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Phenologies of the Citrus Nematode and Citrus Roots Treated with Oxamyl

G.A. HAMID, S.D. VAN GUNDY AND C.J. LOVATT

Citrus Research Center and Agricultural Experiment Station, University of California, Riverside, CA 92521, USA

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Abstract

Field plots of 20-year-old 'Washington' navel orange trees (*Citrus sinensis* L. Osbeck) on Troyer citrange rootstock (*C. sinensis* x *Poncirus trifoliata* L. Raf.) infected with the citrus nematode (*Tylenchulus semipenetrans* Cobb.) and trees previously infected but treated to reduce further nematode infection with oxamyl, applied at a rate of 1.1 kg a.i./ha/3 weeks from April through October were sampled every other week to determine the seasonal distribution of young feeder roots through the top 60 cm of the soil profile in relation to the distribution of citrus nematode larvae and adult females. More than 60% of the feeder roots on nematode-infected trees were concentrated in the top 30 cm of soil. Peaks of root infection were observed at both 30 and 60 cm, once each spring, summer, and winter during both years of the study, suggesting at least 3 generations of citrus nematode per year. The lowest infection occurred in October both years. Within 2 months after initial application, oxamyl reduced the number of nematode females per total roots per kg soil sample by 50% and per g roots by 60% in the top 30 cm of soil to a level below the economic threshold which was maintained throughout the year. As a result of oxamyl treatment, the number of new roots initiated was reduced by 40% with a concomitant 40% increase in root biomass.

Introduction

Prior to 1977, citrus nematode (*Tylenchulus semipenetrans* Cobb.) populations were managed with the fumigant-nematicide 1,2 dibromo-3-chloropropane (DBCP) (2,4,13,14,17). Since then, an active search for suitable alternatives to DBCP has shown that some nonfumigant organophosphates (phenamiphos) and organocarbamates (aldicarb and oxamyl) have promise as management tools (1,7,15). Their success in reducing root infection by *T. semipenetrans* is achieved through the continuous maintenance of small quantities of the nemastatic chemicals near the root system to protect the newly initiated roots from infection by nematode larvae. Eventually, the number of adult females on the roots and larvae in the soil decrease.

Nonfumigant nemastats are relatively expensive, and their commercial use is contingent on developing optimum management practices for fruit production, along with their judicious use as pesticides to reduce environmental contamination. Application timing is critical to protect the new root flushes from nematode infection. The timing and distribution of newly initiated feeder roots through the soil profile in relation to nematodes population dynamics must be understood to ensure optimal placement of these nemastats. Multiple applications at a low rate are more effective and more environmentally sound than one single application at a high rate (16).

For these reasons, we followed the seasonal distribution of young feeder roots of mature nematode--infected citrus trees and the seasonal distribution of *T. semipenetrans* adult females on roots and larvae and males in the soil through the top 60 cm of the soil profile. In addition, we examined the effectiveness of multiple applications of a low rate of oxamyl in protecting newly-initiated roots from infection by citrus nematode and the subsequent reduction of adult females feeding on the roots.

Materials and Methods

Studies were conducted for 2 years, during the period March 1982 through April 1984, at the Citrus Research Center and Agricultural Experiment Station of the University of California, Riverside, on 36 replanted 20-year-old 'Washington' navel orange (*Citrus sinensis* L. Osbeck) scions budded to Troyer citrange (*C. sinensis* x *Poncirus trifoliata* L. Raf.) rootstock. All trees were heavily infected with the citrus nematode (*Tylenchulus semipenetrans* Cobb.) and were studied for root growth and nematode population dynamics for the first year. In the second year, half of the trees were treated with oxamyl (methyl-N,N-dimethyl-N[methyl-carbamyl)oxy]-1-thiooxmimidate) at the rate of 1.1 kg a.i./ha every 3 weeks. The chemical was manually applied by spraying the oxamyl solution in the bottom of the irrigation furrow in a 2.5 to 5cm band on both sides of the tree, using a pressurized sprayer at 10 psi. Each application was followed immediately with a regularly scheduled irrigation at the rate of 3.37 acre–cm of water in 48 hr. Applications were initiated with the first irrigation in April 1983, and continued every 3 weeks through the end of October 1983 for a total of 8 applications (8).

Citrus nematode populations and root growth were sampled every 2 weeks for 2 years, beginning March 1982, by taking soil samples with a 7.5 cm bucket auger at two different soil depths; 0 to 30 and 30 to 60 cm, from the furrow at the dripline of

5 trees per treatment per sampling date. To avoid the secondary effect of root pruning due to sampling, the 36 trees included in the study were sampled so that any individual tree was sampled only once every 12 weeks. The entire block of 36 trees were sampled on the south and north sides of the tree during the first year of the study. During the second year samples were taken from only the south side. All roots were screened from the soil sample with a 16 mesh screen and the number of new root tips and total fresh weight of feeder roots in each sample were recorded. An aliquot of feeder roots was stained with a modified acid fuchsin technique which substituted acetic acid for lactophenol, and the nematode females were extracted using a blender (3). A 1000 g soil sample was extracted for citrus nematode larvae and males using the elutriator technique (5). Soil temperature was recorded continuously at a depth of 30 cm below the dripline of the tree and precipitation was obtained from the campus weather station.

Results

During the growing season of the first year of the study, nematode infection averaged 1786 ± 117 females per g roots and 18,163 ± 1280 larvae and males per kg soil on both the north and south facing sides of the trees through the soil profile to a depth of 60 cm. During the growing season of the second year of the study, nematode infection of the roots of the untreated trees averaged 2493 ± 148 female nematodes per g roots through soil profile to a depth of 60 cm on the south side of the trees, a 30% increase in root infection over the first year of the study ($p < 0.001$). In the same profile, there were more average roots per kg soil and more average adult females per g of root on the south side of the tree throughout the year than on the north side of the tree ($p < 0.001$). On both sides of the tree, there were significantly more roots in the upper 30 cm profile than in the lower 30 cm profile (Table 1). The average number of new roots initiated during the year was not significantly influenced by tree side or soil depth (Table 1).

Root infection measured as number of adult females extracted from 1 g roots in the 0 to 60 cm profile of soil exhibited 3 peaks during the 2 year study; one each in the spring, summer and winter ($p < 0.05$) (Fig. 1 and 2a,b and 3a,b) which corresponded with the 3 major flushes of new roots (Fig. 2c and 3c) during the second year.

Root biomass was lowest in the spring and increased during the summer and reached a peak in the fall (Fig. 2d and 3d). Since the patterns of root growth and infection on the north and south sides of the tree were the same, only the south side was sampled during the second year of the study.

During the two growing seasons studied, more than 60% of the feeder roots of the nematode infected trees were concentrated in the top 30 cm of the soil profile ($p < 0.01$). The number of nematode adult females/g roots, however was significantly less in the top 30 cm profile of soil than in the 30 to 60 cm profile of soil (p <

0.01) (Table 1). Larvae and males were equally distributed to a depth of 60 cm (Table 1) with little variation in number throughout the 2 years of the study (Fig. 1).

Table 1: Distribution of larvae and adult females of the citrus nematode and the amount of root infection, root biomass, and root initiation of the 'Washington' navel orange on Troyer citrange rootstock through the soil profile to a depth of 60 cm for both nematode-infected and oxamyl-treated trees

Treatment	Tree side	Sampling depth	Gram feeder roots/kg soil	No. of root tips/g roots	No. of larvae/kg soil	No. of females per g roots	No. of females per soil sample
				1982–1983			
Nematode-Infected	North	0–30 cm	0.39[3z]	5.40[NS]	20,718[3]	1,551[NS]	1,932[3]
		30–60 cm	0.17	4.19	14,568	1,783	881
Nematode-Infected	South	0–30 cm	0.46[2]	4.98[NS]	17,550[NS]	1,566[2]	2,356[NS]
		30–60 cm	0.28	3.53	19,815	2,184	1,906
				1983–1984			
Nematode-Infected	South	0–30 cm	0.54[3]	2.91[NS]	16,523[NS]	2,252[2]	4,228[1]
		30–60 cm	0.34	2.46	15,117	2,734	2,819
Oxamyl-Treated	South	0–30 cm	0.78[3]	1.84[NS]	8,861[1]	911[2]	2,283
		30–60 cm	0.49	2.19	14,054	1,558	2,311

[z]Not significant (NS) or significant at 5% ([1]), 1% ([2]), or 0.1% ([3]) levels.

Soil temperatures at a depth of 30 cm during the growing year varied between 10.5 and 23°C (Fig. 1) and no correlation was observed with nematode populations on roots or in soil except that larval populations generally decreased during the winter and increased in the spring as soil temperatures rose and as irrigation was initiated to compensate for the lack of rainfall. Neither root infection by the citrus nematode, nor larval and male population densities of this pest, were influenced by the timing of the irrigations nor by the occurrence of precipitation ($r^2 = 0.03$) ($p < 0.001$) (Fig. 1). No correlation was observed between peaks or declines in root infection and changes in population densities of larvae and males ($r^2 = 0.06$) ($p < 0.01$) (Fig. 1).

Two treatments of oxamyl significantly reduced root infection as measured by citrus nematode females in the top 30 cm of the soil profile ($p < 0.05$) (Fig. 2a). Five treatments (4 months) of oxamyl significantly reduced root infection in the 30 to 60 cm profile. After 4 months of treatment, the initial infection level of 3259 ± 739 females/g roots was reduced by 85% to an average level of 642 ± 90 females/g roots for the remainder of the year (Fig. 2a). During the second year, oxamyl reduced the average number of nematode females per total root sample by 50% and per g roots by 60% in the top 30 cm of soil profile (Table 1).

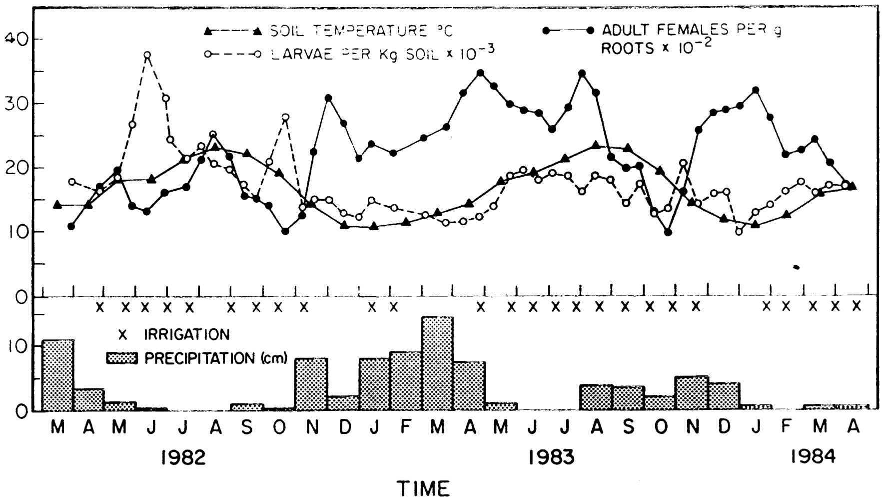

Figure 1: Population densities of adult females (–•–) and larvae (–o–) of *Tylenchulus semipenetrans*, to a soil depth of 60 cm, on the south sides of nematode infected 20-year-old 'Washington' navel orange scions budded to Troyer citrange rootstocks, soil temperature at a soil depth of 30 cm (–Δ–), precipitation (), and dates of irrigation (X) (3.37 acre–cm in 48 hr).

Four months after oxamyl application, the initial number of larvae and males per kg soil was reduced by 50% in the upper 30 cm of the soil profile ($p < 0.05$). This was 60% fewer larvae and males per kg soil than from nematode infected trees for the same sampling date ($p < 0.001$) (Fig. 2b). From then on, the larvae and male populations stabilized at 5814 ± 719 nematodes per kg soil. Oxamyl had little or no effect on the number of larvae and males per kg soil in the 30 to 60 cm of the soil profile (Fig. 3b and Table 1).

Concomitant with the reduction in root infection by the citrus nematode in the 0 to 30 cm profile brought about by the oxamyl treatment, the number of new root tips initiated was reduced by 40%, while root biomass increased by more than 40% by the end of the growing season ($p < 0.001$) (Table 1; Fig. 2c and 2d). At the 30 to 60 cm profile, the high rate of root initiation characteristic of nematode-infected trees was not significantly reduced until November (Fig. 3c), concomitantly, root biomass increased ($p < 0.01$) (Fig. 3d).

Application of oxamyl at a rate of 1.1 kg a.i./ha/3 weeks April through October improved the value of harvested fruit by increasing fruit size (Table 2). The number of boxes of small fruit harvested [i.e. fruit referred to as 163's, and 138's (fruit with an average diameter of 5.8 packed 163 fruit per carton and fruit with an average

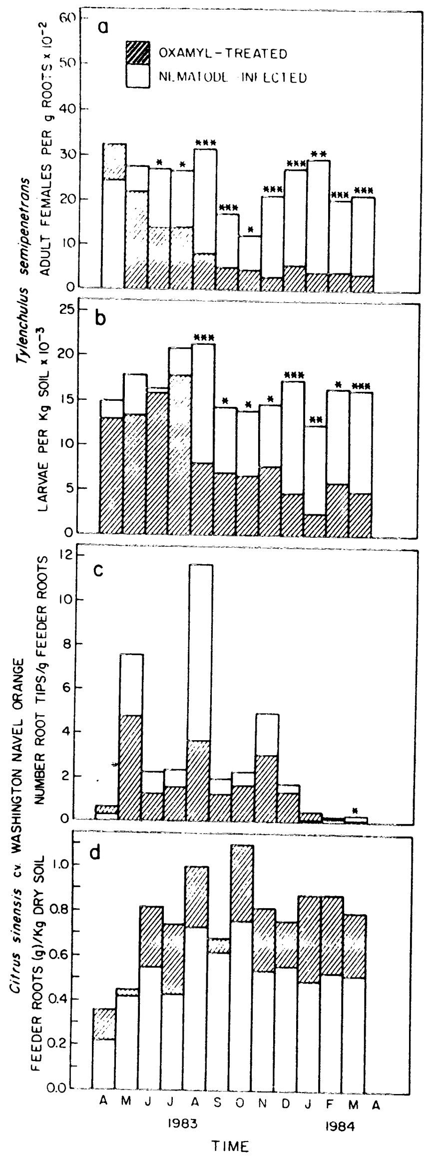

Figure 2: Population densities of adult females (a) and larvae (b) of *Tylenchulus semipenetrans*, number of new roots initiated (c), and biomass of feeder roots (d) at the top 0 to 30 cm of the soil profile on the south sides of nematode-infected ☐ and oxamyl-treated (1.1 kg a.i./ha/3 weeks, starting mid-April 1983) ▨ 20-year-old 'Washington' navel orange scions budded to Troyer citrange rootstocks. The *, **, and *** denote significant differences at the 5%, 1% and 0.1% level, respectively.

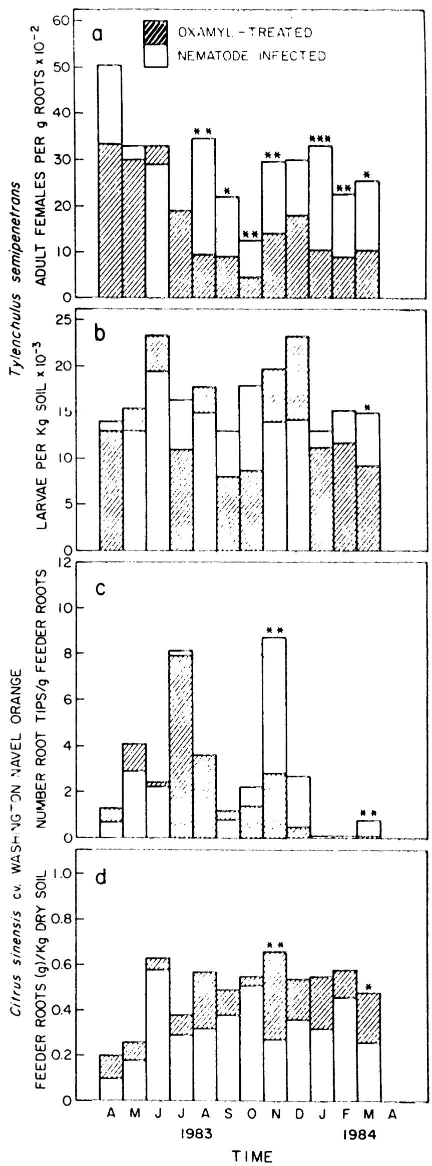

Figure 3: Population densities of adult females (a) and larvae (b) of *Tylenchulus semipenetrans*, number of new roots initiated (c), and biomass of feeder roots (d) at a soil depth of 30 to 60 cm, on the south sides of nematode-infected ☐ and oxamyl-treated (1.1 kg a.i./ha/3 weeks, starting mid-April 1983) ▨ 20-year-old 'Washington' navel orange scions budded to Troyer citrange rootstocks. The *, **, and *** denote significant differences at 5%, 1%, and 0.1% level, respectively.

Table 2: Mean number of fruit and mean number of boxes of fruit per tree in nematode-infected and oxamyl-treated 'Washington' navel orange on Troyer citrange rootstocks

		Mean number of fruit per tree		Mean number of boxes per tree	
Average fruit diameter (cm)	Number of fruit per carton	Nematode-infected	Oxamyl-treated	Nematode-infected	Oxamyl-treated
5.8	163	48.8	11.3[3z]	0.281	0.073[3]
6.1	138	142.6	62.2[2]	1.033	0.452[2]
6.6	113	211.3	199.6[1]	1.870	1.767[1]
7.2	88	177.7	269.5[NS]	2.019	3.062[NS]
7.7	72	74.2	120.9[NS]	1.031	1.679[NS]
	All fruit	710.8	715.9[NS]	6.797	7.851[NS]

[z]Not significant (NS) or significant at 5% [1], 1%[2], or 0.1%[3] levels.

diameter of 6.1 cm packed 138 fruit per carton)] decreased 75 and 56%, respectively. There was a 52% increase in 88's (fruit with an average diameter of 7.2 cm packed 88 fruit per carton) and a 63% increase in 72's (72 fruit per carton with an average diameter of 7.7 cm). The commercial packout yielded one additional box of 88's and 2/3 of a box of 72's from the treated trees. When combined, there is a statistically significant decrease in the number of smaller fruit (163's and 138's) and a statistically significant increase in the number of larger fruit (88's and 72's).

Discussion

The phenology of citrus growth in southern California begins in early spring with flowering in late February or early March and is quickly followed by a foliage flush. The first root flush occurs about 6 weeks following flowering and fruit set. The first root flush also coincides with rising soil temperatures and the initiation of irrigation. The second root flush occurs during mid-summer about 6 weeks following the first root flush. Soil temperatures have reached their peak of about 23°C. The most frequent irrigation schedule also occurs at this time. A third root flush occurs in late fall about 6 weeks following the second root flush. The fruit set in early spring grow and increase in size during the summer growing season and remain on the tree until early spring. An understanding of the phenology of the citrus tree in the field is important to the understanding of the ecology and host parasite relations of the citrus nematode, and its control and effect on crop yield.

This particular study was conducted on a commercial navel orange scion grafted to a tolerant citrus rootstock and planted following a previous citrus crop infected with citrus nematode. During this two year study the number of adult females per g of root ranged from about 1,700 in the first year to 2,400 in the second year. The economic threshold, a level at which there is a significant increase in yield and an economic return for the cost of treatment, is ca. 700 females per g root measured during mid-summer (9). Depending on tree age, scion/rootstock combination and level of nematode infection, under California conditions one should expect a yield response with a 50 to 75% reduction in root infection by the citrus nematode to bring the number of females per g root closer to the economic threshold.

In this study, the use of 8 multiple applications of oxamyl at a rate of 1.1 kg a.i./ha/every 3 weeks from April through October provided enough protection to the three root flushes during the same period to reduce the average root infection to 642 females/g roots in the top 30 cm of soil and 911 females/g roots at the next 30 to 60 cm of soil. The growth response of the citrus fruit was represented by a significant shift in the size distribution towards larger fruit on the oxamyl treated trees. No additional fruit were expected in this study since the number of fruit had been determined at fruit set prior to the first oxamyl treatment. No insect problems were encountered during this study, therefore, the yield increase could not be attributed to insect or mite control by soil applications of oxamyl. The yield response and nematode control obtained in this study were similar to previous research (7) as well as other unreported results obtained from commercial plantings in Southern California. These results further substantiate earlier studies (7) that the number of females infecting roots is a better criteria for measuring economic threshold than the number of larvae and males per unit of soil.

For both nematode-infected and oxamyl-treated trees, root initiation began in early April but was not significant until May (fig. 2c and 3c). The soil temperature at a depth of 30 cm in April was 14°C, which is above the biological threshold for root growth. When total root initiation to a soil depth of 60 cm was determined, trees heavily infected by the citrus nematode initiated new root growth every 4 to 6 weeks, and each root flush had more new root tips per g roots than the trees protected from nematode infection by oxamyl (8). Three root flushes were significantly larger than the others; they occurred in May, July-August and November, with the largest flush occurring in August. The highest amount of root infections by the citrus nematode also occurred on the August flush. On the oxamyl-treated trees the spring root flush was the largest presumably enhancing the trees ability to take up water and nutrients during the period when the fruit were rapidly expanding in growth and may account for the striking increase in fruit size at harvest. Root initiation throughout the rest of the year, June through December, was fairly constant from 0 to 60 cm, averaging 2.5 ± 0.5 root tips per g roots. By December the soil temperature to a depth of 30 cm had reached 10°C and root growth ceased.

We observed three distinct peaks in citrus female populations closely paralleling the three largest root flushes suggesting that in Southern California there are probably three generations of females supported on citrus trees from April through December. The three peaks in root infection by adult female nematodes occurred at different soil temperatures: at a mean maximum soil temperature of 13°C in spring,23°C in summer, and 13°C during the winter (Fig. 1). The winter female peak is somewhat delayed after the fall (November) root flush because of the declining soil temperatures at this time of the year. The development of infective larvae (October soil larval peak) into adult females was also slowed by low soil temperatures. Larval data from Arizona (10) and Florida (11) suggest that there is one generation and two generations, respectively. However, our results demonstrate that it is difficult to project generations from larval data and emphasizes the value of female data.

The general pattern of soil temperature remained the same during the 2-years of investigations, but larval populations varied dramatically from one growing season to the other (Fig. 1). The linear regression coefficient between number of larvae and temperature was calculated. The r^2 was 0.02 ($p < 0.01$). This is consistant with the previous report of Cohn (6) that seasonal changes in established nematode populations was not related to soil temperature changes. Our data show that when the soil temperature reached a maximum of 23°C at a depth of 30 cm in August, in each of two growing seasons, the female population rapidly decreased to a yearly minimum in October (Fig. 1), and then rose sharply to reach a peak in December and January. The sharp decline in females in October may be related to death of females from the two previous generations or the failure of the August generation of females to reproduce due to high temperatures and excessive irrigation (12). The fall root flush infection appears to be the critical carry-over population to maintain high populations of the citrus nematode from growing season to growing season.

These results confirm the previously recommended multiple application of non-fumigants (7,8,15) as well as the strategy that applications should begin in the spring just prior to the first root flush and should continue on a monthly basis until November, thus protecting all new roots produced during the growing season from nematode infection. These data also pinpoint the fall root flush as the most critical control point in the infection cycle for long term control and management of the citrus nematode.

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