Determination of *Tylenchulus semipenetrans* Biotype in Zebediela and Champagne, Republic of South Africa

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ABSTRACT

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The biotype of the citrus nematode (*Tylenchulus semipenetrans*) from Zebediela and Champagne Citrus Estates, Republic of South Africa, was determined using 2-year-old differential hosts, namely, grape (*Vitis vinifera* cv. Richter 110), carrizo citrange (*Citrus sinensis* × *Poncirus trifoliata*), rough lemon (*C. jambhiri*), olive (*Olea europaea* cv. Misson), and trifoliolate orange (*P. trifoliata*). The study was conducted under greenhouse conditions with 10 replicates. Nematodes from Zebediela and Champagne were extracted from roots through mechanical shaking, sieving, and incubation in Baermann trays and inoculated at 10,000 and 40,000 eggs and juveniles per plant, respectively. Results demonstrated that the citrus nematode from Zebediela had reproductive factors greater than one on grape, rough lemon, trifoliolate orange, and carrizo citrange, but lower than one on olive. Reproductive factors were less than one for all differential hosts with the Champagne population, with olive having the lowest value. Differential-host tests using Zebediela and Champagne nematode populations suggested that the *T. semipenetrans* biotype is poncirus.

Additional keywords: citrus biotype, Mediterranean biotype

The citrus nematode (*Tylenchulus semipenetrans* Cobb.) inflicts significant losses in most citrus-growing areas through fruit losses and debilitation of trees (4,5). Resistant rootstocks had been an option of choice in the management of population densities of this nematode (4,12). However, in many instances, the existence of *T. semipenetrans* biotypes has reduced the efficacy of resistant rootstocks. Nematode biotypes are morphologically identical but have different reproductive potentials on different hosts. First evidence of *T. semipenetrans* biotypes emerged in the early 1970s (1). Four biotypes were identified first in California (1) which have since been confirmed in Florida (6,11), Israel (7), Italy (10), and Spain (17).

Based on differential-host tests, the four identified biotypes are the (i) citrus, (ii) mediterranean, (iii) poncirus, and (iv) grass biotypes (1,7,9–11,16). The first three biotypes share citrus as a common host but differ in their ability to infect and reproduce on trifoliolate orange (*Poncirus trifoliata* L.) and olive (*Olea europaea* L.). Trifoliolate orange is parasitized only by the

poncirus biotype which does not infect olive, whereas the citrus biotype infects olive but not trifoliolate orange (12). The mediterranean biotype reproduces very poorly on both *P. trifoliata* and olive (17).

Knowledge of the biotypes in citrus-producing regions is useful in the choice of resistant rootstocks in both breeding and production programs (9). The citrus biotype is widespread in California and Italy (1,9), whereas the poncirus biotype is resident in California, Israel, Florida, and Japan (1,6,7,9). The mediterranean biotype had been reported from countries in the Mediterranean basin (7,17). The citrus nematode biotypes in Florida, Arizona, and Texas may be the mediterranean or citrus biotypes because they infect and reproduce poorly on *P. trifoliata* (12).

Without empirical support, Cohn (2) suggested that the *T. semipenetrans* biotype in the Republic of South Africa was the mediterranean biotype, which implied that the biotype did not reproduce on both *P. trifoliata* and olive. This assumption, cross-referenced by others (9,12), resulted in widespread use of *P. trifoliata* rootstocks in the Republic of South Africa for the management of the citrus nematode. However, over the years, the use of *P. trifoliata* had declined from 21% to approximately 5% because the rootstock was unsuitable for the South African citrus industry (13). Under South African conditions, *P. trifoliata* lacked vigor and had poor tolerance to high soil pH and salinity

(13). The objective of this study was to determine the *T. semipenetrans* biotype in Zebediela and Champagne Citrus Estates using differential-host tests.

MATERIALS AND METHODS

The study was conducted under greenhouse conditions at the Horticultural Unit of the University of Limpopo (23°53'10"S, 29°44'15"E). Differential-host plants included approximately 2-year-old grape (*Vitis vinifera* L.), carrizo citrange (*Citrus sinensis* × *P. trifoliata*), rough lemon (*C. jambhiri* Lush), olive, and trifoliolate orange (*P. trifoliata*). Plant roots and tops were pruned and plants transplanted into 30-cm-diameter plastic pots, filled with 2.8 liters of steam-pasteurized sandy soil and Hygromix (Hygrotech, South Africa) at 3:1 (vol/vol). Pots were placed on the greenhouse bench at 30-cm interrow × 60-cm intrarow spacing. Two days after transplanting, plants were fertilized with 5 g of 2:3:2 N:P:K (22% active) + 0.5% Zn.

Zebediela and Champagne nematode populations were used in two separate experiments which ran concurrently. Populations for inoculation were obtained from citrus roots collected from 25 randomly selected healthy looking trees per hectare on rough lemon rootstocks at Zebediela (24°11'S, 29°01'E) and Champagne Citrus Estates (24°50'S, 31°20'E). Root samples weighing approximately 500 g/tree were collected, composited, and transported to the laboratory in cooler boxes. Roots were cut into small pieces, placed into 1,000-ml bottles containing 1% NaOCl solution (8), and mechanically shaken for 10 min. Contents from bottles were passed through a 45-μm-pore sieve nested on a 25-μm-pore sieve, with contents of the latter being washed into a 250-ml beaker. Baermann trays were used to obtain second-stage juveniles (J2) for inoculation (8,14).

Grape, carrizo citrange, rough lemon, olive, and trifoliolate orange host plants were arranged in a randomized complete block design, with 10 replicates. Two months after transplanting, plants for Zebediela Experiment were inoculated with approximately 10,000 J2/plant. Nematodes were applied into 10-cm-deep holes on the cardinal quadrants of trunks using a 20-ml plastic syringe. Plants for Champagne Experiment were inoculated with approximately 40,000 J2/plant because preliminary studies had indicated that the Champagne population was less virulent

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than the Zebediela population (P. W. Mashela, unpublished data).

Two tensiometers per treatment were inserted 20 and 30 cm deep in randomly selected pots to determine the water potential of the soil. Plants were hand-irrigated with 1,000 ml of tap water when readings averaged from 30 to 50 kPa. Ambient day temperatures in the greenhouse were maintained at 25 and 20°C maximum and minimum, respectively.

At harvest, 120 days after inoculation with nematodes, stems were cut at the crown and pots were emptied to remove root systems and immersed in water to free soil particles. Nematodes were separated from 20 g of roots per plant by maceration and blending for 30 s in 1% NaOCl (8), then passed through a 45-µm-pore sieve onto a 25-µm-pore sieve, and contents of the latter were collected for nematode counting. Soil per pot was thoroughly mixed and a 250-cm³ soil sample was collected. Nematodes were extracted from soil samples using the sugar-floatation and centrifugation method (3). Eggs and juve-

niles were counted from a 10-ml aliquot with the use of a stereomicroscope.

Nematode numbers from roots were converted to nematodes per total root system per host, whereas soil nematode numbers were converted to 28,000 cm³ of soil per pot. Reproductive factors (RFs), described as final population/initial population (Pf/Pi) ratios, were subjected to analysis of variance with SAS software (SAS Institute, Cary, NC). Mean separation was achieved with Fisher's least significant difference test. In order to compare the final population densities from the two populations, initial nematode numbers from Champagne were standardized to 10,000 J2 and analyzed using the independent *t* test procedure.

RESULTS

Analysis of variance for RFs of *T. semipenetans* populations from Zebediela and Champagne Citrus Estates on differential hosts had highly significant differences (Table 1). Differential hosts explained 67 and 55% of total treatment variation in

RFs in Zebediela and Champagne populations, respectively. Differential-host tests demonstrated that the citrus nematode populations from Zebediela and Champagne had different RFs. The *T. semipenetans* biotype from Zebediela had RFs greater than one in grape, rough lemon, carrizo citrange, and trifoliolate orange, whereas the RF in olive was lower than one (Table 2). The RFs obtained with *T. semipenetans* populations from Champagne were less than one on all rootstocks, whereas the RF on olive was significantly lower than those on other differential-host plants.

Comparisons of number of nematodes per differential host across locations suggested that populations from Zebediela were always reproducing significantly more than those from Champagne (Table 3). For instance, the numbers of nematode juveniles from Zebediela were approximately six times more than those from Champagne in grape roots and approximately three times more in the soil.

DISCUSSION

An RF provides a basic measurement of the nematode reproduction potential and, therefore, gives good indication of the host status of a plant to the nematode under study (18). All RF values above one indicate susceptibility of the host, whereas those below one indicate resistance, which is expressed in terms of the inability of the nematode to develop and reproduce in the host plant. Using nematode reaction on differential host, olive was consistently

Table 1. Analysis of variance for reproductive factor of *Tylenchulus semipenetans* populations from two citrus-producing regions of South Africa based on numbers in roots and soil of differential hosts in a greenhouse experiment^a

Source of variation	df	Zebediela		Champagne	
		MS	F value	MS	F value
Replication	9	2.03	1.27	0.01	0.88
Differential host	4	5.09	7.14**	0.11	9.14**
Residual	36	0.46	...	0.08	...

^a Values followed by ** are significant at *P* ≤ 0.01.

Table 2. Mean comparison for reproductive factors of *Tylenchulus semipenetans* from two citrus-producing regions of South Africa within roots and soil of differential hosts (*n* = 10)

Differential host	Zebediela				Champagne			
	Total no. of nematodes		Final ^a	RF ^b	Total no. of nematodes		Final ^a	RF ^c
	Root	Soil			Root	Soil		
Grape	4,935	10,786	15,721	1.57	3,060	14,434	17,494	0.44
Carrizo citrange	1,096	9,038	10,134	1.01	3,194	15,109	18,303	0.46
Rough lemon	4,994	10,046	14,007	1.50	8,802	15,008	23,810	0.59
Olive	1,554	6,059	7,613	0.76	423	6,966	7,389	0.19
Trifoliolate orange	2,071	13,362	15,433	1.54	1,312	14,246	15,558	0.39
LSD _{0.05} ^d	2,064	3,352	...	0.39	880	4,020	...	0.10

^a Final population = nematode number in total root and soil.

^b Reproductive factor (RF) = final population (Pf)/initial population (Pi = 10,000).

^c RF = Pf/Pi(=40,000).

^d Least significant difference at *P* ≤ 0.05.

Table 3. Comparisons of populations from two locations on mean population density of *Tylenchulus semipenetans* within roots and soil of differential hosts 4 months after inoculation^a

Differential hosts	Nematode/total root			Nematode/total soil		
	Zebediela	Champagne	<i>t</i> Value	Zebediela	Champagne	<i>t</i> Value
Grape	4,935	765	3.91**	10,786	3,594	7.24**
Carrizo citrange	1,096	333	4.38**	9,038	3,542	7.37**
Rough lemon	3,961	574	3.09*	10,046	3,752	5.41**
Olive	1,554	106	4.88**	6,059	1,742	3.38**
Trifoliolate orange	2,071	328	1.60 ^{ns}	13,362	3,562	4.34**

^a Initial population (Pi; number of nematodes) for Champagne was 40,000 second-stage juveniles. Thus, values were standardized to Pi = 10,000 for the purpose of comparison against Zebediela. Values followed by * and ** are significant at 5 and 1% probability levels, respectively; ns = nonsignificant difference.

resistant to *T. semipenetrans* populations used in this study, whereas other hosts, including trifoliolate orange, were susceptible.

Generally, *T. semipenetrans* biotypes are distinguishable from their RFs on (i) citrus and their relatives such as carrizo citrange, (ii) trifoliolate orange, and (iii) olive. The citrus nematode biotype reproduces on citrus, its relatives, and olive, whereas the mediterranean biotype does not reproduce on trifoliolate orange and olive (7,9,17). Poncirus biotype reproduces on trifoliolate orange but not olive (9,12). On this basis, the *T. semipenetrans* biotype in this study appears to be poncirus biotype, because it reproduced on *P. trifoliolate* but not olive.

In the Champagne population, the RFs on all differential hosts were less than one, suggesting that the contributing factor was the same in all treatments. A model proposed by Seinhorst (15) suggested that maximum rates of nematode reproduction could be achieved only before equilibrium (E) point ($P_i = P_f$). Generally, before E, RFs are higher than one if the host is suitable whereas, after E, the RFs are lower than one. Based on this model, it appears that, for the Champagne population, the inoculation level of 40,000 nematodes per plant was higher than the E point for *T. semipenetrans* on the tested hosts, whereas that of 10,000 nematodes for Zebediela was less than the E. Despite the discrepancy in P_i in the two independent experiments, results in Champagne indicated that all hosts except olive were suitable for the reproduction of *T. semipenetrans*.

In a survey for the *T. semipenetrans* from various fruit orchards in the Western Cape Province, Republic of South Africa, *T. semipenetrans* was detected in grape, persimmon, and various citrus orchards but

not in olive orchards (P. W. Mashela, unpublished). The present investigation suggested that the biotype of *T. semipenetrans* from Zebediela and Champagne, Republic of South Africa, is poncirus. Further studies with molecular markers are needed to validate results of the differential-host test, and to determine the distribution and prevalence of the poncirus biotype in citrus-producing regions of South Africa. The identification of the *T. semipenetrans* biotype in South Africa will be helpful to recommend the most suitable rootstock and further utilization in citrus improvement programs.

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LITERATURE CITED

1. Baines, R. C., Cameron, J. W., and Soost, R. K. 1974. Four biotypes of *Tylenchulus semipenetrans* in California identified and their importance in the development of resistant rootstocks. *J. Nematol.* 6:63-66.
2. Cohn, E. 1976. Reports on investigations on nematode of citrus and subtropical fruit crops in South Africa. Report Citrus Subtropical Fruit Resource Institute, Nelspruit.
3. Coolen, W. A. 1979. Methods for extraction of *Meloidogyne* spp. and other nematodes from roots and soil. Pages 317-329 in: Root-Knot Nematodes (*Meloidogyne* species) Systematics, Biology and Control. F. Lamberti and C. E. Taylor, eds. Academic Press, New York.
4. De Villiers, E. A., and Milne, D. L. 1976. Sitrusaalwurm. *Tylenchulus semipenetrans* Cobb. Boerdery Suid-Afrika H. 5/1976.
5. Duncan, L. W. 2005. Nematode parasites of citrus. Pages 437-466 in: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. M. Luc, R. A. Sikora, and J. Bridge, eds. CABI Publishing, Wallingford, UK.
6. Duncan, L. W., Inserra, R. N., O'Bannon, J. H., and El-Morshedy, M. M. 1994. Reproduction of a Florida population of *Tylenchulus semipenetrans* on resistant citrus rootstocks. *Plant Dis.* 78:1067-1071.
7. Gottlieb, Y., Cohn, E., and Spiegel-Roy, P. 1986. Biotypes of the citrus nematode (*Tylenchulus semipenetrans* Cobb.) in Israel. *Phytoparasitica* 14:193-198.
8. Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Dis. Rep.* 57:1025-1028.
9. Inserra, R. N., Volvas, N., and O'Bannon, J. H. 1980. A classification of *Tylenchulus semipenetrans* biotypes. *J. Nematol.* 12:283-287.
10. Lo Giudice, V., and R. N. Inserra. 1980. Reaction of citrus and noncitrus rootstocks to *Tylenchulus semipenetrans*. *Nematol. Mediterr.* 8:103-105.
11. O'Bannon, J. H., Chew, V., and Tomerlin, A. T. 1977. Comparison of five populations of *Tylenchulus semipenetrans* to *Citrus*, *Poncirus* and their hybrids. *J. Nematol.* 9:162-165.
12. O'Bannon, J. H., and Ford, H. W. 1977. Resistance in citrus rootstocks to *Radopholus similis* and *Tylenchulus semipenetrans* (Nematoda). *Proc. Int. Soc. Citricult.* 2:544-549.
13. Rabe, E., and Von Broembsen, L. A. 1991. Production Guidelines for Export citrus. Vol. 1, Citriculture-Establishment. Co-op Citrus Exchange, Technical Division, S.A.
14. Rodriguez-Kabana, R., and Pope, M. H. 1981. A simple incubation method for the extraction of nematodes from soil. *Nematropica* 11:175-186.
15. Seinhorst, J. W. 1966. The relationships between population increase and population densities in plant-parasitic nematodes. 1. Introduction and migratory nematodes. *Nematologica* 12:157-169.
16. Stokes, D. E. 1969. *Andropogon rhizomatus* parasitized by a strain of *Tylenchulus semipenetrans* not parasitic to four citrus rootstocks. *Plant Dis. Rep.* 52:882-885.
17. Verdejo-Lucas, S. 1992. On the occurrence of the "Mediterranean biotype" of *Tylenchulus semipenetrans* in Spain. *Fund. Appl. Nematol.* 15:475-477.
18. Windham, G. L., and Williams, W. P. 1988. Reproduction of *Meloidogyne javanica* on corn hybrids and inbreds. *Ann. Appl. Nematol.* 2:25-28.