Population Densities of *Tylenchulus semipenetrans* Related to Physicochemical Properties of Soil and Yield of Clementine Mandarin in Spain

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ABSTRACT

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A survey was conducted from April to June 2002 in 62 orchards of clementine mandarin grafted on Troyer or Carrizo citrange rootstock in Catalonia (northeastern Spain) to determine the relationship between physicochemical soil properties and *Tylenchulus semipenetrans* population densities. Soil was analyzed to determine texture, pH, electric conductivity, organic matter content, N, P, K, Mg, calcium carbonate, and calcium oxide. Also, trials were conducted in 2003 and 2004 in three drip-irrigated orchards of clementine mandarin cv. Clemenules (El Pla and Mariclaire) or cv. Hernandina (Martorella) to determine the relationship between citrus nematode densities and yield of mandarin. *T. semipenetrans* was detected in 77% of the surveyed orchards. The number of second-stage juveniles + males in soil was related to N and K soil content ($3.57953 - 0.001305 N + 0.00113 K$, $R^2 = 0.1620$, $P < 0.0157$). The relationship between relative yield of clementine mandarin cv. Clemenules and densities of females per gram of root in spring was described by the Seinhorst damage function model ($0.035 + 0.965 (0.9995)^{(Pi - 287)}$, $R^2 = 0.4782$, $P < 0.0001$).

Additional keywords: gain threshold, plant-parasitic nematodes

Spain is the largest citrus producer of Europe, with an annual production that exceeds 5 million metric tons per year. Citrus crops are cultivated on about 280,000 ha in coastal areas and river valleys in eastern and southern Spain. Citrus is important socioeconomically because it provides a large source of foreign currency and because, due to the generally small orchard size and labor-intensive nature of the crop, it generates employment for many families.

The citrus nematode, *Tylenchulus semipenetrans* Cobb, is the most important plant-parasitic nematode of citrus worldwide. Mature trees can tolerate large numbers of these nematodes before exhibiting lack of vigor or decline symptoms; however, young trees grow poorly if replanted into nematode-infested soils (9). The identification of factors that regulate popula-

tion densities is needed to estimate the potential of the nematode to damage citrus and to cause yield losses. Although *T. semipenetrans* develops in any soil suitable for citrus growth, some physicochemical properties of soil can favor or inhibit nematode population development in a direct or indirect manner. Thus, moderate amounts of clay and silt favor population development (1,4,25,39). The amount of organic matter in soil can favor (27), have no effect (1,25), or inhibit the increase of nematode population densities (25). The pH has a positive relationship with nematode population densities in soil (1,4,19). In saline soils, nematode population on salt-stressed plants increase when salinity decreases during the rainy season (22). Magnesium content in the soil was directly related to fungal parasitism of nematode eggs which, in turn, will affect population densities of *T. semipenetrans* (13).

Damage thresholds, the minimum nematode population densities that reduce tree growth and yield, are influenced by nematode reproduction rate, susceptibility of the rootstock, climatic conditions, soil properties, presence of other pathogens or microbial antagonists, and orchard management practices (7,9). Therefore, the estimate of

damage thresholds must be based on the factors that affect the nematode in a given region. For example, nematicide treatments are recommended when the populations exceeds 400 females per gram of root in February to April or 700 in May to June in California (12), 100 females per gram of root in South Africa (17), or 20 juveniles/cm³ of soil in Cyprus (29). Most studies estimate yield losses due to *T. semipenetrans* to be in the range of 0 to 30% depending on the infestation level and orchard management (2,3,6,10,14,36,37).

In Spain, *T. semipenetrans* is present in more than 80% of the established orchards in the regions of Valencia and Andalucía (1,25,26) but its distribution in the Catalanian region (northeastern Spain) was unknown. This region is the northernmost territory of the country to grow citrus because of restrictions imposed by climatic conditions. In Catalonia, orchards of clementine mandarin (*Citrus clementina* Hort. ex Tan.) represent 77% of the hectare in the region, of which 52% is cultivated with cv. Clemenules. Moreover, information on yield losses caused by *T. semipenetrans* under the conditions prevailing in Spain is unreported. Therefore, the objectives of this study were to (i) determine population densities of *T. semipenetrans* in clementine mandarin citrus orchards in Catalonia (northeastern Spain), (ii) determine their relationship to physicochemical properties of the soil, and (iii) determine the relationship between citrus nematode densities and clementine mandarin yield.

MATERIALS AND METHODS

Nematode survey. Composite soil and root samples were collected from 62 orchards of clementine mandarin grafted on Troyer or Carrizo citrange rootstocks. Orchards were located in four geographical areas of the Baix Ebre and Montsià counties in Catalonia, Spain, and they were representative of orchards located near the coast (25 orchards), inland (15 orchards), riverside (11 orchards), and new citrus-growing areas previously cultivated with olive or almonds (11 orchards). The age of the orchards ranged from 6 to 26 years. All

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orchards were drip irrigated. The orchards were sampled from April to June 2002. From each orchard, about 3 kg of soil and roots were collected with a spade from the top 25 cm of soil from the area wetted by irrigation drippers surrounding eight arbitrarily selected trees, one sample per tree. Soil and roots were combined and mixed, and a sample of about 2 kg was bagged, labeled, and taken to the laboratory for nematode analysis. The remaining 1 kg was used to determine physicochemical properties of soil. Nematode population densities in both soil and roots were assayed for each orchard. Samples were sieved through a 4-mm aperture screen to remove stones and separate roots from soil. Nematodes were extracted from a 500-cm³ soil subsample on Baermann trays that had a screen surface area of 280 cm². Second-stage juveniles (J2) and males that migrated to the water were collected 1 week later, concentrated on a 25- μ m-pore sieve, counted, and expressed as J2 + males per 250 cm³ of soil. Nematodes and eggs were extracted from roots that were carefully washed free of soil and chopped. A 5-g subsample was placed in a food blender jar (Hamilton Beach 1G58200, WA) containing a 0.05% NaOCl solution. The roots were macerated at maximum speed for two successive 15-s intervals (24). The suspension of nematodes and eggs then was sieved through a 74- μ m screen to remove root debris and concentrated on a 20- μ m screen to collect nematode eggs. The numbers of females and eggs were counted and expressed per gram of fresh root tissue. Other plant-parasitic nematodes from soil and roots samples also were counted.

Physicochemical properties. A 1-kg soil sample from the 3 kg taken from each orchard in the nematode survey study was sent to the Laboratory of Analyses and Soil Fertility, Sidamon, Lleida, Spain to deter-

mine soil texture (United States Department of Agriculture classification), soil pH in water (1:2.5, soil:water), electrical conductivity (dS/m), organic matter (%), and content of N (μ g/liter), P (μ g/liter), K (μ g/liter), Mg (μ g/liter), calcium carbonate (%), and calcium oxide (%).

Yield trial. The study was carried out in three drip-irrigated clementine mandarin orchards: cv. Hernandina was planted at the Martorella orchard in Alcanar, Tarragona; and cv. Clemenules was planted at the El Pla orchard in Alcanar, Tarragona and at the Mariclaire orchard in Traiguera, Castellón (Table 1). The orchards were located in the same area near the coast and were selected from the survey study because they had high infestation levels of the citrus nematode. At each orchard, 10 plots consisting of six trees each were marked in arbitrarily selected rows. These trees were of similar size and trees that appeared unhealthy or undersized were avoided (5). Composite soil and root samples were collected from three trees per plot as described for the nematode survey to determine nematode levels before the beginning of the trials. The orchards were sampled thereafter in March and October (El Pla and Martorella) or only in March (Mariclaire) for two consecutive years, 2003 and 2004. At each sampling time, one composite soil and root sample from three trees per plot, one subsample per tree, were collected from the top 25-cm soil layer with a spade as previously described. Nematodes in soil and roots were extracted as indicated in the survey.

To assess yield, mature fruit were collected and weighed in a single picking. Yield was expressed as kilograms per tree. The economic threshold, defined as the nematode population density at which the value of the damage caused is equal to the cost of control (11), was calculated for

each mandarin cultivar and year considering the gain threshold, the value of harvested mandarin that equals the cost of control (30), and the relationship between nematode densities and yield. The cost of controlling the nematode by any of the nematicides presently registered in Spain for citrus (ethoprophos, oxamyl, carbofuran, and sodium tetrathiocarbonate) ranges from 0.38 to 2.5 euros per tree, considering 500 trees/ha. We considered the lower and upper treatment cost of those nematicides for calculation. The average price to growers of 1 kg of mandarins was 0.25 euros in 2003 and 0.17 euros in 2004.

Statistical analyses. All statistical analysis were done using the SAS system V8 (SAS Institute Inc., Cary, NC). A cluster analysis (proc cluster) was used to group orchards according to soil physicochemical properties by the maximum likelihood clustering method. Orchards grouped by the cluster analysis were submitted to a canonical discriminant analysis using the candisc procedure (proc candisc) to elucidate the main factors that determine the differences between the groups. Data from the survey orchards were subjected to correlation analysis (proc corr) to determine the relationship between each physicochemical property of the soil and population densities of females per g of root or J2 + males of *T. semipenetrans*/250 cm³ of soil. Also, multiple regression analysis was performed to determine those combinations of variables that explained variability in nematode population densities. Only data from orchards infested with the citrus nematode were used for the regression analyses. Nematode counts were transformed to log₁₀ (x + 1) and subjected to analyses using the regression procedure (proc reg) with the backward model selection at a significance level of *P* = 0.05.

Densities of females and eggs per gram of root and J2 + males/250 cm³ of soil from the yield trials were compared between sampling times and years in each orchard. Nematode counts were transformed to log₁₀ (x + 1) and means subjected to Student's *t* tests. Regression analysis was used to determine the relationship between mandarin yield and densities of females in roots or J2 + males in soil in spring. Cultivars were analyzed separately because the alternate bearing behavior of cv. Hernandina (20,21). The relative yield for the same regressor, females or J2 + males, was calculated by dividing actual yield by their intercept from the linear regression analysis. A new regression analysis then was performed by cultivar to determine the goodness of fit using nonlinear regression (proc nlin).

RESULTS

Nematode survey. Eight genera of plant-parasitic nematodes were present in 57 of 62 mandarin orchards surveyed in Catalonia. *T. semipenetrans* was detected

Table 1. Main characteristics, history, and physicochemical properties of the soils of the three orchards of clementine mandarin selected for the yield trial

Characteristics	Orchard		
	Martorella	El Pla	Mariclaire
Cultivar	Hernandina	Clemenules	Clemenules
Rootstock	Carrizo Citrange	Carrizo Citrange	Troyer Citrange
Previous crop	Citrus	Citrus	Citrus nursery
Replanted	1994	1996	1989
Trees per hectare	519	500	800
Soil pH (1:2.5 water)	8.1	8.2	8.1
Electric conductivity (dS/m)	0.35	0.32	0.25
Organic matter (%)	4.2	4.5	3.3
Calcium carbonate (%)	18	33	39
Calcium oxide (%)	4	3	5
Nitrogen (μ g/liter)	31	20	17
Phosphorus (μ g/liter) (Olsen)	170	199	117
Potassium (μ g/liter)	404	526	282
Magnesium (μ g/liter)	573	544	416
Sand (%)	43.2	49.4	53.2
Fine silt (%)	13.0	13.6	14.8
Coarse silt (%)	23.4	14.9	16.1
Clay (%)	20.4	22.2	15.9
Soil texture (USDA) ²	Loam	Loam	Sandy loam

² United States Department of Agriculture.

in 48 of the 62 surveyed orchards, which represented a frequency of occurrence of 77%. An orchard was considered infested if citrus nematode females were recovered from the roots.

The genus *Paratylenchus* was found in 24 of the orchards (39%), with population densities that ranged from 21 to 555 nematodes/250 cm³ of soil (167 ± 177 , mean $\pm$ standard deviation). The nematode was recovered mainly from soil samples but also from citrus roots (five orchards). Other plant-parasitic nematodes found, although in low numbers, in the soil included *Helicotylenchus* spp. in 10% of the orchards, *Tylenchorhynchus* spp. in 6% of the orchards, and a criconematid in 5% of the orchards. *Zygotylenchus guevarai* (Tobar Jiménez) Braun and Loof, *Tetylenchus* spp., and *Longidorus* spp. were found in only 1.6% of the orchards.

Physicochemical properties. The cluster analysis grouped the orchards in seven clusters according to the physicochemical properties of the soil. Cluster 1 included 13 orchards located inland (7 orchards) and near the coast (6 orchards); cluster 2 included 7 orchards located near the coast (5 orchards) and in riverside areas (2 orchards); cluster 3 included 12 orchards located inland (5 orchards), near the coast (5 orchards), in riverside areas (1 orchard), and in new citrus-growing areas (1 orchard); cluster 4 included 13 orchards located in new citrus-growing areas (5 orchards), near the coast (4 orchards), inland (2 orchards), and in riverside areas (2 orchards); cluster 5 included 13 orchards located in riverside (6 orchards) and new citrus-growing areas (5 orchards), inland (1 orchard), and near the coast (1 orchard); cluster 6 included 3 orchards located near the coast; and cluster 7 included only 1 orchard, also located near the coast. The clusters differed for potassium, magnesium, organic matter, and clay content of soil (Table 2). The physical and chemical variables were distributed in two dimensions (Fig. 1) that explained 97% of the total variance, 84.5 and 12.4% for the first and second dimension, respectively. A wide range of population densities occurred in the orchards (Table 3), but they did not differ (females per gram of root, $P = 0.38$; eggs per gram of root, $P = 0.49$; J2 + males/250 cm³ of soil, $P = 0.50$) among orchards grouped by common edaphic characteristics. No correlation was found between single physicochemical variables and population densities of females in roots or J2 + males in soil. However, multiple regression analysis showed that the number of J2 + males in soil was related to N and K soil content ($3.57953 - 0.001305 N + 0.00113 K$, $R^2 = 0.1620$, $P < 0.0157$).

Yield trials. Average infestation levels at the start of the trials (spring 2003 samples) were greatest at El Pla and least at Martorella (Table 4). Citrus nematode population densities differed between

sampling times within and between years. In general, they were higher ($P < 0.05$) in spring than autumn and in 2003 than 2004 (Table 4). Females and eggs per gram of root and J2 + males in soil at Martorella in spring were higher ($P = 0.0002$, 0.0063, and 0.0197, respectively) in 2003 than 2004. In autumn, females per gram of root and J2 + males in soil were higher ($P = 0.0044$ and 0.0309, respectively) in 2003 than 2004. At El Pla, females and eggs per gram of root and J2 + males in soil were higher ($P < 0.0001$, 0.0001, and 0.0001, respectively) in spring 2003 than 2004. In autumn 2004, females per gram of root and J2 + males in soil were lower ($P = 0.0073$ and 0.0031, respectively) than 2003. At Mariclaire, densities of J2 + males in soil were higher ($P = 0.0023$) in spring 2003 than 2004 (Table 4).

Regression between nematode population densities and mandarin yield was performed, combining data over years and locations according to clementine cultivars. Yield of cv. Clemenules was related to densities of females per gram of root in spring (79.28 to 0.02712 females per gram of root; $R^2 = 0.469$; $P = 0.0001$) but unre-

lated to J2 + males in soil. In contrast, yield of cv. Hernandina was not related to either females per gram of root or J2 + males in soil, probably due to the alternate behavior of this cultivar. The Seinhorst damage function model described the relationship between relative yield of clementine mandarin cv. Clemenules and densities of females/g root in spring ($R^2 = 0.4782$; $P < 0.0001$; Fig. 2). The tolerance limit, defined as the minimal density below which no measurable yield loss occurs (33), was 287 ± 183 (mean $\pm$ 95% confidence limits) females per gram of root (Fig. 2). The gain threshold, the value of harvested mandarin that equals the cost of control with ethoprophos, was 1.53 kg/tree in 2003 and 2.24 kg/tree in 2004. For sodium tetrathiocarbonate, the gain threshold was 10.03 kg/tree in 2003 and 14.76 kg/tree in 2004. The estimated economic thresholds for ethoprophos was 330 females per gram of root in 2003 and 350 females per gram of root in 2004. Economic threshold for sodium tetrathiocarbonate was 570 females per gram of root in 2003 and 710 females per gram of root in 2004.

Table 2. Statistical significance (in bold) of the physicochemical properties of the soil that explained differences among the 62 orchards of clementine mandarin sampled in Catalonia, northeastern Spain

Variable	R^2	$R^2/(1 - R^2)$	F value	Pr > F
pH	0.0696	0.0749	0.67	0.6713
Electric conductivity	0.1348	0.1558	1.40	0.2306
Organic matter (%)	0.3530	0.5456	4.91	0.0005
N ($\mu\text{g/liter}$)	0.1091	0.1225	1.10	0.3730
P ($\mu\text{g/liter}$)	0.1481	0.1739	1.56	0.1753
K ($\mu\text{g/liter}$)	0.8717	6.7961	61.17	<0.0001
Calcium carbonate (%)	0.1352	0.1564	1.41	0.2289
Calcium oxide (%)	0.1591	0.1893	1.70	0.1380
Mg ($\mu\text{g/liter}$)	0.8240	4.6802	42.12	<0.0001
Sand (%)	0.0907	0.0998	0.90	0.5033
Fine silt (%)	0.0654	0.0699	0.63	0.7059
Coarse silt (%)	0.0734	0.0792	0.71	0.6410
Clay (%)	0.2629	0.3566	3.21	0.0091

Fig. 1. Canonical discriminant analysis of seven cluster groups grouped by physicochemical properties of soil of 62 orchards of clementine mandarin from Catalonia (northeastern Spain). G1 to G7 indicate cluster groups.

Table 3. Number of *Tylenchulus semipenetrans* females and eggs per gram of root and second-stage juveniles (J2) + males/250 cm³ of soil in clementine mandarin orchards located in four geographical areas of Catalonia (northeastern Spain)

Area, orchard number	Females	Eggs	J2 + males	Other plant-parasitic nematodes
Riverside				
1.1	160	12,608	4,674	...
1.2	600	21,440	11,883	...
1.4	8	216	651	<i>Paratylenchus</i> , criconematid
1.6	52	2,184	3,186	<i>Paratylenchus</i>
1.7	20	857	1,925	<i>Paratylenchus</i>
1.8	1,027	16,472	4,633	<i>Paratylenchus</i>
1.9	189	6,742	6,050	<i>Paratylenchus</i>
1.10	1,920	17,040	10,416	<i>Paratylenchus</i>
1.11	5	738	630	...
New citrus area				
2.1	18	936	259	<i>Paratylenchus</i> , criconematid
2.2	450	10,604	2,242	criconematid
2.3	1,152	12,700	23,147	...
2.5	842	23,057	13,579	...
2.6	1,120	19,360	16,320	...
2.7	1,225	10,942	13,293	...
2.8	12	12	138	...
2.9	12	81	148	...
2.10	1,437	17,900	61,200	...
2.11	1,440	33,720	58,266	...
Inland				
3.2	67	2,554	2,128	<i>Paratylenchus</i>
3.4	764	9,130	12,390	...
3.5	672	11,104	11,200	...
3.6	131	1,148	1,696	...
3.7	58	818	3,993	...
3.8	720	31,500	5,900	...
3.9	39	437	1,316	...
3.11	554	11,981	12,500	...
3.12	954	33,326	10,199	...
3.14	55	82	155	...
Near the coast				
4.1	648	9,493	29,941	...
4.2	422	4,805	9,638	...
4.3	400	9,640	6,740	...
4.4	80	1,960	2,280	<i>Paratylenchus</i>
4.5	1,080	56,880	15,555	...
4.7	110	2,355	4,015	<i>Paratylenchus</i> , <i>Helicotylenchus</i>
4.8	640	13,960	11,036	...
4.10	240	1,350	740	<i>Paratylenchus</i>
4.11	111	5,562	9,856	...
4.13	199	9,030	7,744	...
4.15	64	1,392	9,664	...
4.16	360	16,620	21,480	...
4.18	275	9,460	39,678	...
4.19	221	9,448	18,526	<i>Paratylenchus</i>
4.20	224	5,024	18,120	...
4.21	465	11,753	52,000	...
4.22	2,280	63,960	22,500	...
4.23	672	12,608	19,690	...
4.25	952	16,524	38,637	<i>Paratylenchus</i>

DISCUSSION

T. semipenetrans was the most frequent and abundant plant-parasitic nematode in the orchards of Catalonia, as also happens in other citrus-growing regions of Spain (1,25,26).

In this study, the main factors explaining the variability among orchards were organic matter, clay, K, and Mg soil contents. Although none of the physicochemical properties of soil were correlated singly with nematode populations, the multiple regression explained a small amount of variability (16%) in numbers of J2 + males in soil. Thus, nematode population densities in soil were negatively related to concentration of

N and positively related to K content in soil. Nitrogen fertilization in the citrus-growing area of Catalonia consists mainly of ammonium nitrate. Nitrogen can affect the density of nematodes in soils depending on the specific fertilizer. Ammonia has a nematocidal effect (32) that is enhanced when ammonia-releasing fertilizers are combined with alkaline amendments (28). Mangat and Sharman (18) reported that urea applied at the rate of 160 kg/ha prevented *T. semipenetrans* infection and development on rough lemon in a pot test. Potassium content of the soil was positively related to densities of J2 + males in soil, a relationship known to occur in other crops (31,34).

This is the first study conducted in Spain that relates citrus nematode densities with potential yield losses. Gain threshold and economic threshold were calculated as tools for decision making to estimate the amount of yield gain and the density of nematodes that economically justify the use of control methods. The tolerance limit of cv. Clemenules on the citrange rootstocks is 287 females per gram of root. In all, 40% of the mandarin orchards surveyed in Catalonia (25 of 62 orchards) had population densities that exceeded the tolerance limit and potentially may suffer nematode damage if no other limiting factor were present. However, management should not be considered until the gain equals or exceeds the cost of the management options. The estimated economic threshold, considering the price of mandarins of 2003 and 2004, ranged from 330 to 710 females per gram of root, which indicates that approximately 22% of the cv. Clemenules orchards in Catalonia might find management profitable. Nevertheless, these threshold values should be taken cautiously because the data set is small (three orchards and 2 years) and because numerous factors (7,9) affect the citrus nematode-plant interactions in a given region. Additionally, the price of mandarins is subjected to fluctuation; therefore, it is advisable to use an averaged price of the last several years to estimate economic threshold to reduce variability. The estimated economic threshold was similar to those at which nematicide treatments are recommended in California for samples taken in February to April (12), but higher than those recommended at South Africa (100 females per gram of root; 17).

Assessment of yield loss in mature citrus orchards depends on long-term cumulative stress (8); therefore, we propose density of females per gram of root as the best indicator to predict yield losses because this represents the success of the previous nematode inoculum in soil. Furthermore, densities of females per gram of root have been shown to be more consistent than juveniles in soil for comparing seasonality between years and orchards (35), evaluating the efficacy of nematicides (16), and indicating the damage potential to the tree (5,12).

Although the number of females in roots is the best nematode stage to relate damage to the crop, the damaging effect on the yield will result from the damage the nematode already has caused at sampling (March) because the number of fruit has been determined (16). Subsequently, the effect of nematicides on citrus yield will be shown after the first- (23), second- (2,6,36), or third-year applications (14). The efficacy of nematicides has been estimated at 70% on average (6,15,38). Citrus yield increase due to nematicide treatments has been reported between 15 and 30% (8). Although more research will be necessary

Table 4. Fluctuations of population densities of *Tylenchulus semipenetrans* in two clementine mandarin orchards cv. Clemenules (El Pla and Mariclaire orchards), and one cv. Hernandina (Martorella orchard) for two consecutive years

Orchard, year	Season	Nematode population densities ^z		
		Females	Eggs	Juveniles
Martorella				
2003	Spring	656 ± 391 a*	12,296 ± 5,664 a*	8,327 ± 2,303 a*
	Autumn	297 ± 125 b ⁺	2,606 ± 1,869 b	6,934 ± 3,567 a ⁺
2004	Spring	206 ± 92 a	5,704 ± 2,418 a	5,666 ± 2,051 a
	Autumn	150 ± 229 b	1,505 ± 1,488 b	4,027 ± 3,526 a
El Pla				
2003	Spring	1,557 ± 512 a*	31,015 ± 7,027 a*	27,236 ± 5,714 a*
	Autumn	621 ± 257 b ⁺	6,610 ± 4,406 b	12,956 ± 4,276 b ⁺
2004	Spring	421 ± 146 a	7,475 ± 2,002 a	8,064 ± 2,810 b
	Autumn	296 ± 184 a	4,352 ± 2,942 b	20,265 ± 4,195 a
Mariclaire				
2003	Spring	534 ± 278	na	29,947 ± 16,314*
2004	Spring	425 ± 177	na	13,696 ± 8,383

^z Population is measured per gram of root for females and eggs and per 250 cm³ of soil for juveniles. Data were transformed to log₁₀ (x + 1) before analysis. Values are back-transformed mean ± standard deviation of 10 replications. Data for each orchard and year followed by the same letter are not significantly different (*P* < 0.05) according to Student's *t* tests. Data for each orchard followed by * or + are significantly different (*P* < 0.05) in spring or autumn between years, respectively, according to Student's *t* tests; na = data not available.

Fig. 2. Seinhorst damage function model: relationship between relative yield of clementine mandarin cv. Clemenules and population densities of females of *Tylenchulus semipenetrans* per gram of root in spring.

to substantiate economic threshold values, they can be taken as a benchmark and will be helpful to provide guidance to the growers.

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