DOWNY MILDEW

Test accepted: March 1991
Pathogen: *Peronospora trifoliorum* de Bary **Test author:** Donald L. Stuteville

PLANT CULTURE

Growth Chamber

Container ..... Flats
Medium ..... Fine sand
Seed Depth ..... 1.3 cm
Temp/Light ..... 20°C; continuous light (approximately 100 @ mol $m^{-2}s^{-1}$)
No. of Plants ..... 40 to 50 per replication
No. of Reps ..... 4 minimum

INOCULUM CULTURE AND PREPARATION

Source ..... Conidia (sporangia) from several locations should be represented; conidia from field plants usually are contaminated and germinate poorly; to reduce contaminants add 50 µg nystatin and 10 µg tetracycline/mL of inoculum;[6] only conidia produced in the lab should be used as inoculum for tests.

Storage ..... Conidium viability declines rapidly if harvest is delayed or if conidia are exposed to dry air for more than a few minutes;[4] however, a low percentage of conidia will survive for a few weeks on diseased seedlings stored at -20°C; conidia will remain viable for many years in liquid nitrogen.[1]

Production ..... Conidia form only during darkness at near 100% relative humidity; they will not form in free water;[4] to produce conidia place flats of infected plants (6 days after inoculation) into darkened, near-airtight containers (we use plastic sweater boxes covered with aluminum foil) about 16 hours before conidia are needed for inoculum.

Preparation ..... Remove flats from darkened containers, immediately harvest plants, and place them into a jar containing chlorine-free water;[3] close the jar and shake it vigorously to dislodge conidia; pour the spore suspension through a tea strainer to remove plant debris; adjust concentration to at least 25,000 viable conidia per mL water and use immediately.

INOCULATION PROCEDURE

Inoculation ..... Spray suspension onto seedlings until a drop forms between the cotyledons; to determine viability, spray inoculum onto a slide, place on a filter paper saturated with distilled water in a closed petri dish, incubate in a dark area at 20°C, and 24 hours later determine percent conidia with germ tubes

SCHEDULE

The following schedule requires little attention on weekends.

Day 1 (Th) ..... Plant seeds 1.3 cm deep in rows at least 2.5 cm apart in flats of fine sand
Days 3 to 6 ..... Sprinkle water on flats twice daily to settle the sand around the emerging seedlings
Day 5 (M 4pm) .... Induce sporulation on plants seeded the previous week for inoculum production by placing them in darkened containers
Day 6 (T 8am) ..... Inoculate seedlings and place in darkened containers
Day 7 (W 8am) .... Remove flats from containers and rogue any plants that have emerged since inoculation
Days 8 to 12 ..... Continue roguing newly emerged plants
Day 12 (M 4pm) .. Induce sporulation by placing flats of plants into darkened containers
Day 13 (T 8am) ... Evaluate test

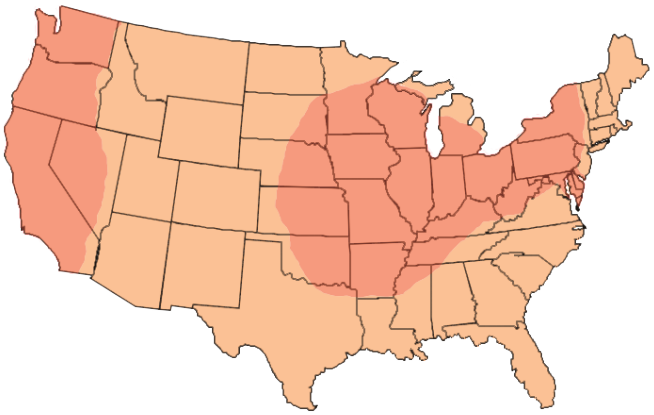
RATING

Evaluation of cultivars is based on the percentage of resistant (symptomless) plants compared with the resistant check cultivar.

CHECK CULTIVARS

	Expected Symptomless Plants (%)	
Resistant		
Saranac**	15-20	isolates 15 and 17
	50-60	isolate 18
KS208[5]**	80-90	all isolates tested
Susceptible		
Kanza**	0-5	all isolates tested

DISTRIBUTION AND SEVERITY OF DOWNY MILDEW



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.

Downy mildew, *Peronospora trifoliorum* de Bary
(Click map to the left for a larger version.)

SOURCE OF INOCULUM

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CORRELATION TO FIELD REACTION

Resistance among cultivars to the same race correlates well, but the percentage of resistant (symptomless) plants is typically much lower in the seedling test than in the field.

RACES

Several races are known.

ALTERNATIVE METHODS

Downy mildew resistance of spaced plants in the field can be evaluated following epiphytotics of downy mildew which may occur during spring and fall.[2]

1 Resistant ..... No symptoms
2 Resistant ..... Small, usually nonsporulating lesions on one or two leaves
3 Susceptible ..... Sporulating lesions on 10 to 25% of the leaves
4 Susceptible ..... General infection over the entire plant.
5 Susceptible ..... Dead

Plants classified as 1 or 2 are considered resistant. Use ASI or percentage of resistant plants to compare cultivars.

REFERENCES

1. Bromfield, K.R., and C.G. Schmitt. 1967. Cryogenic storage of conidia of *Peronospora tabacina*. Phytopathology 57: 1133.
2. Elgin, J.H., JR., and D.K. Barnes, Eds. 1984. In: Standard tests to characterize pest resistance in alfalfa cultivars. USDA, ARS, Misc. Pub. No. 1434.
3. Fried, P.M., and D.L. Stuteville. 1975. Effect of chlorine on Peronospora trifoliorum sporangial production and germination. Phytopathology 65:929-930.
4. Fried, P.M., and D.L. Stuteville. 1977. *Peronospora trifoliorum* sporangium development and effects of humidity and light on discharge and germination. Phytopathology 67:890-894.
5. Sorensen, E.L., D.L. Stuteville, and E.K. Horber. 1989. Registration of KS208 Alfalfa germplasm with resistance to five diseases and three insects. Crop Sci.29: 1094-1095.
6. Stuteville, D.L. 1977. Antibiotics that selectively inhibit bacteria and fungi antagonistic to *Peronospora trifoliorum*. Proc. Am. Phytopathol. Soc. 4:167-]68.