

Aphanomyces Root Rot Resistance (Races 1 and 2)

Aphanomyces euteiches Drechs.

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 to 16 hour day length

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

Temperature..... 4-12 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

Concentration 100 to 1000 zoospores or 1 Ml comminuted mycelium per

Method Add water to the surrounding reservoir to saturate the entire root zone, then drench inoculum over the seedlings into the upper root zone

INCUBATION

Location Environmentally controlled chamber or greenhouse Plant

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.

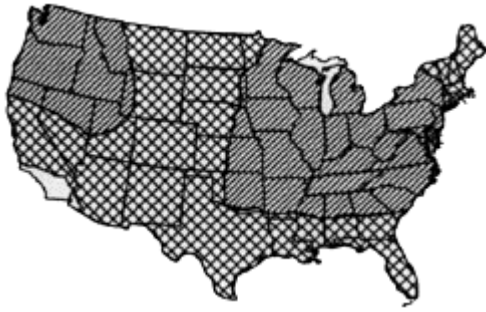
- 1 Resistant..... No necrosis of roots and hypocotyls
- 2 Resistant..... Slight necrosis of roots and hypocotyls
- 3 Susceptible Necrosis of roots and lower hypocotyl, slight chlorosis of cotyledons, and moderate stunting of stem(s)
- 4 Susceptible Extensive necrosis of roots, hypocotyls and cotyledons,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

CHECK CULTIVARS

	Approximate Expected Resistance (%)	Acceptable Range of Reaction (%)
Race 1		
Resistant WAPH-1 (1)	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.

DISTRIBUTION AND SEVERITY OF APHANOMYCES ROOT ROT



Aphanomyces Root Rot (*Aphanomyces euteiches* Drechs)

(Click on the map for a larger version; see also the [key](#))

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

Name Craig R. Grau
 Address..... Department of Plant Pathology
 University of Wisconsin Madison
 1630 Linden Drive
 Madison, WI 53706-1598
 Phone..... (608) 262-6289
 FAX..... (608) 263-2626
 E-mail..... cg6@plantpath.wisc.edu

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

A. euteiches can be maintained on agar, but requires frequent subculturing every 2-4 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, self-pollinated 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.

Acknowledgments:

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