PLANT CULTURE

Greenhouse

Container...............Bench or flat deep enough to allow root development.
Media.....................Steam pasteurized sandy loam soil mixture
Seed Prep.................Scarify, surface sterilize, and germinate on filter paper or seed may be directly planted and plant counts taken.
Planting................Spacing 3.0 x 1.5 cm; may be direct seeded and counted prior to inoculation, particularly for selection.
Temp/Light...............25°C; 12 to 16 hour daylength; supplemental winter light required.
No. of plants ...........100 plants per replication.
No. of Reps..............3 to 5 replications.
Other.....................Inoculate with *Rhizobizlm meliloti* Dang; fertilize and spray as necessary; care in choice of insecticides is needed because some have nematicidal activity.

INOCULUM CULTURE

Source...................Nematodes growing on sterile callus tissue; nematodes are extracted and prepared as a water suspension.
Storage Temp ...........0 to 5°C.
Storage Life..........Maximum of 7 days in water; callus cultures can be refrigerated for several months if callus is in good condition.
Other...................Use a container with a large surface area to provide oxygen exchange.

INOCULATION PROCEDURE

Plant Age..............2 weeks.
Type of Inoc ..........All stages. Concentration 200 nematodes per plant.
Method....................Spray on using mist atomizer or if low concentration is available they may be put on with an eye dropper directly on the cotyledonary node or leaf axil; a second inoculation helps to ensure infection.

INCUBATION

Location...............Greenhouse flats or bench.
Culture...............Maintain soil moisture; encourage crown bud development by trimming top growth several times during the test period. Age to Rate 12 weeks.

RATING

1 Resistant..........No swelling or distortion
2 Resistant..........Slight swelling but no distinct symptoms.
3 Susceptible........Moderate swelling and distortion.
4 Susceptible........Severe swelling and distortion.
5 Susceptible........Severe necrosis or death.

CHECK CULTIVARS

<table>
<thead>
<tr>
<th></th>
<th>Approximate Resistance(%)</th>
<th>Acceptable Range of Reaction(%)</th>
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</thead>
<tbody>
<tr>
<td>Resistant</td>
<td></td>
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<tr>
<td>Vernema**</td>
<td>60</td>
<td>45-70</td>
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<tr>
<td>Lahontan**</td>
<td>40</td>
<td>30-50</td>
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<tr>
<td>Lew</td>
<td>32</td>
<td>25-45</td>
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<tr>
<td>Susceptible</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranger**</td>
<td>5</td>
<td>0-12</td>
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<tr>
<td>Moapa 69</td>
<td>1</td>
<td>&lt;6</td>
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</tbody>
</table>

Values for resistant standards include totals of 1's and 2's.

DISTRIBUTION AND SEVERITY OF STEM NEMATODE

Stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev

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

SOURCE OF INOCULUM

Name....................R. N. Peaden
Address...............USDA-ARS Rt.2 Box 2953A
                     Prosser, WA 99350
Phone ..................509-786-9218
Fax .....................509-786-9370 or 509-786-9277
CORRELATION TO FIELD REACTION

Field reactions will be similar to greenhouse tests. However, results may be more variable or take longer to develop.

RACES

Races of Ditylenchus dipsaci are known to occur. The alfalfa race can parasitize and increase the mortality rate of non-host plants, but no population of the alfalfa race has been found that can reproduce on plants other than alfalfa and sanfoin.

CULTURE OPTIONS

Nematode culture for inoculation can be obtained from plants growing in the greenhouse or the field. It may be necessary to surface sterilize before proceeding with inoculation (3).

INOCULATION AND RANGE OF CONDITIONS

Best results are obtained in a high humidity environment. This can be obtained by covering containers with wetted cheesecloth or clear plastic germination domes. A plastic covering over the cheesecloth minimizes evaporation and creates a high humidity atmosphere. A second inoculation with 200 nematodes per plant should be made two weeks after the initial inoculation.

HELPFUL INFORMATION

Evaporation retarding agents may be useful in the nematode suspension for inoculation preparation. An environment minimizing loss of soil moisture should be maintained. If flood irrigation is used, the soil surface should be as flat and uniform as possible. Avoid uneven watering in flats. Nematodes will become concentrated in low areas.

ALTERNATIVE METHODS

Inoculate with the nematode suspension directly into the row at the time of seeding before covering the seed. This will result in some resistant seedling death when the nematodes feed at the growth point of the seedling embryo.

Mature plants can be screened by adding inoculum directly over the crown buds and covering with soil. Maintain good soil moisture.

REFERENCES

