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Stagonospora Leaf Spot and Crown Rot Resistance

Stagonospora meliloti (Lasch) Petr.

Donald Erwin, David Gilchrist, Larry Teuber, and L. D. Satterlee

PLANT CULTURE

Greenhouse

Container	Permanent sand benches for direct seeding, plastic pots, or Conetainers (Container Nursery, Canby, OR); method should permit deep vertical tap root growth to 25 to 35 cm
Media	Fertilized sand or UC soil mixture (50% silt; 50% peat moss)
Temp/Light	21 to 27°C; 14 to 16 hour daylength
No. of Plants	35 to 50 plants per rep
No. of Reps	3 minimum
Culture	Control insect populations; supplemental fertilizer (0-10-10) added once a month with water applied from below the crown.
Other	Inoculate seed with *Rhizobium meliloti* Dang; seed directly into sand bench or into Conetainer; place cones in the sand (permits water and nutrient control and avoids excess water at crown)

INOCULUM CULTURE

Source	Pure cultures are isolated from alfalfa stems or crowns onto acidified potato dextrose agar or dilute V8 juice agar amended with streptomycin (30 pmm); radial growth is slow (2 mm per day); incubate infected leaves or stems containing pycnidia in a moist petri dish for 48 hours and streak pycnidiospores onto plates
Storage	Pure cultures on V8JA slants at 4°C; transfer pycnidiospores (not mycelium) to maintain pycnidial type
Storage Life	6 to 10 weeks; renew by pycnidiospore transfer to fresh medium (avoid mycelium)

INOCULATION PROCEDURE

Age of Plant	Earliest stage for reproducible infection using CA isolates is two-week regrowth on plants cut at first flowering (30 to 45 days)
Type of Inoc	Pycnidiospore suspension from 10 to 14 day old V8JA plates that have been flooded with spores
Concentration	10^6 spores per mL +0.01% Tween 20 (wetting agent); blended water agar may increase adherence

Methods

Foliar	Spray foliage until just before runoff, allow foliage to dry prior to misting
Crown	Inject spores (10^6 per mL) into upper tap root with PS #19 hypodermic needle with side orifice (Pupper and Sons, Inc., New Hyde Park, NY) or pour inoculum on scalpel wounds to prevent escapes

INCUBATION

Location	For leaf spot inoculate in mist chamber or greenhouse with mist system for 48 to 72 hours at 20 to 22°C and 16 hour daylength
Spacing	2.5 to 5 cm apart
Length	Rate foliar lesions at 14 to 21 days after inoculation; rate crown rot at 2 to 6 months

RATING

Foliar

1 Resistant	Free of symptoms
2 Resistant	Lesions on leaves and petioles but none on stems
3 Resistant	Lesions on leaves and up to 25% of stem tissue
4 Susceptible	Lesions on leaves and 25% to 60% of stem tissue
5 Susceptible	Lesions on leaves and 60% to 100 % of stem tissue

Root and crown

0 Resistant	No necrotic tissue at inoculation site
1 Resistant	Local superficial lesion
2 Resistant	Necrotic tissue (reddish flecks) with 1 to 5 mm lesion
3 Susceptible	Necrotic tissue 5 to 10 mm laterally and 10 to 20 mm down from inoculation site
4 Susceptible	Necrotic tissue 10 to 20 mm laterally and 20 to 30 mm down from inoculation site
5 Susceptible	Necrotic throughout, dead plant

CHECK CULTIVARS

	Approximate Expected Resistance (%)	Acceptable Range of Reaction (%)
Moderately Resistant		
UC 129*	25	20-30
Susceptible		
Moapa 69	5	0-10

*Seed of UC 129 is available from D.C. Erwin. Values for resistant standards are the totals of all resistant categories.

DISTRIBUTION AND SEVERITY OF STAGONOSPORA CROWN ROT

Stagonospora stem and crown rot, *Stagonospora meliloti* (Lasch) Petr.

(Click on the map above for a larger version. See also the KEY)

SOURCES OF INOCULUM

Name ..... Ann Martinsen
Address..... Dept. of Plant Pathology, Univ. of CA
Davis, CA 95616
Phone..... 916) 752-7043

Name ..... D. C. Erwin, and R. A. Khan
Address..... Dept. of Plant Pathology, Univ. of CA
Riverside, CA 92521
Phone..... 714) 787-4128

SCIENTISTS WITH EXPERTISE

Name ..... D. G. Gilchrist
Address..... Dept. of Plant Pathology, Univ. of CA
Davis, CA 95616
Phone..... (916)752-6938

Name.....D. C. Erwin
Address .....Dept. of Plant Pathology, Univ. of CA
Riverside, CA 92521
Phone .....(714) 787-4128

Name.....J.A.G. Irwin
Address .....Dept. of Botany
University of Queensland
St. Lucia, Australia 4067

CORRELATION TO FIELD REACTION

Greenhouse selections made at UC Davis and evaluated at three field locations in CA ranked similarly under all conditions.

CULTURE OPTIONS AND RANGE OF CONDITIONS

Pure cultures are grown on V8 Juice Agar at 22 to 24°C for pycnidial formation, and at 10 to 16°C for mycelial growth. Streaking of pycnidial spores or pouring a spore suspension on V8 plates will produce a "lawn" of pycnidia in 10 to 14 days. Light enhances pycnidial production.

HELPFUL INFORMATION

Temperatures may vary 21 to 27°C but higher temperatures reduce severity. All seedling plants tested in CA have been highly resistant to infection prior to emergence of the first flower buds. Reliable rating of genotypes requires regrowth following the first flowering. Foliar and stem infection is highly correlated with crown and root infection.

ALTERNATIVE METHODS

Since foliar inoculation is easier, faster, reliable and closer to natural conditions it is the method of choice, but crown inoculation also can be done by uprooting young plants after cutting the foliage, rinsing the roots with water, soaking the roots and crowns in a spore suspension then replanting. The incubation period for this method is two to three months.

REFERENCES

1. Erwin, D.C., R.A. Khan, O.K. Ribeiro, and W.F. Lehman. 1987. Growth sporulation and pathogenicity of *Stagonospora meliloti* and selection for resistance to crown and leaf spot of alfalfa. Plant Dis. 71: 181 - 185.

2. Erwin, D.C., R.A. Khan, and W.F. Lehman. 1987. Registration of UC 129 alfalfa germplasm with moderate resistance to Stagonospora leaf spot and root rot. Crop Sci. 27:820.

3. Graham, J.H., F.I. Frosheiser, D.L. Stuteville, and D.C. Erwin, eds. 1979. *In* A compendium of alfalfa diseases. American Phytopath. Soc. St. Paul, MN 65 pp.

4. Stuteville, D.L., and D.C. Erwin. 1990. *In* A compendium of alfalfa diseases. Amer. Phytopath. Soc. St. Paul, MN, 2nd ed. 84 pp.