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Seasonality of *Tylenchulus semipenetrans* Cobb and *Pasteuria* sp. in Citrus Orchards in Spain

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Abstract: Population densities of the Mediterranean biotype of *Tylenchulus semipenetrans* were monitored in soil and citrus roots at 3-month intervals for 3 consecutive years in four citrus orchards in the provinces of Tarragona (Amposta and Xalamera) and Valencia (Moncada and Cárcer). Nematode population densities in soil peaked once a year in April or July depending on the orchard and year. Numbers of females per gram of root increased once or twice each year. The maximum density of eggs per gram of root was recorded in April at Xalamera and Cárcer orchards, but there was no recognizable peak in the other two orchards. Numbers of nematodes in soil, females or eggs per gram of root, or eggs per female were correlated with either temperature or rainfall in the sampling month, 1 month before sampling, or in the second preceding month, depending on the orchard. Members of the *Pasteuria* group were present in the four citrus orchards, and a positive relationship occurred between nematodes in soil and bacterial parasitism ($r^2 = 0.75$) in the orchard at Amposta.

Key words: citrus nematode, *Pasteuria* spp., population dynamics, *Tylenchulus semipenetrans*.

Spain is the largest producer of citrus in Europe. About 280,000 ha of citrus is cultivated in this region, with an annual production that exceeds 5 million tons per year. Oranges, mandarins, and lemons, produced mainly for fresh-market consumption, account for 93% of the citrus production. The citrus nematode, *Tylenchulus semipenetrans* Cobb, causes the disease known as citrus slow decline, which limits citrus production across a range of edaphic and environmental conditions, particularly in replant situations (Duncan and Cohn, 1990). This nematode is abundant in citrus orchards in Spain and infests more than 80% of the established orchards (Bello et al., 1985; Martinez Beringola et al., 1987; Navas et al., 1992; Ortuño Martinez et al., 1969; Tuset and García, 1986; Verdejo-Lucas et al., 1995), although its economic importance has not been determined. It most likely may be assumed, however, that damage to citrus in Spain due to this nematode is similar to that reported in other countries with similar climatic and environmental conditions where yield losses have been estimated to be around 14% (Bello et al., 1996).

Characterizing the population dynamics of the citrus nematode in a production area is necessary to estimate the potential of the nematode to damage citrus, identify factors that regulate population densities, and optimize control strategies. Factors that regulate nematode densities interact and include plant host, tree phenology, antagonistic organisms, climatic conditions, and physical and chemical properties of the soil (Baines et al., 1978; Duncan and Cohn, 1990; Duncan, 1999). This study describes seasonal fluctuations in population densities of *T. semipenetrans* in four naturally infested citrus orchards during 3 consecutive years. Density-dependent abundance of microorganisms that parasitize nematodes has been reported (Verdejo-Lucas, 1992). Therefore, we also investigated seasonality in number and proportion of juveniles with spores of *Pasteuria* spp.

Materials and Methods

Seasonal fluctuations in population densities of *T. semipenetrans* in soil and roots were monitored in four commercial citrus orchards naturally infested with the Mediterranean biotype of the nematode (Verdejo-

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Lucas et al., 1997c). The orchards were in eastern Spain—two in the province of Tarragona (Amposta and Xalamera) and the other two in the province of Valencia (Moncada and Cárcer), about 200 km south of Tarragona. Orchard characteristics and history are indicated in Table 1. The Cárcer orchard was also an experimental site used to test the agronomic performance of new citrus hybrid rootstocks from the breeding program of the Instituto Valenciano de Investigaciones Agrarias (IVIA). In this orchard, the sampled trees were on rootstock selections from crosses of Cleopatra mandarin (*Citrus reshni* Hort.) x Troyer citrange [*C. sinensis* (L.) Osbeck. x *Poncirus trifoliata* (L.) Raf.] (03.02.12), Troyer citrange x Cleopatra mandarin (02.03.49), Cleopatra mandarin x *P. trifoliata* (03.01.09), and of Troyer citrange x common mandarin (*C. deliciosa* Ten.) (02.04.18). The grafted scions were Navelina, except that Washington Navel was grafted to the selection 03.02.12. The selected rootstocks had shown susceptibility to the citrus nematode in greenhouse tests (Verdejo-Lucas et al., 1997b, 2000), and nematode analysis before the start of the study revealed that they supported similar nematode densities at the orchard (date not shown) and were considered as replications.

Composite soil samples were collected with a shovel from the top 20-cm soil layer from the same five trees, which were arbitrarily selected, in January, April, July, and October for 3 consecutive years starting in April 1994. In the Cárcer site, only three trees of each selection were sampled due to the limited number of available trees. Samples from individual trees consisted of about 1 kg of soil and roots collected at the edge of the canopy in the flood-irrigated orchards or at the inner edge of the bulb area wetted by the drippers in the drip-irrigated orchards. The sampled areas were marked to avoid resampling the same spot in successive samples. Samples from each orchard were combined, mixed, and sieved through a 4-mm screen to remove stones and separate soil from roots. Nematodes in soil (second-stage juveniles and males) were extracted from a 250-cm^3 subsample by differential sieving and centrifugation and sugar flotation (Jenkins, 1964). Roots were washed carefully, blotted dry, and weighed. Nematodes were extracted from a 5-g root subsample by macerating the roots in a food blender at maximum speed for two successive 15-second intervals (McSorley et al., 1984). The nematode suspension was then sieved through a 74-µm screen to remove root debris, and nematodes were concentrated and collected on a 25-µm screen. The

TABLE 1. Characteristics and history of four citrus orchards sampled to monitor seasonal fluctuations in population densities of *Tylenchulus semipentrans* for 3 consecutive years.

	Province			
	Tarragona		Valencia	
Characteristics	Amposta	Xalamera	Moncada	Cárcer
Rootstock	Troyer citrange	Sour orange	Troyer citrange	03.02.12, 02.03.49, 01.03.9, 02.04.18[a]
Scion	Navelina	Clementina Nules	Washington Navel	Navelina
Soil texture	Sandy clay loam	Loam	Sandy loam	Clay loam
pH	7.83	7.93	8.29	8.37
Orchard age	12	25	12	12
Irrigation system	Drip	Flooding	Drip	Flooding
North latitude	40°41′	40°55′	39°32′	39°04′
East longitude	0°35′	0°30′	0°24′	0°34′
Average annual temperature	17.3	18.0	16.7	19.3
Average rainfall (mm/year)	493	501	313	428
Juveniles/250 cm^3 soil[b]	11,100	23,300	1,980	5,000

[a] Selections from crosses of Cleopatra mandarin × Troyer citrange (03.02.12), Troyer citrange × Cleopatra mandarin (02.03.49), Cleopatra mandarin × *P. trifoliata* (03.01.09), and of Troyer citrange × common mandarin (02.04.18).

[b] Before initiation of the study.

number of females and eggs were counted and expressed per gram of root.

Seasonality of *T. semipenetrans* with spores of the *Pasteuria* group was determined in the Amposta orchard. A random sample of 30 nematodes (J2 and males) collected from the soil samples was microscopically examined at ×400 at each sampling date, and nematodes with spores adhering to the cuticle and number of spores per nematode were recorded.

The orchards were managed by growers following standard practices in the area for tree management and disease and pest control. Nematicides were not applied during the study period in any of the orchards. Citrus trees received supplemental drip or flood irrigation (Table 1) as needed from spring to autumn. Meteorological information was provided by weather stations located near each orchard.

Statistical analysis: SAS statistical procedure (SAS Institute Inc., Cary, NC) was used for data analyses. Data on number of nematodes in soil, females and eggs in roots, and eggs per female were transformed to [log $(x + 1)$] and subjected to correlation analysis to determine if seasonal fluctuation in the number of nematodes in soil, females or eggs in roots, or eggs per female were related between orchards. Correlation analysis was also used to determine the relationship between seasonal changes in densities of each nematode life stage and either mean monthly temperature (T) or rainfall (R) in the sampling month, 1 month before sampling (T-1, R-1), or in the second preceding month (T-2, R-2) to the month in which sampling was conducted. Rainfall data were transformed to [log $(x + 1)$] before analysis. Analysis of variance was performed to detect significant seasonality in the population dynamics of *T. semipenetrans* in each orchard from year to year (April to January). The relationship between total nematodes in soil and the number with *Pasteuria* spores was determined by regression analysis.

Results

Tylenchulus semipenetrans was the only plant-parasitic nematode detected in soil from the orchards at Moncada or Xalamera. In addition to the citrus nematode, the soil from the Amposta orchard contained *Tylenchorhynchus* spp. and *Ditylenchus* spp., and the soil from Cárcer contained *Gracilacus* spp., *Helicotylenchus* spp., *Heterodera* spp., *Pratylenchus* spp., *Paratylenchus* spp., and *Tylenchorhynchus* spp. Seasonal fluctuations in densities of these nematodes are not reported because specimens occurred infrequently and in low numbers.

Numbers of nematodes in soil generally increased in April or July in three of the orchards in the first 2 years of this study (Fig. 1A). No seasonality was observed at Cárcer. Densities of nematodes in soil changed very little during the third year of the study. Seasonal changes in nematode population density in soil were correlated between orchards only at Xalamera and Moncada ($r = 0.628$, $P = 0.028$). Numbers of females per gram of root peaked in July in all four orchards in 1994 (Fig. 1B). In 1995, the number of females increased in April in the orchards at Moncada and Xalamera, and in July at Amposta and Cárcer. Densities of females in roots peaked twice (April and October) in 1996 at the four orchard sites, although the proportional increase was lower than in previous years. This increase was more pronounced in the orchards at Moncada and Xalamera.

Seasonal fluctuation in the number of females per gram of root and eggs per female were positively correlated between orchards (Table 2). Numbers of eggs per gram of root increased consistently in April at Xalamera and at Cárcer, although to a much lower extent (Fig. 1C). Peaks were detected in October (1994 and 1996) and January (1996) at Amposta; there was no recognizable peak in egg production at Moncada. These differences in patterns were most likely the reason that seasonal changes in the number of eggs per gram of root were not correlated between orchards. An inverse relationship was found between the number of females per gram of root and the number of eggs produced per female at Moncada ($r = -0.927$, $P = 0.0001$), Cárcer ($r = -0.85586$, $P = 0.0004$),

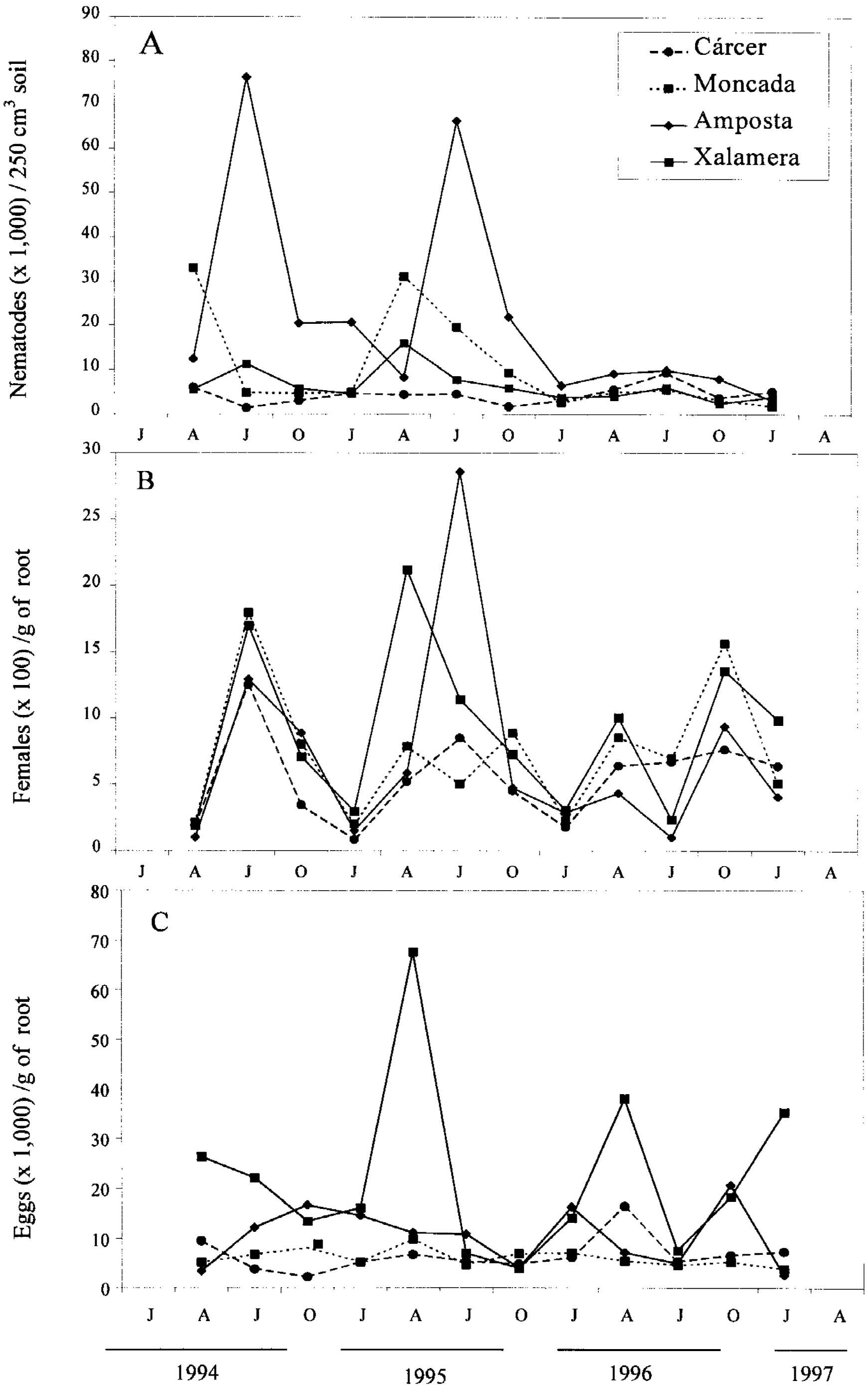

FIG. 1. Changes in population densities of *Tylenchulus semipenetrans* in four citrus orchards in Spain from April 1994 to January 1997. A) Seasonal fluctuations of second-stage juveniles in soil. B) Seasonal fluctuation of females per gram of root. C) Seasonal fluctuations of eggs per gram of root.

Amposta ($r = -0.752$, $P = 0.0048$), and Xalamera ($r = -0.580$, $P = 0.0478$).

Nematode densities in soil and roots from year to year did not differ significantly at any of the four orchards although some trends and relative differences were observed (Fig. 2). Lower numbers of nematodes in soil were observed at Amposta at the end of this

TABLE 2. Pearson correlation coefficients (*r*) and probability (*P*) of seasonal population fluctuations of females per gram of root and eggs per female in four citrus orchards in Spain during 3 consecutive years.

	Cárcer	Amposta	Xalamera
		Females/g root	
Moncada	$r = 0.829$, $P = 0.001$	$r = 0.559$, $P = 0.059$	$r = 0.736$, $P = 0.006$
Cárcer	—	$r = 0.633$, $P = 0.027$	$r = 0.710$, $P = 0.009$
Amposta		—	$r = 0.776$, $P = 0.003$
		Eggs/female	
Moncada	$r = 0.798$, $P = 0.002$	$r = 0.565$, $P = 0.055$	$r = 0.613$, $P = 0.033$
Cárcer	—	$r = 0.656$, $P = 0.024$	$r = 0.754$, $P = 0.004$
Amposta		—	$r = 0.662$, $P = 0.019$

3-year study. Eggs in roots tended to increase at Xalamera, but not clear trend was observed in the number of females. In general, there was little change in the number of females in the four orchards during this study. Densities of *T. semipenetrans* tended to be lower in the orchard with the experimental rootstocks (Cárcer) than in the others, and there were few annual changes in this site.

Juveniles, males, females, and eggs of the citrus nematode were recovered from soil and root samples throughout the year. In general, the mean monthly temperature was above 16 °C from April–May to October–November, and the lowest temperature was always recorded in January in the four sites (Fig. 3). The mean temperature tended to be higher at Cárcer than in the other sites; the lowest values were registered at Amposta. The rainfall season usually occurred during the fall, although abundant precipitation was recorded in winter 1995 and both spring and winter 1996 (Fig. 3). The longest

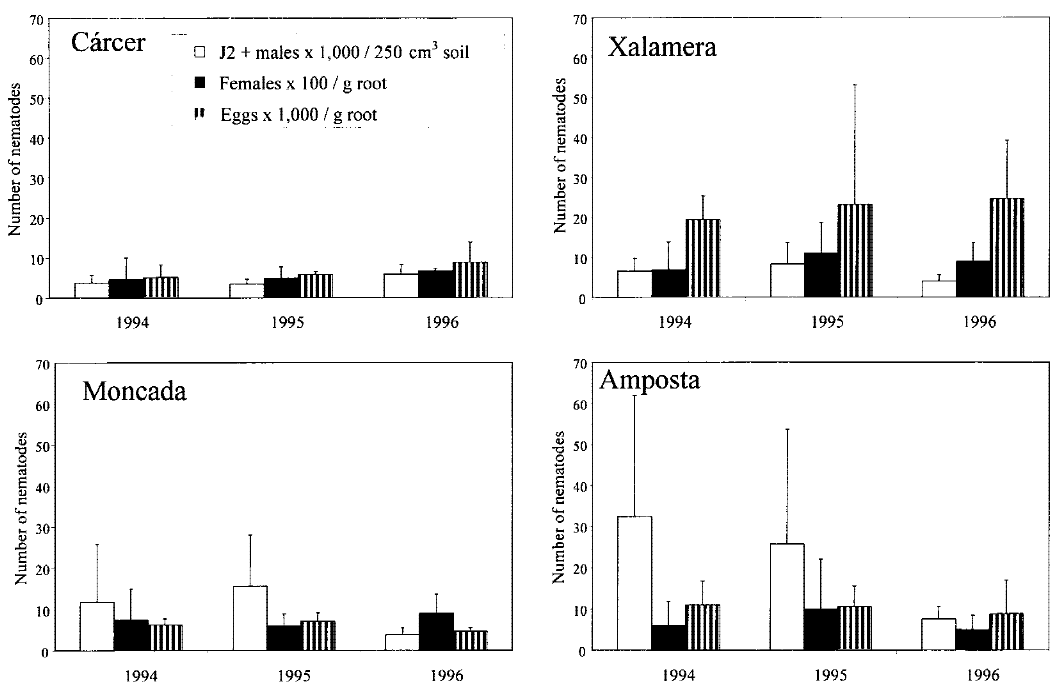

FIG. 2. Annual progression of the population densities of *Tylenchulus semipenetrans* in soil and roots from year to year in four citrus orchards in Spain for 3 consecutive years starting in April 1994. Each value is mean ± standard deviation of four sampling dates per year.

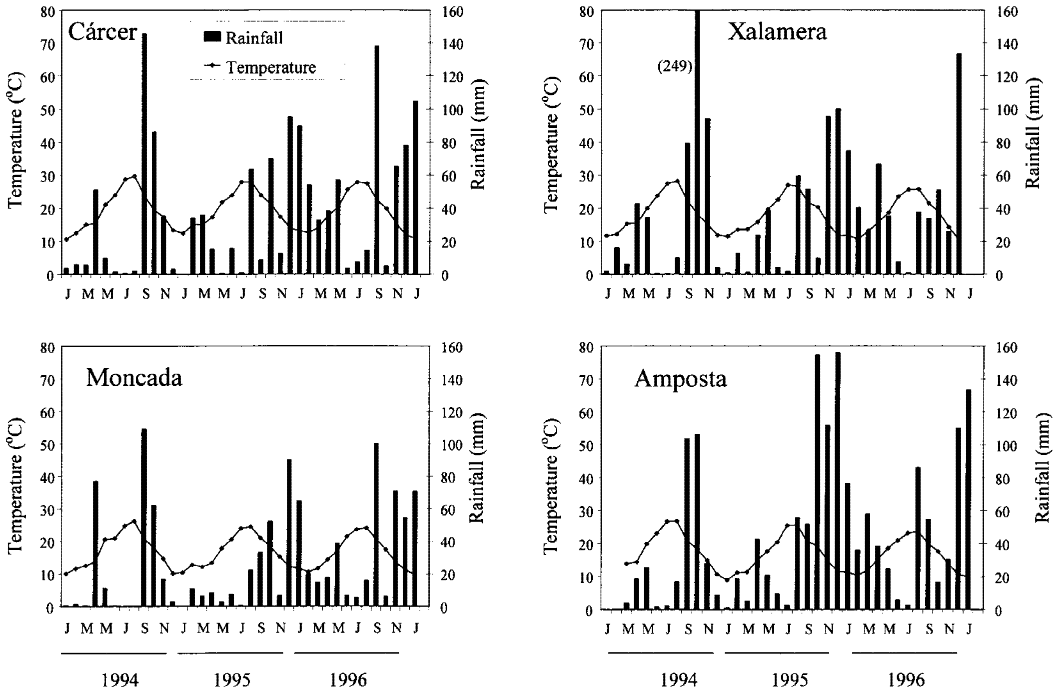

FIG. 3. Mean monthly temperature and monthly rainfall recorded in four citrus orchards in Spain for 3 consecutive years.

dry period was recorded at Moncada; the shortest dry period occurred at Amposta (Fig. 3).

The mean monthly temperature during the sampling month (T) or either 1 month (T-1) or 2 months (T-2) prior to sampling was related to the population dynamics of the citrus nematode in some, but not all, of the orchards, depending on the nematode life stage. Seasonal fluctuation in number of nematodes in soil was positively correlated with T and T-1 at Amposta (Table 3), but nematodes in roots were not correlated with these temperature means. At Moncada, sea-

TABLE 3. Pearson correlation coefficients (r) and probability (P) of seasonal changes in densities of nematode life stages and temperature[a] in four citrus orchards in Spain during 3 consecutive years.

	Temperature[b]		
	T	T-1	T-2
J2 + males/250 cm^3 soil			
Amposta	$r = 0.72$, $P = 0.009$	$r = 0.64$, $P = 0.026$	
Females/g root			
Moncada		$r = 0.61$, $P = 0.033$	
Cárcer	$r = 0.69$, $P = 0.013$		
Eggs/g root			
Cárcer		$r = -0.59$, $P = 0.040$	$r = -0.74$, $P = 0.006$
Xalamera		$r = -0.60$, $P = 0.038$	$r = -0.63$, $P = 0.028$
Eggs/female			
Moncada	$r = -0.58$, $P = 0.045$	$r = -0.62$, $P = 0.032$	
Cárcer	$r = -0.74$, $P = 0.005$	$r = -0.76$, $P = 0.004$	$r = -0.59$, $P = 0.039$
Xalamera	$r = -0.65$, $P = 0.021$	$r = -0.69$, $P = 0.012$	$r = -0.65$, $P = 0.029$

[a] Only significant correlations included.
[b] Mean monthly temperature at the sampling month (T), 1 month before sampling (T-1), or in the second preceding month (T-2) to the month in which sampling was conducted.

sonal fluctuation in the number of females per gram of root was positively correlated with T-1, whereas the number of eggs per female was negatively correlated with T and T-1. At Cárcer, seasonal fluctuation in the number of females and eggs per gram of root was positively correlated with T, and inversely correlated with T-1 and T-2, respectively. Also, the number of eggs per female was inversely related with T, T-1, and T-2. At Xalamera, seasonal fluctuation in the number of eggs per gram of root was inversely related to T-1 and T-2, and the number of eggs per female was inversely related to T, T-1, and T-2. The rainfall recorded the month before sampling was inversely related to nematodes in soil at Xalamera ($r = -0.723$, $P = 0.0079$) and Moncada ($r = -0.59$, $P = 0.043$).

Spores of *Pasteuria* were found on the cuticle of the vermiform stages of *T. semipenetrans* at Amposta on 9 of the 12 sampling dates, and the percentage of individuals with spores tended to be higher in July all 3 years (Table 4). There was a positive relationship between number of nematodes in soil and those with spores attached ($r^2 = 0.75$, $P = 0.00027$). Bacterial microcolonies and spores were found filling the body cavity of J2 and males in July 1995 and in October 1994, 1995, and 1996. More than 75% of the citrus nematodes with spores had 1 to 2 spores attached. In the remaining orchards, spores attached to the nematode cuticle were observed only three times at Cárcer (April 1994, January and April 1996) and once at Moncada (April 1996). In the Xalamera orchard, spores filling the body cavity of J2 were found twice (April and October 1995), but they were not observed attached to the nematode cuticle at any other sampling date. Apparently, this *Pasteuria* population completes its life cycle within the vermiform stages of the nematode because no spores were observed on the cuticle or within the adult female stage on any sampling date in any orchard.

Discussion

The results of this study in orchards with different characteristics and environmental conditions show the complexity of the population dynamics of *T. semipenetrans*. The population from Moncada showed higher infectivity and reproductive potential than the others on sour orange and Carrizo citrange in a pot test (Verdejo-Lucas et al., 1997c). However, the four populations have been identified as Mediterranean biotypes and did not show different behavior in the field.

The life cycle of *T. semipenetrans* is regulated by host phenology and seasonal changes in the soil environment (Duncan and Cohn, 1990; Duncan, 1999). One or two periods of nematode population growth per year were detected in this study depending

Table 4. Population densities of *Tylenchulus semipenetrans* and percent of individuals with *Pasteuria* spores adhering to the nematode cuticle in a citrus orchard at Amposta, Tarragona, Spain, from April 1994 to January 1996.

Year	Month	No. J2 + males/ 250 cm^3 soil	No. individuals with spores adhered	Percentage with spores (%)
1994	April	12,310	1,230	10
	July	76,330	12,213	16
	October	20,440	1,635	8
	January	20,660	620	3
1995	April	8,230	0	0
	July	66,360	22,560	34
	October	21,870	875	4
	January	6,380	0	0
1996	April	9,070	1,810	20
	July	9,900	4,650	47
	October	7,930	2,220	28
	January	3,080	0	0

on nematode life stage, year, and orchard. Three nematode life stages—J2 in soil, females in root, and eggs in roots—were recorded as an indication of the nematode inoculum present in the orchards at each sampling date. Of these, females in root was the best indicator of seasonal activity of *T. semipenetrans* between orchards. This life stage increased once (1994 and 1995) or twice (1996) annually, depending on the year, indicating an important relationship between nematode population dynamics and environmental factors. The number of females infecting roots is also considered the best indicator for evaluating the efficacy of nematicides (Hamid et al., 1988).

Numbers of juveniles in soil showed a consistent peak once each year in either April or July depending on the orchard and year. The period of maximum egg production was April at Xalamera and Cárcer; there was no recognizable peak in egg production in the other two orchards. Eggs per female followed a similar seasonal pattern of fluctuation to that of females in root. The inverse correlation between females per gram of root and fecundity has been found in a number of citrus rootstocks (Verdejo-Lucas et al., 2000), but an increase in the number of eggs per gram of root following the highest annual levels of females was not observed in this study.

The 3-month sampling intervals may have prevented us from detecting distinct peaks in egg production. Quarterly samples would not have detected population trends that may have occurred in the intervening months. Nevertheless, the correlations demonstrated that sampling frequency was adequate to reveal some important associations between citrus nematode and environmental factors. Several studies have described one peak (Prasad and Chawla, 1965; Husain et al., 1981, Bello et al., 1986), two peaks (O'Bannon et al., 1972; Salem 1980; Baghel and Bhatti, 1982; Duncan et al., 1993), or three peaks (Hamid et al., 1988) of maximum population development per year. Hamid et al. (1988) showed that population peaks measured in number of adult females per gram of root corresponded with major flushes of new roots. An increase in root density could affect the density of females or eggs per gram of root, even if the nematode population remained constant. In Florida, nematode population density paralleled feeder root density, and the number of *T. semipenetrans* per gram of feeder root did not vary significantly by horizontal location (Duncan, 1986).

Major variations in seasonal population density between orchards in Valencia (Moncada and Cárcer) and those in Tarragona (Amposta and Xalamera), about 200 km farther north were not observed, but drip-irrigated orchards (Moncada and Amposta) tended to have higher population densities than those irrigated by flooding (Cárcer and Xalamera). Environmental and edaphic factors such as soil moisture, temperature, pH, soil type, and $CaCO_3$ content of the soil may affect nematode population densities and seasonal fluctuations (Husain et al., 1981; Bello et al., 1986; Navas et al., 1992). Additionally, citrus rootstock (O'Bannon et al., 1972; Salem, 1980; Al Sayed et al., 1993, tree vigor (Duncan and Noling, 1987), available carbohydrate (Duncan and Eissenstat, 1993; Duncan et al., 1993), and lignin concentration in roots (Duncan et al., 1993) have been associated with nematode abundance and seasonal fluctuations. This study identifies rainfall, temperature, and the bacterial antagonist *Pasteuria* as other factors affecting seasonal fluctuations of the citrus nematode in some orchards. For instance, J2 and males in soil and the mean monthly rainfall the month before sampling were negatively correlated in the orchards with soils containing a higher content of silt (Xalamera) and sand (Moncada), but no correlation was found in the heavier soils of Amposta or Cárcer.

The negative relationship between nematode abundance and soil moisture has been found by others, and several explanations have been offered. The general response of the citrus nematode to soil moisture appears to be a preference for lower water potentials but a poor ability to survive severe drought. Because *T. semipenetrans* is a poor anhydrobiote (Tsai and Van Gundy, 1989), nematode populations increase with low humidity but decline in very dry soils (Husain et al., 1981; Baghel and Bhatti, 1982; Duncan et al., 1993). The citrus nematode responded differently to the same water deficit, depending on whether the drought-affected area enclosed all or part of the rhizosphere. Water would become available from other portions of the root system via root xylem by hydraulic lift (Duncan and El-Morshedy, 1996). However, in well-drained soils, nematodes may be washed into deeper soil layers after heavy rains and escape detection by standard soil-sampling procedures.

The length of life cycle of *T. semipenetrans* is temperature-dependent and is completed in 6 to 8 weeks at 25 °C on a susceptible host (Van Gundy, 1958). The generation time is longer at lower temperature (O'Bannon et al., 1966), on poor hosts (Cohn, 1965), and in alkaline, calcareous, and saline soils (Sweelan and Abo-Taka, 1989). In addition, reproduction rates of the nematode differ between species of *Citrus* and their hybrids (Cohn et al., 1965; O'Bannon, et al., 1972; Husain et al., 1981) and are affected by tree age (Cohn et al., 1965; Bello et al., 1986). In the study orchards, rootstocks were susceptible to *T. semipenetrans,* temperatures above the infection threshold occurred for about 4 to 5 months of the year, and a single peak of maximum nematode activity was detected per year. Population increase of the citrus nematode occurs at a low rate, and the maximum population levels are usually attained about 12 years after planting for trees planted in virgin soils (Cohn et al., 1965). Apparently, the population dynamics of *T. semipenetrans* in these mature orchards have reached an equilibrium because population densities did not significantly increase or decrease during 3 consecutive years.

Members of the *Pasteuria* group were present in the four citrus orchards, but the one at Amposta appeared to be more conductive to the antagonist than the others. Although nematode densities in soil tended to decrease, the corresponding reduction in the root populations was not observed. Parasitism of *T. semipenetrans* by *Pasteuria* spp. has been reported to occur in Samoa (Sturhan, 1985); Corsica, France (Sturhan, 1988); Iraq (Fattah et al., 1989); Florida (Walter and Kaplan, 1990); Italy (Ciancio et al., 1994); Turkey (Elekçioglu, 1995); and Spain (Verdejo-Lucas et al., 1997a). The bacterial parasite produces mature spores inside the vermiform stages of the nematode, as found in this and previous studies (Fattah et al., 1989; Walter and Kaplan, 1990; Kaplan, 1994). This particular *Pasteuria* population may attach, infect, and develop to mature spores in 18 days during the migration stage of the nematode, when the citrus nematode J2 and males remain in the soil for extended time periods (Kaplan, 1994). Therefore, the bacterial parasitism will prevent nematodes from invading roots. Populations of *Pasteuria* that are able to complete their life cycle in the infective J2 would be more effective in regulating nematode densities than those needing the adult female (i.e., *Pasteuria penetrans* attacking *Meloidogyne*) because they will suppress the first generation of the nematode. This specialized nematode parasite was the only antagonist monitored in this study, but generalist antagonists such as nematode-trapping and parasitic fungi were observed occasionally.

The most severe effects of nematode infestation by *T. semipenetrans* in Spanish citrus are generally noted when young trees are interplanted in old orchards and in replant situations. Pre-plant soil fumigation provides adequate nematode control (O'Bannon and Tarjan, 1973). In established orchards, population densities of *T. semipenetrans* at a given time of the year have been used as a guideline for nematode management. Action thresholds have been set at about 5,000 J2 per 250 cm^3 soil and 1,200 females per gram feeder root in spring samples (Garabedian et al., 1984). The application of management strategies will be most effective during periods of active root development because conditions that favor root growth may favor the buildup of *T. semipenetrans* (Duncan and Noling, 1987). The applications should begin in the spring, just prior to the first root flush, and continue during the growing season to protect all new roots from nematode infection (Hamid et al., 1988). Consequently, the optimum time of nematicide application in the study orchards should begin in mid-April, when soil temperatures are above the infection threshold and high infective inoculum levels (J2) are detected in soil.

Literature Cited

Al Sayed, A. A., S. H. Abdel-Hameed, and H. I. El-Nagar. 1993. Population dynamics of *Tylenchulus semipenetrans* in relation to citrus species and soil temperature. Bulletin Faculty of Agriculture, University of Cairo 44:183–190.

Baghel, P. P. S., and D. S. Bhatti. 1982. Vertical and horizontal distribution of phytonematodes associated with citrus. Indian Journal of Nematology 12:339–344.

Baines, R. C., S. D. Van Gundy, and E. P. DuCharme. 1978. Nematodes attacking *Citrus*. Pp. 321–345 *in* W. Reuther, E. Clair Calavan, and G. E. Carman, eds. The citrus industry, vol. 4. Oakland, CA: Division of Agricultural Sciences, University of California.

Bello, A., A. Navas, and C. Belart. 1986. Nematodes of citrus groves in the Spanish Levante. Ecological study focused to their control. Pp. 211–216 *in* R. Cavalloro and E. Di Martino, eds. Integrated pest control in citrus groves. Rotterdam, The Netherlands: Balkema Publications.

Bello, A., A. Navas, C. Belart, and M. P. Alvira. 1985. Los nematodos de los citricos. Castellón de la Plana: Excelentísimo Ayuntamiento Castellón de la Plana.

Bello, A., M. Escuer, and M. A. Pastrana. 1996. Nematodos fitoparásitos y su control en ambientes mediterráneos. Pp. 1039–1069 *in* G. Llácer, M. M. López, A. Trapero, and A. Bello, eds. Patología Vegetal, vol 2. Madrid: Sociedad Española de Fitopatología.

Ciancio, A., R. Bonsignore, N. Vovlas, and F. Lamberti. 1994. Host records and spore morphometrics of *Pasteuria penetrans* group parasites of nematodes. Journal of Invertebrate Pathology 63:260–267.

Cohn, E. 1965. The development of the citrus nematode on some of its hosts. Nematologica 11:593–600.

Cohn, E., G. Minz, and S. P. Monselise. 1965. The distribution, ecology, and pathogenicity of the citrus nematode in Israel. Israel Journal of Agriculture Research 15:187–200.

Duncan, L. W. 1986. The spatial distribution of citrus feeder roots and of the citrus nematode, *Tylenchulus semipenetrans*. Revue de Nematologie 9:233–240.

Duncan, L. W. 1999. Nematode diseases of citrus. Pp. 136–148 *in* L. W. Timmer and L. W. Duncan, eds. Citrus health management. St. Paul, Minnesota: APS Press.

Duncan, L. W., and E. Cohn. 1990. Nematode parasites of citrus. Pp. 321–346 *in* M. Luc, R. Sikora, and J. Bridge, eds. Plant-parasitic nematodes in subtropical and tropical agriculture. Wallingford, UK: Commonwealth Agricultural Bureau International.

Duncan, L. W., and D. M. Eissenstat. 1993. Responses of *Tylenchulus semipenetrans* to citrus fruit removal: Implications for carbohydrate competition. Journal of Nematology 25:7–14.

Duncan, L. W., and M. M. El-Morshedy. 1996. Population changes of *Tylenchulus semipenetrans* under localized vs. uniform drought in the citrus root zone. Journal of Nematology 28:360–368.

Duncan, L. W., J. H. Graham, and L. W. Timmer. 1993. Seasonal patterns associated with *Tylenchulus semipenetrans* and *Phytophthora parasitica* in the citrus rhizosphere. Phytopathology 83:573–581.

Duncan, L. W., and J. W. Noling. 1987. The relationship between development of the citrus root system and infestation by *Tylenchulus semipenetrans*. Revue de Nématologie 10:61–66.

Elekçioglu, I. H. 1995. Occurrence of *Pasteuria* bacteria as parasites of plant-parasitic nematodes in the east Mediterranean region of Turkey. Nematologia Mediterranea 23:213–215.

Fattah, F. A., H. M. Saleh, and H. M. Aboud. 1989. Parasitism of the citrus nematode, *Tylenchulus semipenetrans,* by *Pasteuria penetrans* in Iraq. Journal of Nematology 21:431–433.

Garabedian, S., S. D. Van Gundy, R. Mankau, and J. D. Radewald. 1984. Nematodes. Pp. 129–131 *in* Integrated pest management for citrus. Berkeley CA: Division of Agriculture and Natural Resources Publications, University of California.

Hamid, G. A., S. D. Van Gundy, and C. J. Lovatt. 1988. Phenologies of the citrus nematode and citrus roots treated with oxamyl. Pp. 993–1004. *in* Goren and K. Mendel, eds. Proceedings of the 6th International Citrus Congress, Tel Aviv, Israel, March 6–11. R. Philadelphia/Rehovot: Balaban Publishers.

Husain, S. I., H. Y. Mohammad, and A. J. Al-Zarari. 1981. Studies on the vertical distribution and seasonal fluctuations of the citrus nematode in Iraq. Nematologia Mediterranea 9:7–19.

Jenkins, W. R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. Plant Disease Reporter 48:69.

Kaplan, D. T. 1994. Partial characterization of a *Pasteuria* spp. attacking the citrus nematode, *Tylenchulus semipenetrans,* in Florida. Fundamental and Applied Nematology 17:509–512.

Martinez Beringola, M. L., A. Carceles, and M. P. Gutierrez. 1987. Ensayos de nematicidas contra el nematodo de los agrios, *Tylenchulus semipenetrans*. Boletin Sanidad Vegetal Plagas 13:261–271.

McSorley, R., J. L. Parrado, and W. H. Dankers. 1984. A quantitative comparison of some methods for the extraction of nematodes from roots. Nematropica 14: 72–84.

Navas, A., G. Nombela, and A. Bello. 1992. Caracterización de la modalidad de distribución de *Tylenchulus semipenetrans* en el levante español. Nematropica 22: 205–216.

O'Bannon, J. H., J. D. Radewald, and A. T. Tomerlin. 1972. Population fluctuation of three parasitic nematodes in Florida citrus. Journal of Nematology 4:194–199.

O'Bannon, J. H., H. W. Reynolds, and C. R. Leathers. 1966. Effects of temperature on penetration, development, and reproduction of *Tylenchulus semipenetrans.* Nematologica 12:483–487.

O'Bannon, J. H, and A. C. Tarjan. 1973. Preplant fumigation for citrus nematode control in Florida. Journal of Nematology 5:88–95.

Ortuño Martinez, A., J. Gomez Gomez, and F. Canovas Candel. 1969. Poblaciones nematológicas fitoparásitas en los suelos de la huerta de Murcia. Anales de Edafología y Agrobiología 28:389–398.

Prasad, S. K., and M. L. Chawla. 1965. Observations on the population fluctuations of citrus nematode, *Tylenchulus semipenetrans* Cobb 1913. Indian Journal of Entomology 27:450–454.

Salem, A. A.-M. 1980. Observations on the population dynamics of the citrus nematode, *Tylenchulus semipenetrans,* in Sharkia Governorate. Egyptian Journal of Phytopathology 42:31–34.

Sturhan, D. 1985. Untersuchungen über verbreitung und wirtr des nematodenparasiten *Bacillus penetrans.* Mitt. Biol. Bundesanst. Land-Forstwirtsch., Berlin-Dahelm 226:75–93.

Sturhan, D. 1988. New host and geographical records of nematode-parasitic bacteria of the *Pasteuria penetrans* group. Nematologica 34:350–356.

Sweelan, M. E., and S. M. Abo-Taka. 1989. Biology and reproduction of citrus nematode (*Tylenchulus semipenetrans*) under five soil types. Indian Journal of Agricultural Sciences 59:374–376.

Tsai, B. Y., and S. D. Van Gundy. 1989. Tolerance of protoanhydrobiotic citrus nematode to adverse conditions. Revue de Nematologie 12:107–112.

Tuset, J. J., and J. García. 1986. Problemática en el control del nematodo de los agrios (*Tylenchulus semipenetrans* Cobb). Tratamientos con nematicidas solidos. Levante Agricola 267–268:73–78.

Van Gundy, S. D. 1958. The life history of the citrus nematode *Tylenchulus semipenetrans* Cobb. Nematologica 3:283–294.

Verdejo-Lucas, S. 1992. Seasonal population fluctuations of *Meloidogyne* spp. and the *Pasteuria penetrans* group in kiwi orchards. Plant Disease 76:1275–1279.

Verdejo-Lucas, S., M. Español, C. Ornat, and F. J. Sorribas. 1997a. Occurrence of *Pasteuria* spp. in northeastern Spain. Nematologia Mediterranea 25:109–112.

Verdejo-Lucas, S., F. J. Sorribas, J. B. Former, and A. Alcaide. 1995. Niveles poblacionales del nematodo *Tylenchulus semipenetrans* en plantaciones de cítricos. IV Congreso Sociedad Española Ciencias Hortícolas, Barcelona, Spain. (Abstr).

Verdejo-Lucas, S., F. J. Sorribas, J. B. Forner, and A. Alcaide. 1997b. Screening hybrid citrus rootstocks for resistance to *Tylenchulus semipenetrans* Cobb. Hortscience 32:1116–1119.

Verdejo-Lucas, S., F. J. Sorribas, J. B. Forner, and A. Alcaide. 2000. Resistance of hybrid citrus rootstocks to a Mediterranean biotype of *Tylenchulus semipenetrans* Cobb. Hortscience 35:269–273.

Verdejo-Lucas, S., F. J. Sorribas, J. Pons, J. B. Forner, and A. Alcaide. 1997c. Biotypes of *Tylenchulus semipenetrans* from Spanish citrus orchards. Fundamental and Applied Nematology 20:399–404.

Walter, D. E., and D. T. Kaplan. 1990. Antagonists of plant-parasitic nematodes in Florida citrus. Journal of Nematology 22:567–573.