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The mediterranean biotypes of *Tylenchulus semipenetrans* in Spanish citrus orchards

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Summary - The reproductive potentials of fourteen citrus nematode (*Tylenchulus semipenetrans*) populations, collected from the principal citriculture areas of Spain, were compared on sour orange and Carrizo citrange. All citrus nematode populations were identified as the mediterranean biotype. Reproductive potentials varied greatly among nematode populations. The infectivity and reproduction of populations collected from the rhizosphere of Troyer or Carrizo citrange rootstocks were higher ($P \geq 0.05$) than those of populations collected from sour orange.

Résumé - ***Le biotype méditerranéen de* Tylenchulus semipenetrans *dans les vergers espagnols de citrus*** - Les capacités de reproduction de quatorze populations du nématode des citrus (*Tylenchulus semipenetrans*) collectées dans la principale zone de citriculture de l'Espagne ont été comparées sur oranger amer et citrange Carrizo. Toutes les populations ont été identifiées comme appartenant au biotype méditerranéen. La capacité de reproduction varie considérablement en fonction des populations. Le pouvoir infestant et le taux de reproduction des populations collectées dans la rhizosphère des porte-greffes citranges Troyer ou Carrizo sont plus élevés ($P \geq 0,05$) que ceux des populations collectées sur oranger amer.

Key-words: biotype, citrus nematode, differential hosts, *Tylenchulus semipenetrans*.

Tylenchulus semipenetrans Cobb occurs worldwide in citrus orchards (O'Bannon & Ford, 1977; Bello *et al.*, 1986; Duncan & Cohn, 1989; Inserra *et al.*, 1994). The nematode has been detected in more than 90% of the Spanish orchards surveyed (Ortuño *et al.* 1969; Tuset & García, 1986; Martinez Beringola *et al.*, 1987; Navas *et al.*, 1992; Verdejo-Lucas *et al.*, 1995). An effective mean of combating this pest is the use of nematode-resistant citrus rootstocks (Baines *et al.*, 1974; Duncan & Cohn, 1989). However, the existence of physiological races or biotypes of *T. semipenetrans* may limit the usefulness of some resistant rootstocks. Biotypes with different reproduction potentials on different plant hosts have been recognized within the species (Baines *et al.*, 1969, 1974; Lamberti *et al.*, 1976; O'Bannon *et al.*, 1977; Inserra *et al.*, 1980; Gottlieb *et al.*, 1986). At present, three biotypes are recognized: citrus, mediterranean and *Poncirus* (Inserra *et al.*, 1980, 1994). The citrus biotype infects many genera in the Rutaceae family, including *Citrus* spp., Troyer and Carrizo citrange (*Citrus sinensis* L. × *Poncirus trifoliata* [L.] Raf.), as well as olive (*Olea europaea* L.), grape (*Vitis vinifera* L.) and persimmon (*Diospyros* spp.), but reproduces poorly on *Poncirus trifoliata* and some hybrids of this genus. The host range of the mediterranean biotype is very similar to the citrus biotype, with the exception of olive. The *Poncirus* biotype reproduces on *Citrus* spp., *P. trifoliata* and hybrids of *P. trifoliata*, as well as grape, but not olive. This biotype has been identified in California, Japan and Israel (Baines *et al.*, 1974; Gottlieb *et al.*, 1986). The citrus biotype occurs in California and Italy (Baines *et al.*, 1974; Lamberti *et al.*, 1976; Inserra *et al.*, 1980). The mediterranean biotype has been identified in mediterranean countries and South Africa (Inserra *et al.*, 1980; Lo Giudice & Inserrra, 1980; Gottlieb *et al.*, 1986; Verdejo-Lucas, 1992). The citrus populations that occur in Argentina, Australia, Brazil, India, and Venezuela may be either the citrus or the mediterranean biotype because they reproduce poorly on *P. trifoliata*, but they have not been tested on olive (Inserra *et al.*, 1994). The continuous cultivation of resistant rootstocks may enhance the development of nematode populations able to reproduce on such rootstocks. For instance, a population of *T. semipenetrans* that reproduced on Swingle citrumelo (*C. paradisi* Macf. × *P. trifoliata*) has been recently reported in Florida (Duncan *et al.*, 1994). This rootstock was reported as resistant to most citrus nematode populations (O'Bannon & Ford, 1977; Lo Giudice & Inserrra, 1980; Kaplan & O'Bannon, 1981).

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Citrus is cultivated in about 268 000 ha in the east and south of Spain with an annual production of approximately 5 million tons of fruit (Anon., 1995). Sour orange (*Citrus aurantium* L.) was formerly the most widespread citrus rootstock in Spain, but the rapid spread of citrus tristeza virus forced the replacement of sour orange with other rootstocks more tolerant to the virus (Cambra, 1994). Troyer and Carrizo citranges, used to establish new orchards, and to replace dead trees, have not adapted well to Spanish soils because of their intolerance of calcareous soils and salinity (Forner & Pina, 1992). Subsequently, a breeding program was initiated at Instituto Valenciano de Investigaciones Agrarias (IVIA), Moncada, Spain, to identify new citrus rootstocks resistant or tolerant to tristeza virus, and adapted to Spanish soils (Forner & Alcaide, 1994).

Information on biotypes of *T. semipenetrans* in Spain is necessary because of its relevance to breeding programs, the choice of rootstock selection, and management practices for use in replant areas. In a previous study, the mediterranean biotype of *T. semipenetrans* was identified in Spain (Verdejo-Lucas, 1992), but only a limited number of populations were tested in that study. The present study expands the previous one with the results of a differential host preference test carried out to identify the biotype of fourteen additional populations of *T. semipenetrans* collected from citrus orchards in Spain. The reproductive potential of the nematode on sour orange and Carrizo citrange also was compared.

Materials and methods

Seeds of *P. trifoliata* cv. Rubidoux, sour orange, Carrizo citrange, and Swingle citrumelo 4475 were germinated in seedbeds. Cuttings of olive cv. Arvequina were provided by a commercial nursery. Seedlings and rooted plants were transplanted singly to 1.5 dm^3 black plastic bags containing a steam sterilized potting mixture of sphagnum peat moss (60%) and quartz sand (40%) (v/v) with the addition of superphosphate at 1.47 g/l and microelements (50 mg Cu SO_4/l, 27.5 mg Zn SO_4/l, 27.5 mg Mn SO_4/l, 46.6 mg Fe SO_4/l). The pH of the potting mixture was adjusted to 6.5 with $CaCO_3$. Plants were maintained for six months in a greenhouse before adding nematodes.

Nematode-infected citrus roots were collected from roots under the canopies of trees in fourteen orchards known to be infested by *T. semipenetrans* (Verdejo *et al.*, 1995). These orchards were located in six provinces representing different citriculture areas in Spain (Table 1). Eight nematode populations were obtained from sour orange, four from Troyer citrange, one from Carrizo citrange, and one from an experimental hybrid of Troyer citrange × Cleopatra mandarin. Roots were washed free of soil, cut into 0.5 cm sections, and eggs and second stage juveniles (J2) extracted by macerating the roots in a food blender at

Table 1. *Origin of the populations of* Tylenchulus semipenetrans *and main characteristics of the citrus orchards.*

Population code	Province	Cultivar / Rootstock	Population densities*	Orchard age	Soil texture	pH
ALM	Almeria	Navel / Sour orange	1900	22	Loam	8.1
CAR	Valencia	Navelina / Troyer citrange x Cleopatra mandarin	5000	12	Clay loam	8.4
CEM	Tarragona	Navelina / Troyer citrange	11100	12	Clay sandy loam	8.2
ELA	Alicante	Navelina/ Sour orange	1000	8	Loam	8.1
GEN	Valencia	Salustiana / Troyer citrange	49400	12	Sandy loam	7.8
MU1	Murcia	Lemon / Sour orange	1335	20	Loam	8.7
MU2	Murcia	Lemon / Sour orange	3060	20	Loam	8.7
MU4	Murcia	Lemon / Sour orange	7140	25	Loam	8.7
SEV	Sevilla	Navelina / Sour orange	15790	25	Clay sandy loam	8.1
SIL	Valencia	Navelina / Sour orange	3770	30	Clay loam	8.4
TEL	Alicante	Navelina/ Troyer citrange	3480	8	Loam	8.3
TRY	Valencia	Washington navel / Troyer citrange	8570	12	Sandy loam	8.3
VAL	Valencia	Salustiana / Carrizo citrange	1980	12	Clay loam	8.6
XAL	Tarragona	Clementina Nules / Sour orange	23300	25	Loam	8.3

* Numbers of eggs + J2/g root in the orchards when root samples were collected.

approximately 1000 rpm for two successive 15 s intervals (McSorley *et al.*, 1984). Eggs + J2 used as inoculum were introduced into the soil near the roots, and plants were inoculated with approximately 10 000 eggs + J2. Nematode eggs accounted for 80% of the total nematodes added. Plants were maintained in a greenhouse for 6 months, and each nematode population-indicator plant combination was replicated five times. The temperature of the greenhouse ranged from 16 to 26 °C during the duration of the test. At harvest, roots were washed free of soil, weighed, and frozen at -20 °C until proceded. When required, roots were thawed at room temperature and all fibrous roots from each plant were processed to extract all life stages of the nematode by blender maceration. Nematodes collected on a 25 µm screen were subjected to centrifugation and sugar flotation (Jenkins, 1964) to remove root debris. The numbers of females and eggs + J2 were expressed per gram of fresh root tissue. The infectivity (females/g root) and reproductive potential (eggs + J2/g root) of the nematode populations on sour orange and on Carrizo citrange were analyzed by analysis of variance. Populations were grouped and compared according to the population source, either sour orange or Troyer / Carrizo citrange. Data were *log (x + 1)* transformed and means were subjected to a Tukey test ($P > 0.05$).

Results and discussion

All nematode populations consisted of the mediterranean biotype, because they all reproduced on sour orange and Carrizo citrange, but not on *P. trifoliata*, olive or Swingle citrumelo (Tables 2, 3). These results confirm a previous report on the occurrence of this biotype, the only one identified to date in Spanish citrus orchards (Verdejo-Lucas, 1992). Nematodes failed to complete their life cycle on olive and Swingle citrumelo. However, a few individuals reached the female stage on *P. trifoliata* inoculated with populations TRY, VAL, ALM, and MU4 but egg production was not observed on this host species. The number of females produced ranged from 0.3 to 1.8 females/g root, and these females were found only in one out of the five replicated plants tested (data not shown). In contrast, populations infected and reproduced on both sour orange and Carrizo citrange, although variability was high and differed between rootstocks (Table 3). In general, more nematodes were recovered from Carrizo citrange than from sour orange roots. The populations TRY and GEN consistently reproduced well on both rootstocks (Table 3).

Populations of *T. semipenetrans* collected from orchards planted on Troyer citrange or Carrizo

Table 2. *Numbers of eggs + juveniles of* Tylenchulus semipenetrans *per g of root on four differential plant hosts 6 months after nematode inoculation.*

Population code	*Poncirus trifoliata*	*Olea europea*	Swingle citrumelo	*Citrus aurantium*
ALM	0	0	0	140 *e*
ELA	0	0	0	114 *ed*
MU1	0	0	0	130 *cde*
MU2	0	0	0	735 *abcd*
MU4	0	0	0	520 *bcde*
SEV	0	0	0	224 *cde*
SIL	0	0	0	420 *abcde*
XAL	0	0	0	2990 *abc*
CEM	0	0	0	60 *de*
GEN	0	0	0	4340 *a*
TEL	0	0	0	334 *bcde*
TRY	0	0	0	2300 *ab*
VAL	0	0	0	115 *de*
CAR	0	0	0	360 *bcde*

Values are mean of five replicated plants. Means in the same column followed by the same letter are not significantly different according to Tukey test ($P \geq 0.05$).

citrange produced higher ($P \geq 0.05$) numbers of females/g root and eggs + J2/g root on greenhouse grown Carrizo citrange than populations collected from sour orange. However, there was no difference in infectivity or reproduction when these populations, collected from either citrange or sour orange, were tested on sour orange in the greenhouse (Table 3). Troyer and Carrizo citrange rootstocks are considered to be susceptible to *T. semipenetrans* (Hutchinson *et al.*, 1972; Lamberti *et al.*, 1976; O'Bannon & Ford, 1977; McCarty *et al.*, 1979; Lo Giudice & Inserra, 1980) although early work described these rootstocks as moderately resistant to the nematode (Baines *et al.*, 1960; Van Gundy & Kirkpatrick, 1964). Baines *et al.* (1969) reported Troyer citrange as being moderately susceptible to the citrus biotype of *T. semipenetrans* in California. The field and greenhouse data provided in this study (Tables 1, 3) suggest that the mediterranean biotype of the nematode has adapted to reproduce well on Troyer and Carrizo citranges. The moderate resistance of the citrange rootstocks may have changed over time in areas where these rootstocks have been widely planted. Both rootstocks have been used in Spain since the early seventies to replace trees on sour orange (Forner & Pina, 1992). The continuous exposure of the nematode to partially resistant rootstocks may enhance its ability to infect and reproduce on these citranges rootstocks.

Although only one biotype of *T. semipenetrans* has been detected so far in Spain, other biotypes may

Table 3. *Infectivity and reproduction of fourteen Spanish populations of* Tylenchulus semipenetrans *on sour orange and Carrizo citrange 6 months after the inoculation of the nematode.*

Population source	Population code	Sour orange		Carrizo citrange	
		Females/g root	Eggs+ J2 /g root	Females/g root	Eggs + J2/g root
Sour orange	ALM	2 *e*[1]	140 *e*	nt[2]	nt
	ELA	22 *bcde*	114 *de*	105 *cd*	1110 *b*
	MU1	15 *bcde*	130 *cde*	nt	nt
	MU2	32 *abcd*	735 *abcd*	90 *bcd*	820 *bd*
	MU4	94 *ab*	520 *bcde*	nt	nt
	SEV	20 *bcde*	224 *cde*	100 *bcd*	1450 *b*
	SIL	12 *bcde*	420 *abcde*	77 *cd*	790 *b*
	XAL	118 *ab*	2990 *abc*	120 *bcd*	1490 *b*
Troyer/Carrizo	CEM	6 *cde*	60 *de*	125 *bcd*	1620 *b*
citrange	GEN	90 *abc*	4340 *a*	1050 *a*	7410 *a*
	TEL	13 *bcde*	334 *bcde*	344 *abc*	1150 *b*
	TRY	185 *a*	2300 *ab*	684 *ab*	25460 *a*
	VAL	3 *de*	115 *de*	72 *d*	515 *b*
	CAR	44 *abc*	360 *cde*	94 *bcd*	430 *b*
	Mean	45.5	904	263.5	3892
	C V	31.7	19.3	17.1	10.8
	Rootstock source[3]				
	S *vs* C	40 *vs* 54	672 *vs* 1394	99 *vs* 456*	1133 *vs* 7232*

[1] Values are mean of five replicated plants. Means in the same column followed by the same letter are not significantly different according to Tukey test ($P \geq 0.05$).

[2] Not tested.

[3] Contrast of host suitability for populations grouped according to whether the source rootstock was sour orange (S) or Troyer/Carrizo citrange (C).

* indicate a significant difference between the compared means at $P \geq 0.05$.

appear since biotype distribution is mainly determined by the host plant rather than by climatic, edaphic or other environmental factors (Baines *et al.*, 1974; Gottlieb *et al.*, 1986). The citrus biotype may appear in areas where new citrus orchards are established on land previously occupied by olive, although the association of the nematode with olive has not been reported in the country. The *Poncirus* biotype is less likely to appear since *P. trifoliata* is not used as a rootstock in Spain due to its sensitivity to high soil pH. Swingle citrumelo is used only in restricted areas of the country because of its poor performance in calcareous soils (Forner & Pina, 1992). The failure of reproduction of the nematode populations tested on Swingle citrumelo 4475 may be due to the lack of exposure of the nematode to this rootstock.

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