

# ROOT-LESION NEMATODE RESISTANCE

March 1995

*Pratylenchus penetrans* Cobb, Filipjev and Schur-Stekhoven; 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 (conetainers)

**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+ replicates

**Other** ..... Inoculate with *Rhizobium meliloti* Dang

### 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<sup>2</sup>

## INOCULUM CULTURE AND PREPARATION

**Source** ..... Maintain *Pratylenchus penetrans* in monoxenic alfalfa callus<sup>(5)</sup> 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 noninoculated 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<sup>3</sup> 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<sup>(7)</sup> and staining nematodes in the roots with acid fuchsin<sup>(2)</sup>.

### 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<sup>(7)</sup>.

**Susceptible** ..... Baker

