucky, Maryland, Michigan, Minnesota, Mississippi, Nevada, New York North Carolina, Ohio, Oklahoma, Pennsylvania, Tennessee, Virginia, Wisconsin, and Ontario and Quebec, Canada. Race 2 alfalfa strains have been confirmed in Idaho, Maryland, Minnesota, North Carolina, Iowa, Tennessee, Virginia and Wisconsin.[2]

Aphanomyces can cause severe stunting and death of seedlings, and can cause a chronic disease of lateral roots of established plants. It frequently is recovered from fields where Phytophthora root rot and Pythium damping off are found. *A. euteiches* and *Phytophthora medicaginis* may cause a root disease complex. Aphanomyces root rot is favored by warm, saturated soil conditions.

SOURCE OF INOCULUM AND SCIENTIST WITH EXPERTISE

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CORRELATION TO FIELD REACTION

There is a good correlation between results of this test and visual root scores, plant vigor and forage yield in naturally infested fields.[4]

RACES

There are two recognized races of *A. euteiches*. Isolates are known which may belong to additional races, as yet not defined.

PLANT GROWTH OPTIONS AND RANGE OF CONDITIONS

Controlled environmental conditions (20 to 24°C) are optimum for separation of resistant and susceptible reactions. However, warmer conditions (28 to 32°C) are more favorable for pathogen activity, and will give a more severe test. A more qualitative (dead or alive) reaction occurs at temperatures equal to, or greater than, 28°C.

HELPFUL INFORMATION

Different brands of corn meal agar may produce different results. Best pathogen growth is obtained with BBL corn meal agar from Becton, Dickinson and Company, Sparks, MD 21152 USA. *A. euteiches* can be maintained on agar, but requires frequent subculturing every 2 to 4 months. Isolates commonly lose aggressiveness after about 1 year in culture, therefore, isolates recently recovered from alfalfa seedlings should be used. Use actively growing cultures for zoospore production, as zoospore production declines for mycelial mats older than 5 days of age. Agitation will induce zoospores to encyst to a non-motile stage allowing an accurate enumeration with a hemacytometer.

Seedlings in classes 1 and 2 are considered resistant, however, selfpollinated class 3 plants frequently produce resistant progeny.[3]

ALTERNATIVE METHODS

The seedlings assay using zoospores is the most effective and preferred method for characterizing alfalfa populations for reaction to Aphanomyces root rot. Older plants (6 to 12 weeks old) may be used to screen alfalfa for reaction to *A. euteiches* under controlled conditions. However the chronic root symptoms that develop are difficult to characterize into severity classes. Four to six week old plants can be inoculated with *A. euteiches* and then clipped back, and the amount and rate of foliage regrowth can be used to score plants for their reaction to the pathogen.

REFERENCES

1. Grau, C. R. 1992. Registration of WAPH-1 alfalfa germplasm with resistance to Aphanomyces root rot. Crop Science 32:287-288
2. Grau, C. R., A. M. Muehlchen, J.E. Tofte, and J. E. Smith. 1991. Variability in virulence of *Aphanomyces euteiches*. Plant Disease 75:1153-1156.
3. Mitchell, J. E., and C.Y. Yang. 1966. Factors affecting growth and development of *Aphanomyces euteiches*. Phytopathology 56:917-922.
4. Wiersma, D. W., C. R. Grau, and D. J. Undersander. 1995. Alfalfa cultivar performance with differing levels of resistance to *Phytophthora* and *Aphanomyces* root rots. Journal of Production Agriculture 8:259-264.

PRODUCTION OF APHANOMYCES EUTEICHES ZOOSPORES

1. Culture A. euteiches on corn meal agar CMA plates for 7 days (whole plate colonization). It is very important to pour the agar relatively thin to about 1/3 depth of the plate and to incubate cultures at 25°C so that oospores form. At cooler temperatures, the culture is not as developed and will not form many zoospores.
2. Remove 25 to 30 7-mm diameter plugs (cork borer #5) from the edge of culture and place in 50 mL liquid PG medium for approximately 24-48 h (250 mL sterile Erlenmeyer flasks, in the dark, no shaking, 20-22°C).
3. PG medium removal and repeated washing cycles trigger asexual reproduction.
   - 0 h: Pour off PG removing every last drop! Add ~25 ml autoclaved spring water (see note below) and let sit several minutes. Swirl and pour off. Add ~100 ml spring water (to 100 ml mark on flask). Let stand in the dark for 1 hr.
   - 1 h: Pour off spring water. Remove every last drop! Add ~100 ml spring water.
   - 2 h: Repeat 1 hr washing.
   - 4 h: Repeat 1 hr washing.
   - 6 h: Final resuspension of plugs in limited volume of spring water (typically 15 mL for 250 mL flasks, just enough to immerse the mycelium).
4. Incubate in the dark at 20-22° C overnight.
5. Zoospore harvest and numeration 16-18 h after final resuspension.

Notes:

Flame flasks opening for 3-4 seconds before and after each washing step, flame before harvesting (let it cool down to avoid heat-shocking the zoospores!)

Expect about 105 zoospores/ml. Place a 11 ul drop of zoospores on the hemocytometer and check for zoospores. If there are many swimming spores, to facilitate counting, place the hemocytometer on a hot plate for about 5 seconds to cause spores to encyst. Over-heating the slide will cause spores to lyse, so heat carefully.

PG medium: 20 g Bactopeptone, 5 g glucose, 1 L ddH2O

Spring water: Ice Mountain brand works well but other brands may be used. Autoclave for 30 minutes.