

A Classification of *Tylenchulus semipenetrans* Biotypes

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Abstract: The presence of two biotypes of the citrus nematode (*Tylenchulus semipenetrans*) in Italian citrus and olive orchards has been confirmed by comparing host specificity. Host reaction to California biotypes C1 and C3 and to three populations from Arizona, Texas, and Florida indicates that of these five United States biotypes, all except C3 consistently fit biotype C1. These findings, and the results of host-range studies in other countries, show that four biotypes of *T. semipenetrans* are distributed worldwide: the "Poncirus biotype," the "Citrus biotype," the "Mediterranean biotype," and the "Grass biotype." **Key Words:** citrus-root nematode, host specificity, olive tree, *Citrus* spp., *Poncirus trifoliata*.

Six biotypes of *Tylenchulus semipenetrans* Cobb have been identified: four in California (1), one in Florida (11), and one in Italy (5). Five of these biotypes reproduce on *Citrus* spp. and *Poncirus* sp., but the sixth, from Florida, is reported to parasitize only *Andropogon rhizomatus* (11). O'Bannon et al. (8) studied the host range of California biotypes C1 and C3 (1) and three populations from Arizona, Texas, and Florida on *Citrus*, *Poncirus*, and their hybrids. They separated the C3 biotype and called it the "Poncirus biotype" because it readily reproduces on *Poncirus trifoliata* (L.) Raf. According to Baines (1), the California C3 biotype infects grape (*Vitis vinifera* L.) but not olive (*Olea europaea* L.); whereas the C1 and C2 biotypes reproduce on grape; and C1, C2, and C4 reproduce on persimmon (*Diospyros* sp.) and olive, but poorly on trifoliolate orange (*P. trifoliata*). A citrus biotype from Italy does not reproduce on olive or trifoliolate orange but does infect grape and persimmon (5). Recently *T. semipenetrans* was found infecting olive roots in the coastal areas of northern Italy where citrus is not grown.

Attempts to characterize this population in Italy by using differential host plants are reported. Comparative histological observations were also made on the reactions caused by the California C1 and C3, Arizona, Texas, and Florida populations on *Citrus* spp., *Poncirus* sp. and their hybrids, and those caused by an Italian population on

olive. A new classification of *T. semipenetrans* biotypes is proposed.

MATERIALS AND METHODS

To compare the two Italian citrus nematode populations from olive and citrus, each of six 5-month-old sour orange (*Citrus aurantium* L.) and 'Moraiolo' olive seedlings in individual pots were inoculated separately with about 5,000 second-stage juvenile and male nematodes per pot. The seedlings were grown in 20-cm-d clay pots containing a 1:1 v/v mixture of steam-pasteurized alluvial coastal fine sand and peat moss. The inoculum in a water suspension was pipetted into five holes made in the potting medium near the seedling trunk base. The pots were randomized on a greenhouse bench and given normal maintenance until harvest. Eight months after inoculation all seedlings were removed from the pots, and their roots were washed in running tap water to remove adhering soil. Three grams of feeder roots were cut from each seedling and incubated in jars for nematode extraction (13). After 2 days 50 ml of water was added to each jar; the jar was roiled; and a 5-ml aliquot was withdrawn, placed in a dish, and counted. The number of nematodes per gram of fresh root was computed.

The reproductive potential of the olive population was studied with the following hosts: 'Valencia' sweet orange [*C. sinensis* (L.) Osb.]; four trifoliolate orange (*P. trifoliata*)—'Argentina,' 'Pomeroy,' 'Rubidoux,' and 'Rubidoux' Palermo; two hybrids—'Carriizo' citrange (*C. sinensis* x *P. trifoliata*) and 'Swingle' citrumelo (*C. paradisi* Macf. x *P. trifoliata*); *Severinia buxifolia* (Poir.) Ten.; grape (*Vitis rupestris* Scheele); and 'Moraiolo' olive. Seedlings were transplanted in a completely randomized design

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of five replicates in a soil bin (1 m x 1.5 m) infested with four juveniles/ml soil. Eleven months after transplanting, the seedlings were harvested and active juvenile and male nematodes were extracted from 3 g of feeder roots by root incubation. Mature females were removed from roots by comminuting for 30 s in a blender. Then females were collected on a 45- μ m sieve and counted. The number of nematodes per gram of fresh root was computed.

Histology was studied in hosts infected with California biotypes C1 and C3 and the Arizona, Texas, and Florida populations, and in olive seedlings infected with the Italian population. The hosts used were one citrus variety, 'Chase' rough lemon [*C. limon* (L.) Burm. f.]; three varieties of *P. trifoliata*—'Large Flower,' 'Small Flower,' and 'Rubidoux'; two hybrids—'Carrizo' and 'Troyer'; *S. buxifolia*; and 'Moraiolo' olive. Twenty feeder root pieces about 0.5 cm long with adult females attached were selected from each seedling, or, if no mature females were found, root pieces with juveniles were selected. After being fixed in FAA (formaldehyde solution, acetic acid, alcohol) for 48 h, dehydrated in TBA (tertiary butyl alcohol), and embedded in paraffin, the root pieces were sectioned 15 μ m thick and stained with safranin-fast green according to the standard microtechnique procedures (4).

RESULTS AND DISCUSSION

Eight months after inoculation the olive and sour orange seedlings were found infected with the *T. semipenetrans* population from olive (Table 1). Nematode den-

Table 1. Density of *Tylenchulus semipenetrans* detected on olive and sour orange seedlings 8 months after inoculation with Italian populations from olive and citrus.

Experimental host	Number 2° juveniles-males/g of fresh root*	
	Population from olive	Population from citrus
'Moraiolo' olive	200	0
Sour orange	800†	430†

*Values are mean of six replicates.

†Different from 'Moraiolo' olive ($P = 0.05$).

sity was higher ($P = 0.05$) on sour orange than on olive seedlings. The Italian population from citrus reproduced on sour orange seedlings but not on olive seedlings (Table 1), which confirms previous reports (2,5,6,10).

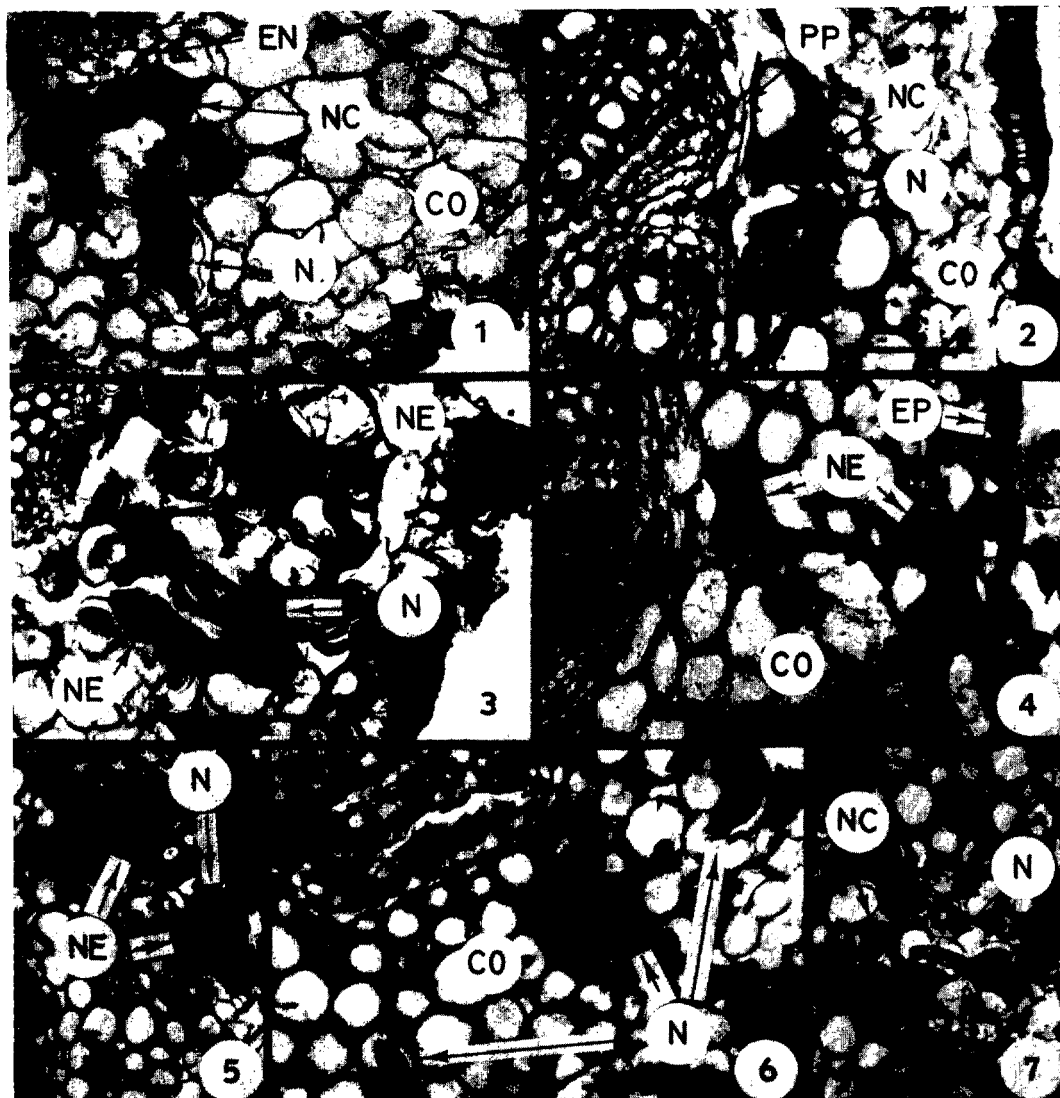
Reproduction by the population from olive was greatest on sweet orange and next on olive and citrange. Reproduction was lowest on *P. trifoliata* selections, 'Swingle' citrumelo, *Severinia buxifolia*, and grape (Table 2). The population from olive has the same *Citrus* spp. and *Poncirus* sp. host range reported by Baines et al. (1) and O'Bannon et al. (8) for biotype C1 and the populations from Arizona, Florida, and Texas. However, it differed with respect to its ability to reproduce on grape.

The parasitic habits of the California, Arizona, Texas, and Florida populations did not vary from that previously described (2,12). Feeding affected the cortical parenchyma, the endodermis, and the pericycle and/or pericyclic periderm, depending upon the root age (Figs. 1, 2). No necrotic reaction to nematode feeding was observed in the cortical nurse cells of rough lemon or 'Carrizo' or 'Troyer' citrange infected with any of the nematode biotypes. Necrosis was observed only in roots harboring dying

Table 2. Reproduction of a *Tylenchulus semipenetrans* population from olive on citrus and non-citrus selections.*

Host	Number of 2° juveniles + males/g of fresh root	Number of mature females/g of fresh root
'Valencia' sweet orange	1554 a	135 a
Trifoliolate orange		
'Argentina'	30 c	1 c
'Pomeroy'	84 c	1 c
'Rubidoux'	16 c	0 c
'Rubidoux'		
Palermo	78 c	2 c
'Carrizo' citrange	678 b	59 b
'Swingle' citrumelo	58 c	3 c
<i>Severinia buxifolia</i>	38 c	0 c
<i>Vitis rupestris</i>	0 c	0 c
'Moraiolo' olive	941 b	46 b

*Values are means of five replicates. Within each column values with different letters are different ($P = 0.05$), according to Duncan's multiple-range test.



Figs. 1-7. Anatomical changes induced by *Tylenchulus semipenetrans* biotypes C1 and C3 and populations from Arizona, Texas, and Florida on rough lemon, *Poncirus trifoliata* selections, and *Severinia buxifolia*. Bar represents 50 μ m. 1) Cross section of rough lemon primary root infected by a Florida citrus nematode population (N = nematode) showing nurse cells (NC) in the cortex (CO) adjacent to endodermal (EN) cells with thick walls. 2) Cross section of rough lemon secondary root infected by a Florida citrus nematode population (N = nematode) showing nurse cells (NC) in the cortex (CO) and necrosis of adjacent pericyclic periderm (PP) cells. 3) Cross section of 'Rubidoux' trifoliate orange root infected by an Arizona citrus nematode population showing necrosis (NE) of epidermal and cortical cells surrounding the nematode (N) (ST = stele). 4) Cross section of *S. buxifolia* root infected by a Texas citrus nematode population showing necrosis (NE) of the epidermal (EP) and cortical (CO) cells at level of nematode penetration points. 5) Cross section of 'Small Flower' trifoliate orange root infected by a juvenile citrus nematode biotype C1, showing necrosis (NE) of the epidermis and cortex around the nematode (N). 6-7) Cross sections of 'Small Flower' trifoliate orange and *S. buxifolia* roots infected by citrus nematode biotype C3, showing several specimens (N) and healthy nurse cells (NC) in the cortex (CO).

mature females, as previously reported by Cohn (2). *Poncirus trifoliata* selections and *S. buxifolia* infected with biotype C1 and the Arizona, Florida, and Texas populations showed necrosis of epidermal and cortical

cells surrounding the nematode body (Figs. 3, 4). This necrotic reaction was observed also in roots inhabited by juvenile stages (Fig. 5) and may have resulted in the nematode's failure to complete its life cycle. In

contrast, fully developed females and normal nurse cells were observed in the roots of these hosts infected with biotype C3 (Figs. 6, 7). Histological changes in parasitized olive roots (Figs. 8, 9) were similar to the host reaction to parasitism observed in the California C1 and the other populations, unlike the California biotype C3 host reactions. Except for biotype C3, the populations from citrus in the United States and those found on olive trees in northern Italy could all be considered to belong to biotype C1 or the "Citrus biotype." From these findings, and on the basis of additional host-range studies with the citrus nematode in several countries (9), it is possible to distinguish four *T. semipenetrans* biotypes distributed worldwide.

The "Poncirus biotype" = biotype C3 (1) reproduces actively on *Citrus* spp. *P. trifoliata*, their hybrids, and grape, but not on olive. This biotype, which probably originated in Japan, appears to be present in California and possibly elsewhere.

The "Citrus biotype" = biotype C1 (1) reproduces very poorly on *P. trifoliata* but infects *Citrus* spp., 'Carrizo' and 'Troyer' citrange, olive, grape, and persimmon. We also include in this biotype California C2 and C4, because they do not consistently re-

produce on all *P. trifoliata* selections (1,7), and the populations from Arizona, Texas, Florida, and the population found on olive in Italy.

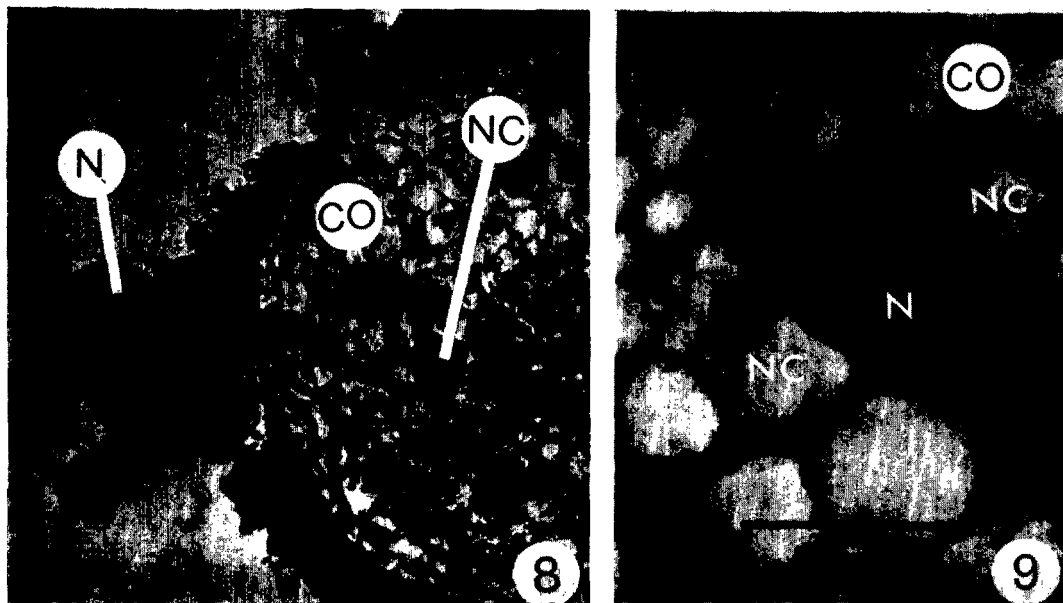
The "Mediterranean biotype" is very close to the "Citrus biotype" and has the same host range, except for olive, which is not a host. This biotype is in all citrus-producing countries of the Mediterranean region and South Africa and perhaps in India.

The "Grass biotype" reproduces only on *Andropogon rhizomatus* and has only been reported in Florida (11).

Probably other *T. semipenetrans* biotypes are present in other areas of the world, and further host-range tests with the plant selections used in this experiment would give a more complete picture of the host specificity of these other biotypes. Knowledge of the biotypes present in the citrus-growing countries is useful in the choice of resistant rootstocks and control measures for use in citrus and olive replant areas.

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Figs. 8-9. Cross section of 'Moraiolo' olive infected with "Citrus biotype" of *Tylenchulus semipenetrans*. Bar represents 30 μ m. 8) Cross section of olive primary root with a sacciform female nematode (N) attached and nurse cells (NC) in the cortex. 9) Cross section of olive primary root with nurse cells (NC) in the cortex (CO) around the nematode (N) head.

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