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. trifoliata*, 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 cm^3 soil; 1,250 eggs, 3,700 juveniles, 590 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; Geraci *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 (J_2) nematodes/100 cm^3 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 J_2/100 cm^3 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 cm^3 soil (processed by Cobb's sieving and decanting 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; Barker *et al.*, 1985) to indicate the minimal population density beyond which the nematode would suppress plant growth. Cohn *et al.* (1965) showed that this level was 4,000 J_2/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 J_2/100 cm^3 soil and were extracted by the modified Baermann funnel technique. However, the highest population density recovered by maceration of the roots, was 2,800 J_2 and females/g, while 27,000 J_2/100 cm^3 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 trifoliate*; 'Troyer citrange'-hybrid, *C. sinensis* x *P. trifoliata*; 'Manzanillo' olive, *Olea europaea* 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 J_2s 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 (0.8 cm diameter x 5 cm deep) made with a glass rod adjacent to roots in the potting medium around the seedling stem base (Baines *et al.*, 1969). Individual pots were inoculated with 10,000 J_2 *T. semipenetrans* per 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 J_2/100 cm^3 soil using a combination of Baermann trays and sieving methods (Stephan *et al.*, 2000); and number of J_2 and females per g fibrous roots < 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 J_2/pot, using newly hatched J_2s 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 J_2/100 cm^3 soil was determined. Reproduction factor (Rf) was calculated by dividing the final nematode population (Pf = J_2 in soil per plant + eggs, J_2, 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 (cm^2), number of leaves/plant, stem diameter (mm), root weight (g), and fibrous root mass density or biomass (mg/cm^3 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 J_2/100 cm^3 soil; and number of eggs, J_2, females, and total stages (eggs, J_2, 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. trifoliata* or 'Manzanillo' olive roots. However, roots of 'Valencia' sweet orange, 'Troyer citrange' and 'Thompson seedless' grape supported relatively large number of J_2s (1,350-2,350/g root) and females (75-250/g root) of all three populations (Table I). Also, relatively large number of J_2s (227-700/100 cm^3 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. trifoliata*, and 'Manzanillo' olive were very low (5-7 J_2/100 cm^3 soil). The very low numbers of J_2 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 J_2s 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. trifoliata* appeared to be resistant. Stokes (1969) and Inserra *et al.* (1980, 1994) also reported that the 'Mediterranean' biotype reproduced very poorly on *P. trifoliata* 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 J_2/pot, was accompanied by a gradual increase in soil J_2 (3,000/100 cm^3 soil), eggs (1,625 /g root), and Pf (78,862). At 20,000 J_2/pot, however, these values declined, while J_2, 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 J_2/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.

Differential hosts	North population			Central population			South population		
	J_2/g root	Females/ g root	J_2/100 cm^3 soil	J_2/g root	Females/ g root	J_2/100 cm^3 soil	J_2/g root	Females/ g root	J_2/100 cm^3 soil
'Valencia' sweet orange *Citrus sinensis*	1400 a	175[1] a	700 a	2350 a	250a	682 a	1600 a	150 a	560 a
'Troyer citrange' (hybrid) *C.sinensis* x *Poncirus trifoliata*	600 b	150 ab	402 b	350 c	100 b	350 c	650 b	75 b	227 c
'Pomeroy' trifoliate orange *P. trifoliate*	0 c	0 c	7 c	0 d	0 c	7 d	0 c	0 c	7 d
'Rubidoux' trifoliate orange *P. trifoliata*	0 c	0 c	7 c	0 d	0 c	5 d	0 c	0 c	7 d
'Manzanillo' olive *Olea europea*	0 c	0 c	5 c	0 d	0 c	7 d	0 c	0 c	5 d
'Thompson seedless' grape *Vitis vinifera*	1350 a	100 b	341 b	1600 b	150 b	481 b	1500 a	75 b	402 b

[1] 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.

Treatment (J_2/pot) (Pi[1])	J_2/ 100 cm^3 soil	No. of developmental stages per g fibrous root.				Final population (Pf[2])	Reproduction factor (Rf[3])
		Eggs	J_2	Females	Total		
Control	0[4] d	0 e	0 e	0 e	0 e	0 e	0.00 f
500	720 c	325 d	820 d	142 d	1287 d	20714 d	41.4 b
1,000	1596 b	872 c	2300 c	310 c	3482 c	46950 c	47.0 a
5,000	2450 a	1250 b	3700 b	590 b	5540 b	71578 b	14.3 c
10,000	3000 a	1625 a	3920 ab	640 ab	6185 a	78862 a	7.9 d
20,000	2700 a	1420 ab	4215 a	710 a	6345 a	67608 b	3.4 e

[1] Pi = Initial population/pot (Kg soil).
[2] Pf = Final population of soil J_2 per pot, in addition to the total number of eggs, J_2, and females in the plant root.
[3] Rf = Reproduction factor; final nematode population divided by the initial population.
[4] 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.

Treatment (J_2/pot)	Foliage weight (g)	Leaf surface area/plant (cm^2)	No. leaves/ plant	Stem diameter (mm)	Root weight (g)	Root biomass (mg/cm^3 soil)
Control	38.8[1] ab	980.2 ab	74.5 a	7.0 a	20.6 ab	9.5 a
500	41.9 a	1028.0 a	79.0 a	7.1 a	23.1 a	10.5 a
1,000	35.2 abc	890.8 abc	67.5 b	6.0 b	18.6 bc	8.9 ab
5,000	33.3 bc	802.0 bc	64.5 bc	6.0 b	17.0 bcd	8.5 ab
10,000	29.7 c	783.5 bc	59.0 cd	5.4 bc	15.1 cd	7.9 ab
20,000	28.0 c	715.3 c	53.0 d	4.9 c	14.2 d	6.4 b

[1] 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_2/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_2/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, 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 was evident that, five months after inoculation with 5,000 J_2/pot and with final infestation levels of 2,450 J_2/100 cm^3 soil, 1,250 eggs, 3,700 J_2, 590 females, and total of 5,540 (eggs, J_2 and females)/g root, little damage was inflicted on sour orange seedlings. Each of these infestation levels represents the damage threshold of this nematode, above which decline symptoms become apparent. Corresponding levels were 4,000 J_2/g root on sour orange in Palestine (Cohn *et al.,* 1965), and 500 juveniles/100 cm^3 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.

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