RACE IDENTITY, PATHOGENICITY AND DAMAGE THRESHOLD OF
TYLENCHULUS SEMIPENETRANS ON SOUR ORANGE IN JORDAN

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Summary. Three populations of Tylenchulus semipenetrans, collected from Northern, Southern, and Central Jordan Valley, were differentiated on 'Valencia' sweet orange, 'Troyer citrange', 'Pomeroy' and 'Rubidoux' Poncirus trifoliata, 'Thompson seedless' grape, and 'Manzanillo' olive. The three populations did not infect olive or P. trifoliate, and consequently were identified as the 'Mediterranean' race. Pathogenicity tests showed that inoculation of 500 juveniles/pot (kg soil) did not affect the growth of sour orange seedlings. However, initial population densities (Pi) of 1,000 to 20,000 juveniles/pot progressively reduced vegetative growth by 9.1 to 30.3% and root weight by 9.7 to 30.9%. Also, Pi exceeding 5,000 juveniles/pot resulted in severe decline symptoms. Plant growth was not adversely affected as long as the resulting nematode infestation stayed below certain critical levels, i.e., 2,450 juveniles/100 cm³ soil, 1,250 eggs, 3,700 juveniles, 390 females, or 5,540 total developmental stages/g root. These levels of infestation represent damage thresholds of this nematode on sour orange seedlings under growth chamber conditions.

Three races of Tylenchulus semipenetrans Cobb, 'Citrus', 'Mediterranean', and 'Poncirus' are currently recognized (Duncan and Cohn, 1990; Whitehead, 1997). They share citrus species as common hosts, but differ in their ability to infect and reproduce on trifoliate orange and olive. Poncirus trifoliata (L.) Raf. is only parasitized by the 'Poncirus' race which reproduces also on grape but not on olive. Olive is infected only by the 'Citrus' race, which also reproduces on the hybrids 'Carrizo' and 'Troyer citrange' as well as on grape. The 'Mediterranean' race, similar to the 'Citrus' race, reproduces very poorly on P. trifoliata, but does not infect olive (Stokes, 1969; Inserra et al., 1980, 1994; Getaci et al., 1981; Verdejo-Lucas et al., 1997).

Pathogenicity of T. semipenetrans and manifestation of decline symptoms are closely related to nematode population density (Cohn, 1972). Citrus nematode populations estimated during the peak growth period of May-July in California, and extracted by Baermann funnel technique, showed that counts of juvenile (J1) nema­todes/100 cm³ soil < 800, > 1,600, and > 3,600 represent low, moderate, and high ranges. Female nema­todes/g root were also used in California to define damage levels, with counts of < 300, > 700 and > 1,400 representing low, moderate, and high levels. If the population exceeds the medium level, tree growth and fruit production are likely to be reduced (Van Gundy, 1984). A population density of T. semipenetrans, greater than 500 J1/100 cm³ soil, as extracted by modified Baermann funnel technique, was considered sufficient to cause economic damage in Pakistan, (Maqbool and Ghaffar, 1986). In India, the maximum population density of T. semipenetrans in citrus orchards was 3,811 nema­todes/100 cm³ soil (processed by Cobb's sieving and de­canteing technique), and 207 females/g root that was stained and dissected (Singh, 1999). Lamberti (1984) reported large numbers of females (1,500/g root) in declining orange trees in Syria where aliquots of roots were stained and dissected.

The terms threshold density and critical infestation rate were used (Cohn, 1972; Bark et al., 1985) to indicate the minimal population density beyond which the nematode would suppress plant growth. Cohn et al. (1969) showed that this level was 4,000 J1/g root, below which the host plant was not affected by T. semipenetrans.

Tylenchulus semipenetrans was found in moderate densities in most citrus groves in the Jordan Valley. According to Al-Qasem and Abu-Gharbieh (1995), most field popu­lation densities, sampled in October and March, were between 488 and 1,352 J1/100 cm³ soil and were extracted by the modified Baermann funnel technique. However, the highest population density recovered by maceration of the roots, was 2,800 J1, and females/g, while 27,000 J1/100 cm³ soil (extracted by the sieving technique) were report­ed by Yousef and Jacob (1994).

The objectives of this research were to determine the race(s) of T. semipenetrans occurring in citrus groves in the Jordan Valley, and infestation levels that represent the damage threshold of the nematode on the sour orange rootstock.

MATERIALS AND METHODS

The local citrus nematode (Tylenchulus semipenetrans) race was determined in a growth chamber by a differential host reaction test. Six different hosts were planted in 15 cm diameter pots containing one kg of sterilized soil
(35% sand, 10% silt, 55% clay; pH 7.5; organic matter 2.05%) and perlite 2:1 v/v, one seedling per pot. The differentials were: 'Valencia' sweet orange, Citrus sinensis (L.) Osb; 'Pomeroy' and 'Rubidoux' trifoliate orange, Poncirus trifoliata; 'Troyer citrange'-hybrid, C. sinensis x P. trifoliata; 'Manzanillo' olive, Olea europea L.; and 'Thompson seedless' grape, Vitis vinifera L.

Three citrus nematode populations were obtained from three separate citrus orchards located in the Northern, Central and Southern Jordan Valley, respectively. These populations were maintained on sour orange (Citrus aurantium L.) seedlings in the growth chamber and used for race determination.

Infected sour orange roots were gently washed to remove adhering soil and placed in aerated water for 24 hours. The newly hatched J₅s were collected on a 325-mesh sieve, washed off, and used for inoculation (Singh and Sharma, 1994). The inoculum in water suspension was pipetted equally into five holes of a 5-cm diameter pot, and placed in a growth chamber at 27±1°C with a photoperiod of sixteen hours light and eight hours darkness for 70 days. The experiment consisted of four replicates in a completely randomized design. At termination, data were taken on the number of J₅/100 cm³ soil using a combination of Baermann trays and sieving methods (Stephan et al., 2000); and number of J₅ and females per g fibrous root < 2 mm diameter comminuted in a blender in 100 ml of water for 10 seconds (Duncan et al., 1993; Singh, 1999).

For the pathogenicity test, three month-old seedlings of sour orange were transplanted into 15 cm diameter pots containing one kg of sterilized soil, as described earlier. The pots were inoculated with different initial population levels (Pi) 500, 1,000, 5,000, 10,000, and 20,000 J₅/pot, using newly hatched J₅s that were surface-disinfected by suspending in 1:1000 w/v copper sulphate solution for 30 minutes (Van Gundy, 1958), while non-inoculated pots served as control.

Treatments were replicated four times in a completely randomized design and pots were maintained in the growth chamber. Plants in all treatments were harvested five months after nematode inoculation. The infestation level for each treatment, based on counting the numbers of various stages of T. semipenetrans per g fibrous root, as well as J₅/100 cm³ soil was determined. Reproduction factor (Rf) was calculated by dividing the final nematode population (Pi = J₅ in soil per plant + eggs, J₅, and females in root per plant) over the Pi (Reddy et al., 1987).

The following horticultural parameters were evaluated for each plant: foliage weight (g), leaf surface area/plant (cm²), number of leaves/plant, stem diameter (mm), root weight (g), and fibrous root mass density or biomass (mg/cm³ soil). The relationship between the citrus nematode infestation levels at termination, and growth decline of sour orange seedlings, were determined. Damage threshold levels for number of J₅/100 cm³ soil; and number of eggs, J₅, females, and total stages (eggs, J₅, and females) per g fibrous root were estimated by determining the level of nematode infestation above which the decline symptoms became severe (Cohn et al., 1965).

RESULTS AND DISCUSSION

At the termination of the differential test, no second stage juveniles or mature females of the three citrus nematode populations were found in the roots of 'Pomeroy' and 'Rubidoux' P. trifoliate or 'Manzanillo' olive roots. However, roots of 'Valencia' sweet orange, 'Troyer citrange' and 'Thompson seedless' grape supported relatively large number of J₅ (1,350-2,350/g root) and females (75-250/g root) of all three populations (Table I). Also, relatively large number of J₅ (227-700/100 cm³ soil) of the three nematode populations, were found in the soil planted to 'Valencia' sweet orange, 'Troyer citrange', and 'Thompson seedless' grape. However, those in the soil of 'Pomeroy' and 'Rubidoux' P. trifoliate, and 'Manzanillo' olive were very low (5-7 J₅/100 cm³ soil). The very low numbers of J₅ encountered in the soil of 'Manzanillo' olive, 'Pomeroy' and 'Rubidoux', in conjunction with absence of female development of the three citrus nematode populations on these hosts, suggest that these J₅s may have survived from the original inoculum used in the test. These findings suggest that the three populations of T. semipenetrans actually belong to the Mediterranean race. Similar results were also obtained by other workers, such as Geraci et al. (1981), and Verdejo-Lucas et al. (1997) who found that many Citrus spp. and 'Troyer citrange' supported high populations of the Mediterranean biotype, whereas P. trifoliate appeared to be resistant. Stokes (1969) and Inserra et al. (1980, 1994) also reported that the Mediterranean biotype reproduced very poorly on P. trifoliate and did not infect olive.

At the termination of the pathogenicity test, results (Table II) indicated that increasing the Pi of T. semipenetrans up to 10,000 J₅/pot, was accompanied by a gradual increase in soil J₅ (3,000/100 cm³ soil), eggs (1,625/g root), and Pf (78,862). At 20,000 J₅/pot, however, these values declined, while J₅, females and total developmental stages in the root increased in proportion to each Pi. Rf ranged from 41.4 at Pi 500, to 47 at Pi 1,000, due to abundance of food, and apparently reduced or absence of competition between nematodes. Then, the Rf gradually decreased to 14.3, 7.9, and 3.4.

The influence of different inoculum densities of T. semipenetrans was also reflected in the various horticultural parameters of sour orange seedlings harvested five months after inoculation (Table III). At termination, the low inoculum density (500 J₅/pot) increased (P = 0.05) the foliage weight, total leaf surface area, number of leaves per plant, stem diameter, root weight, and the
Table I. Reaction of differential hosts to three populations of *Tylenchulus semipenetrans* in the Jordan Valley, 70 days after inoculation.

<table>
<thead>
<tr>
<th>Differential hosts</th>
<th>North population</th>
<th>Central population</th>
<th>South population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>J</em>&lt;sub&gt;2&lt;/sub&gt;/g root</td>
<td>Females/ g root</td>
<td><em>J</em>&lt;sub&gt;2&lt;/sub&gt;/100 cm&lt;sup&gt;3&lt;/sup&gt; soil</td>
</tr>
<tr>
<td>'Valencia' sweet orange <em>Citrus sinensis</em></td>
<td>1400 a</td>
<td>175&lt;sup&gt;1&lt;/sup&gt;a</td>
<td>700 a</td>
</tr>
<tr>
<td>'Troyer citrange' (hybrid) <em>C. sinensis x Poncirus trifoliate</em></td>
<td>600 b</td>
<td>150 ab</td>
<td>402 b</td>
</tr>
<tr>
<td>'Pomery' trifoliate orange <em>P. trifoliate</em></td>
<td>0 c</td>
<td>0 c</td>
<td>7 c</td>
</tr>
<tr>
<td>'Rubidoux' trifoliate orange <em>P. trifoliate</em></td>
<td>0 c</td>
<td>0 c</td>
<td>7 c</td>
</tr>
<tr>
<td>'Manzamillo' olive <em>Olea europea</em></td>
<td>0 c</td>
<td>0 c</td>
<td>5 c</td>
</tr>
<tr>
<td>'Thompson seedless' grape <em>Vitis vinifera</em></td>
<td>1350 a</td>
<td>100 b</td>
<td>341 b</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test at *P* = 0.05.

Table II. Reproduction of *T. semipenetrans* on sour orange seedlings, harvested five months after inoculation with different *Pi* levels.

<table>
<thead>
<tr>
<th>Treatment (J&lt;sub&gt;2&lt;/sub&gt;/pot) (Pi&lt;sup&gt;1&lt;/sup&gt;)</th>
<th>J&lt;sub&gt;2&lt;/sub&gt;/100 cm&lt;sup&gt;3&lt;/sup&gt; soil</th>
<th>No. of developmental stages per g fibrous root</th>
<th>Final population (Pi&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Reproduction factor (Pi&lt;sup&gt;3&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0&lt;sup&gt;4&lt;/sup&gt; d</td>
<td>0 e</td>
<td>0 e</td>
<td>0 e</td>
</tr>
<tr>
<td>500</td>
<td>720 c</td>
<td>325 d</td>
<td>820 d</td>
<td>142 d</td>
</tr>
<tr>
<td>1,000</td>
<td>1596 b</td>
<td>872 c</td>
<td>2300 c</td>
<td>310 c</td>
</tr>
<tr>
<td>5,000</td>
<td>2450 a</td>
<td>1250 b</td>
<td>3700 b</td>
<td>590 b</td>
</tr>
<tr>
<td>10,000</td>
<td>3000 a</td>
<td>1625 a</td>
<td>3920 ab</td>
<td>640 ab</td>
</tr>
<tr>
<td>20,000</td>
<td>2700 a</td>
<td>1420 ab</td>
<td>4215 a</td>
<td>710 a</td>
</tr>
</tbody>
</table>

<sup>1</sup> Pi = Initial population/pot (Kg soil).
<sup>2</sup> Pi = Final population of soil *J*<sub>2</sub> per pot, in addition to the total number of eggs, *J*<sub>2</sub>, and females in the plant root.
<sup>3</sup> Ri = Reproduction factor; final nematode population divided by the initial population.
<sup>4</sup> Values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test at *P* = 0.05.

Table III. Decline of sour orange seedlings inoculated with different density levels of *T. semipenetrans* and harvested five months after inoculation.

<table>
<thead>
<tr>
<th>Treatment (J&lt;sub&gt;2&lt;/sub&gt;/pot)</th>
<th>Foliage weight (g)</th>
<th>Leaf surface area/plant (cm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>No. leaves/plant</th>
<th>Stem diameter (mm)</th>
<th>Root weight (g)</th>
<th>Root biomass (mg/cm&lt;sup&gt;3&lt;/sup&gt; soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.8&lt;sup&gt;1&lt;/sup&gt;ab</td>
<td>980.2 ab</td>
<td>74.5 a</td>
<td>7.0 a</td>
<td>20.6 ab</td>
<td>9.5 a</td>
</tr>
<tr>
<td>500</td>
<td>41.9 a</td>
<td>1028.0 a</td>
<td>79.0 a</td>
<td>7.1 a</td>
<td>23.1 a</td>
<td>10.5 a</td>
</tr>
<tr>
<td>1,000</td>
<td>35.2 abc</td>
<td>890.8 abc</td>
<td>67.5 b</td>
<td>6.0 b</td>
<td>18.6 bc</td>
<td>8.9 ab</td>
</tr>
<tr>
<td>5,000</td>
<td>33.3 bc</td>
<td>802.0 bc</td>
<td>64.5 bc</td>
<td>6.0 b</td>
<td>17.0 bc</td>
<td>8.5 ab</td>
</tr>
<tr>
<td>10,000</td>
<td>29.7 c</td>
<td>783.5 bc</td>
<td>59.0 cd</td>
<td>5.4 bc</td>
<td>15.1 cd</td>
<td>7.9 ab</td>
</tr>
<tr>
<td>20,000</td>
<td>28.0 c</td>
<td>715.3 c</td>
<td>53.0 d</td>
<td>4.9 c</td>
<td>14.2 d</td>
<td>6.4 b</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values in columns followed by the same letter do not differ significantly according to Duncan's multiple range test at *P* = 0.05.
root biomass, by 8, 4.9, 6, 0.7, 12, and 10.5% over the non-inoculated control, respectively. However, the increase of Pi to 1,000; 5,000; 10,000; and 20,000 J/pot was associated with a progressive decline in vegetative and root growth of sour orange seedlings. The greatest deleterious effect was at 20,000 J/pot, where the reductions in foliage weight, total leaf surface area and number of leaves per plant, root weight, and biomass were 27.8, 27.8, 28.9, 30.3, 30.9, and 32.1%, compared to controls, respectively (Table III).

An association was found between nematode infestation levels for all T. semipenetrans developmental stages (Table II) and growth decline of sour orange seedlings (Table III). From these data it is evident that, five months after inoculation with 5,000 J/pot and with final infestation levels of 2,450 J/100 cm³ soil, 1,250 eggs, 3,700 J, 590 females, and total of 5,540 (eggs, J, and females)/g root, little damage was inflicted on sour orange seedlings. Each of these infestation levels represent the damage threshold of this nematode, above which decline symptoms become apparent. Corresponding levels were 4,000 J/g root on sour orange in Palestine (Cohn et al., 1965), and 500 juveniles/100 cm³ soil in Pakistan (Maqbool and Ghaffar, 1986).

It could be concluded that the 'Mediterranean race' of T. semipenetrans is prevalent in Jordan and it constitutes one of the important nematode pathogens on citrus, and consequently effective control measures should be carried out in the infected groves to sustain a longer life span and productivity of citrus trees. Also, the role of the 'Mediterranean race' of T. semipenetrans in citrus decline and control strategies should be further investigated.

LITERATURE CITED


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