ROOT-LESION NEMATODE RESISTANCE

Test accepted: March 1995
Pathogen: *Pratylenchus penetrans* Cobb, Filipjev and Schur-Stekhoven **Test authors:** D.K. Barnes, J.A. Thies, L.A. Wanschura and J.L. Townsend

PLANT CULTURE

Growth Chamber (Antibiosis) Test

Container..... 3.8 cm x 19.0 polyethylene tubes (cone-tainers)
Medium..... Autoclaved 1:1 sand to soil mixture
Temp/Light..... 25° C; 16 hour daylength
No. of Plants..... 3 per tube (overplant and thin), 6 or more replicates
Other..... Inoculate with *Sinorhizobium meliloti*

Field Screening Methods

Planting Rate..... 75 seeds per m of row or broadcast plots (1 m x 8 m plots) at approximately 55 viable seeds per 0.1 m^2

INOCULUM CULTURE AND PREPARATION

Source..... Maintain *Pratylenchus penetrans* in monoxenic alfalfa callus[5] or in corn root explants at 25° C.
Maintenance..... Transfer alfalfa callus at 6 week intervals and corn root explant cultures at 3-4 month intervals.

INOCULATION PROCEDURE

Growth Chamber Test

Age of Plant..... 12-14 days
Inoc. Type..... Suspension in tap water; extract nematodes from alfalfa callus or corn root tissue for 48 hours using shaker method (described in RATING section).
Concentration.... About 40 *P. penetrans* per mL
Method..... Inject 4 mL nematode suspension (150 nematodes per tube) into soil at 4 cm depth using a microliter pipette. Repeat inoculation 1 week later. Total = 300 nematodes/tube.
Other..... Include non-inoculated control.

INCUBATION

Growth Chamber Test

Culture..... Use insect free plants. Use of systemic insecticides is not advised. Do not allow soil to become dry. Avoid splashing of soil between tubes when watering. Clip plants 6 weeks after the second inoculation and evaluate after 4 additional weeks.
Spacing..... Place polyethylene tubes in alternate spaces in rack to allow for air circulation and ease of watering.

Field Screening Methods

Location..... Field naturally infested with population densities of 3 or more nematodes per cm^3 soil.
Culture..... In severely infested soil, spray prepared seedbed with carbofuran (2,3-dihydro-2,2-dimethyl-7 benzofuranyl) methylcarbamate, flowable formulation) at 2.2 kg per ha a.i. before planting to allow plant establishment; based on Minnesota conditions, plots should be harvested 2X in the seeding year and 3X in the second year.
Rating..... In mid-September of the second year, plants are undercut and rated for root damage.

RATING

Growth Chamber Test

Shoot dry weight, fibrous root dry weight, tap root dry weight (not including crown), and numbers of nematodes within the roots are recorded. Nematode numbers within roots per tube are the most important data. Nematode numbers are obtained by cutting fibrous roots into 1 cm sections. A 1.5 g fresh weight sample is placed in a 10.0 cm x 2.5 cm petri dish containing 20 mL of distilled water, and placed on a horizontal shaker. After 7 days, water is decanted and the nematodes are counted using a stereomicroscope. Alternatives are pan extraction[7] and staining nematodes in the roots with acid fuchsin[2].

Field Screening Methods

Resistant plants (scored 1 or 2) have a large amount of top growth, good crown development, and many fibrous roots with few lesions.

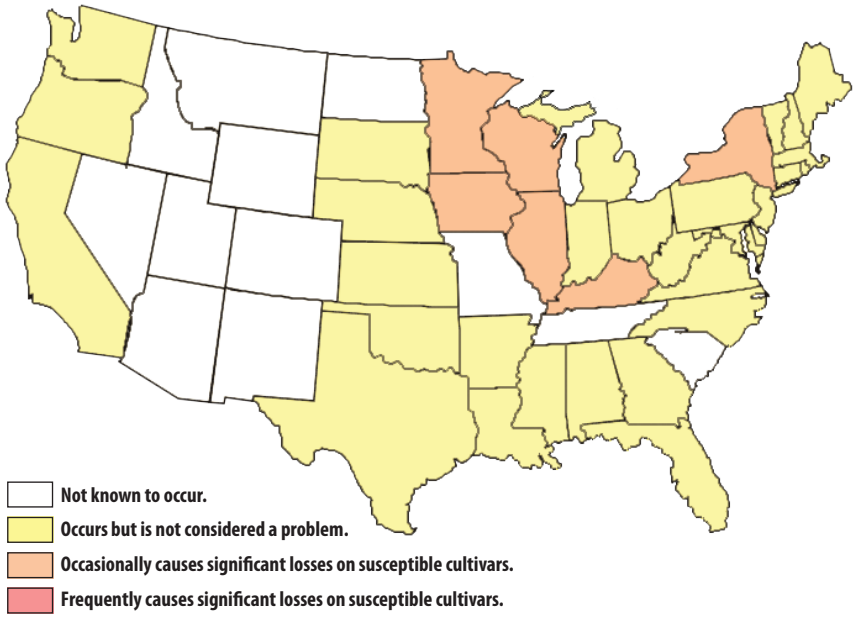
Root Damage Score

1 Resistant..... Normal, healthy root system with abundant fibrous roots
2 Resistant..... Small reduction in the amount of fibrous roots
3 Susceptible..... Moderate reduction in fibrous roots
4 Susceptible..... Total loss of fibrous root system as well as lesions on taproot
5 Susceptible..... Plant dead

CHECK CULTIVARS

Resistant..... MNGRN-16 has an antibiosis level that supports about 60% fewer root lesion nematodes than Baker.
Mod. Resistant... MNGRN-4 is tolerant and has a low level of antibiosis. Germplasm supports about 20% fewer root lesion nematodes per g fresh root weight than Baker[1].
Susceptible..... Baker

DISTRIBUTION AND SEVERITY OF ROOT-LESION NEMATODE



Root-lesion nematode, *Pratylenchus penetrans* Cobb, Filipjev and Schur-Stekhoven[3,9].
(Click on the map above for a larger version.)

HELPFUL INFORMATION

Growth chamber and field studies[4,6] have both been used to characterize resistance. Because antibiosis and tolerance mechanisms are both important, comparing resistance among cultivars is difficult. Field procedures can be used effectively for selecting resistant germplasm but growth chamber tests are required for making claims about antibiosis resistance mechanisms. Nematode number data is inherently variable, thus requiring many replicates (6 to 15) to statistically separate entries.

ALTERNATIVE METHODS TO COMPARE CLONES[8]

Grow rooted clonal stem cuttings for 8 weeks in sterile soil. Transplant plants to 12.5 cm pots containing soil infested with approximately 1,000 *P. penetrans* per pot. Ten weeks later extract nematodes from each root system for 1 week in a mist chamber, and record as nematodes per g fresh root.

REFERENCES

1. Barnes, D.K., J.A. Thies, D.L. Rabas, D.L. Nelson, and D.M. Smith. 1990. Registration of two alfalfas selected for tolerance to the root-lesion nematode. Crop Science 30:751-752.
2. Byrd, D.W., Jr., T. Kirkpatrick, and K.R. Barker. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology 15:142-143.
3. Norton, D.C. (Chairman). 1984. Distribution of plant-parasitic nematode species in North America. A project of the Nematode Geographical Distribution Committee of the Society of Nematologists. 205 pp.
4. Nelson, D.L., D.K. Barnes, and D.H. MacDonald. 1985. Field and growth chamber evaluations for root-lesion nematode resistance in alfalfa. Crop Sci. 25:35-39.
5. Riedel, R.M. 1985. Establishing *Pratylenchus* spp. in monoxenic culture on alfalfa (*Medicago sativa*) callus tissue. In: Zuckerman, B.M., W.F. Mai, and M.B. Harrison (eds.). Plant nematology laboratory manual. pp. 159-162. University of Massachusetts Agric. Expt. Stn., Amherst, MA.
6. Thies, J. A., D. Basigalup, and D. K. Barnes. 1994. Inheritance of resistance to *Pratylenchus penetrans* in alfalfa. Journal of Nematology 26:452-459.
7. Townshend, J.L. 1963. A modification and evaluation of the apparatus for the Oostenbrink direct cottonwool filter extraction method. Nematologica 9: 106-110.
8. Townshend, J.L., and H. Baenziger. 1976. Evidence for resistance to root-knot and root-lesion nematodes in alfalfa clones. Canadian Journal of Plant Science 56:977-979.
9. Townshend, J.L., J.W. Potter, and C.B. Willis. 1978. Ranges of distribution of species of *Pratylenchus* in Northeastern North America. Canadian Plant Disease Survey 58:80-82.