Journal of Nematology 16(3):313–317. 1984.

Distribution of *Tylenchulus semipenetrans* in a Texas Grapefruit Orchard[1]

R. M. Davis[2]

Abstract: Distribution of the citrus nematode (*Tylenchulus semipenetrans*) was studied over 18 months in a 6-year-old orchard of grapefruit (*Citrus paradisi* cv. Ruby Red) on sour orange (*C. aurantium*) rootstock. The 1.8-ha orchard was under chemical weed control, no tillage, and flood irrigation. Highest numbers of nematodes were found in the top 15 cm of the soil profile. The nematode population peaked in April and declined to lowest levels in August and September. Numbers of nematodes were negatively correlated ($r = -0.95$) with soil temperatures above 29 C. Soil populations of nematodes were not correlated with soil moisture. The distribution of the nematode in the field was highly skewed and was described by a negative binomial. In this 1.8-ha block, five soil samples of 12 cores each would provide an estimate within 20% of the true nematode population mean with 95% confidence.

Key words: citrus nematode, population dynamics, sampling, sour orange.

The citrus nematode (*Tylenchulus semipenetrans* Cobb) is a widespread pest in citrus orchards in Texas (6). Decisions to implement control measures against the citrus nematode rely on estimates of nematode populations and on cost of nematicides (10), but little has been done to describe patterns of infestations or to provide a basis for interpreting mean estimates of a nematode population. Distribution of nematodes in a field is usually nonuniform (4,7), and there is a need for accurate descriptions of nematode distributions for the development of reliable soil sampling schemes (5,7). The purpose of this study was to describe some aspects of the distribution of the citrus nematode in a South Texas citrus orchard and to develop an optimum soil sampling method for estimating *T. semipenetrans* populations.

MATERIALS AND METHODS

Soil samples for the nematode distribution study were collected from a 6-year-old orchard of grapefruit (*Citrus paradisi* Macf. cv. Ruby Red) on sour orange (*C. aurantium* L.) rootstock located at the Texas A&I University Citrus Center in Weslaco. The part of the orchard used in the study was 1.8 ha of relatively uniform Willacy fine sandy loam (60% sand, 10% silt, 30% clay, pH 7.8, and 1% organic matter). Trees were spaced 4.6 m apart in rows 8.5 m apart with the rows established east to west. The orchard was under chemical weed control (diuron and simazine) with no tillage; it received applications of dicofol, methidathion, and spray oil to control insects and mites. The orchard was flood irrigated and had permanent soil borders.

The soil was overturned with a balling shovel, and soil samples were collected from the top 10–15 cm of the soil profile unless otherwise noted. One core consisted of approximately 200 cc of soil. The soil was gently homogenized by hand before a 50-cc sample was placed on tissue paper in a Baermann funnel (2). After 24 hours incubation in the dark, a 15-ml aliquot was drawn. The citrus nematodes were counted under a dissecting microscope in each of three 0.5-ml subsamples. The counts were averaged and expressed as the number of nematodes in 100 cc of soil to conform with earlier studies (3,9–11). To determine extraction efficiency, a suspension of 2,500 citrus nematodes was added to each of three 50-cc aliquots of sterilized field soil and extracted as above. The mean recovery was 26%. Longer incubation increased recovery less than 5%.

Distribution of the citrus nematode: Soil samples were collected at least bimonthly over an 18-month period (April 1982 to September 1983) to determine changes in the nematode population in the 1.8-ha block. On each sampling date at least 20 soil cores were collected 10–15 cm deep at the drip line of randomly selected trees as the block was traversed in a zig-zag fashion. The cores were divided into five or more groups (samples) of four cores each. After

---

Received for publication 5 December 1983.

[1] The author thanks Howard S. Wilhite for technical assistance.

[2] Associate Professor, Texas A&I University Citrus Center, Weslaco, TX 78596.

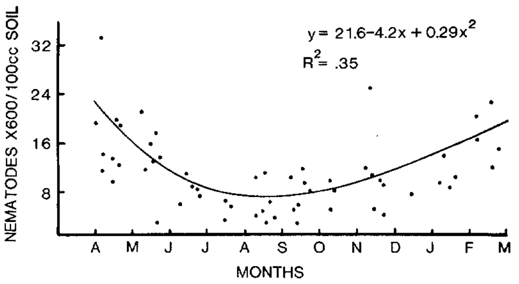

FIG. 1. Relationship between numbers of *Tylenchulus semipenetrans* and months of the year in a grapefruit orchard in Texas. Each point represents the average number of nematodes in five or more samples consisting of four cores each. All soil cores were collected 10–15 cm deep at the drip line of the trees. Significant at $P = 0.05$.

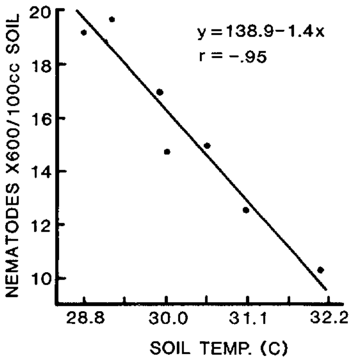

FIG. 2. Relationship between numbers of *Tylenchulus semipenetrans* in a grapefruit orchard and soil temperature 15 cm deep. Each point represents the average number of nematodes in at least 10 samples consisting of four cores each collected 10–15 cm deep at the drip line of the trees. Significant at $P = 0.01$.

the cores in each sample were gently homogenized, the nematodes were extracted as described above. The mean number of nematodes extracted from the samples represented the nematode population on each sampling date. Soil temperature 15 cm deep was measured on most sampling dates by averaging four readings equidistant around one tree using thermocouples and a potentiometer. Soil moisture 10–15 cm deep was also determined on many sampling dates by weighing 10 soil samples of about 200 g each before and after oven drying at 100 C for 24 hours. Regressions were used to evaluate statistical relationships between soil temperatures, moisture, and nematode counts.

To determine the influence of distance from tree trunks on the nematode population, numbers of the citrus nematode were determined in samples collected at varying distances away from the trunks of five trees. Sampling began 33 cm away from the tree trunk and continued at 33-cm intervals for a total distance of 3 m. Three samples of four cores each were collected at each increment from each tree in June 1982 and February 1983.

The distribution of the nematode at increasing depth was studied on three randomly selected trees in the block in June 1982 and February 1983. Soil samples were collected in the undisturbed sides of a trench dug 90 cm deep at the drip line of each tree. Three samples of four cores each were collected 5, 10, 15, and 20 cm deep at each tree and continued at intervals of 10 cm for a final depth of 90 cm. The mean number of nematodes extracted from the nine samples represented the nematode population at each increment.

Statistical analyses and optimum sample size: Within a 2-week period in June 1982 nematodes were extracted from 625 individual soil cores collected from the 1.8-ha block. The soil cores were collected at random throughout the block 10–15 cm deep at the drip line of the trees. Often more than one soil core was collected from an individual tree. Nematodes were extracted from each soil core as previously described.

To determine how the number of cores in a sample and the sample size influence the variability in sampling for the citrus nematode, nematode counts were chosen at random from the data base of the 625 counts from the individual cores and incorporated into various sampling schemes. For example, the coefficient of variation was determined for the means among four samples, each consisting of the counts of four cores. This was repeated for four samples consisting of 8, 12, or 16 cores each. Similarly, the coefficients of variation were calculated for the means of 8, 12, or 16 samples consisting of 4, 8, 12, or 16 cores each. In each sampling scheme the determination of the coefficient of variation was repeated 50 times with counts always chosen at random from the 625 counts. The mean for the 50 replicates represented the coefficient of variation for each sampling scheme. The optimum sample size was cal-

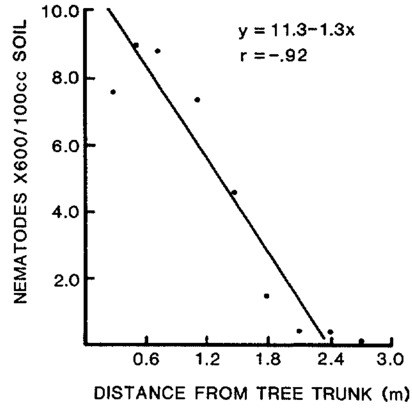

FIG. 3. Relationship between distance from the trunks of trees in a grapefruit orchard and numbers of *Tylenchulus semipenetrans*. Each point represents the average number of nematodes in 15 samples consisting of four cores each collected 10–15 cm deep. Significant at $P = 0.01$.

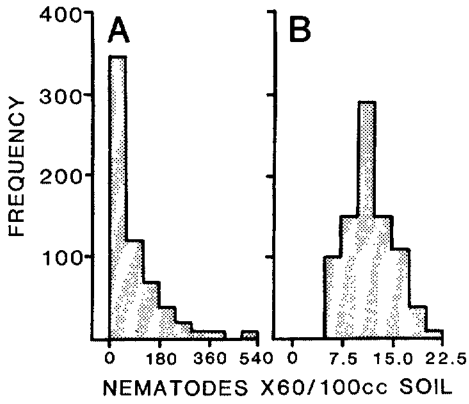

FIG. 5. Distributions of A) actual citrus nematode counts from 625 individual soil cores collected at random at the drip line of trees in a 1.8-ha citrus orchard in Texas and B) the log (X + 100) transformation of the counts.

culated with the knowledge of the standard deviation and mean in 50 replicates of 12 cores each (8).

RESULTS

Population dynamics of the citrus nematode: Numbers of the citrus nematode peaked in April and were lowest in August and September (Fig. 1). The data presented include nematode counts from April 1982 to May 1983. Data obtained outside this period confirmed this trend. The nematode population was not correlated with soil temperature below 29 C; however, the population was negatively correlated with higher temperatures (Fig. 2). The soil temperature at 15 cm deep ranged from 23.4 C to 32.2 C. The relationship between nematode numbers and soil moisture was not correlated.

In June 1982 numbers of nematodes declined sharply with increasing distance from the trunks of the trees (Fig. 3). There was no relationship between numbers of nematodes and distance from the tree trunks in February 1983 (data not shown). At both sampling dates (only data from June 1982

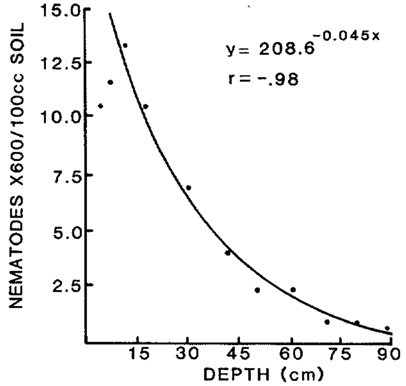

FIG. 4. Relationship between sampling depth and numbers of *Tylenchulus semipenetrans* in a grapefruit orchard. Each point represents the average number of nematodes in nine samples consisting of four cores each. Significant at $P = 0.01$.

CV%

NO. OF CORES				
16	28	37	31	21
12	27	31	28	31
8	49	48	40	30
4	69	57	49	47
	4	8	12	16

NO. OF SAMPLES

FIG. 6. Effect of the number of cores in a sample and number of samples on the coefficient of variation (CV) among repeated samples from a Texas citrus orchard.

presented) numbers of nematodes declined in samples collected deeper than 15 cm (Fig. 4).

Statistical analyses and optimum sample size: A histogram of the frequency distribution of the numbers of nematodes from 625 individual cores was positively skewed, indicating a clumped distribution (Fig. 5A). The distribution did not differ significantly from a negative binomial (*k*-value of 0.6) at $P < 0.05$ according to the chi-square goodness of fit test (1). Because most parametric statistical procedures depend on a normal distribution, these data were transformed log (X + 100) (Fig. 5B).

The coefficient of variability among sample means decreased as the number of samples increased from 4 to 16 at four or eight cores per sample (Fig. 6). With 12 or more cores in a sample, variability did not appreciably decrease with increasing sample size. Similarly, increasing the number of cores beyond 12 in a sample did not generally decrease variability. The coefficients of variation was greater than 100% with fewer than four cores or samples (data not shown). Reliability improved only marginally by taking more than four samples of 12 cores each. In the 1.8-ha block under study, five samples were necessary for the mean estimate to be within 20% of the true mean with 95% confidence (Table 1). The true mean was the average number of nematodes (3,760) in 625 individual soil cores.

TABLE 1. Number of soil samples from a 1.8-ha citrus orchard in South Texas necessary for the mean estimate of *Tylenchulus semipenetrans* to be within certain limits of the true mean.*

Amount of error in the estimate		Number of samples (12 cores/ sample) required, $P = 95\%$
No. of nematodes/ 100 cc soil	% of true mean	
± 188	5	185
± 376	10	46
± 564	15	20
± 752	20	5

* The true mean is the average number of nematodes in 625 soil cores, or 3,760 nematodes/100 cc soil.

DISCUSSION

The citrus nematode causes significant losses to the citrus industry in Texas; therefore, measures that reduce the nematode populations are likely to increase yields (3,9–11). Increased knowledge of the distribution of the nematode may maximize control efforts. For example, the population of the nematode in this study declined in the summer and peaked in the spring, possibly causing the greatest damage in the spring. Thus, optimum timing for nematicide application may be determined by studying changes in the soil nematode population. Other distributional patterns may explain past success or failure in controlling the nematode. For example, control of the citrus nematode in Texas citrus has been obtained despite poor downward movement of nematicides through the fine-textured soils of South Texas (9). Apparently, the nematicides were concentrated in the upper profile of soil where the nematodes are most abundant.

The negative binomial distribution approximates the distribution of populations of many soil-borne plant parasitic nematodes (1,4). The citrus nematode may increase in numbers quickly, a characteristic suggested by the clumped distribution in the orchard under study. The nematode may respond to root growth cycles and increase rapidly in numbers following root growth flushes. These and other factors influencing nematode reproduction may result in nonuniform distributions of citrus nematodes.

An optimum sampling scheme is usually a compromise between accuracy constraints and the cost of obtaining the estimate of a nematode population. While cost limitations will often dictate the optimum sampling procedure for the desired confidence levels, accuracy was the only limiting factor considered in determining sample size in this study. Others have proposed that pest management decisions can be made with population estimates within 20% of the true mean (5,7), and I assumed for the present that this level could suffice for management decisions in citrus orchards. In the 1.8 ha of orchard under study, five soil samples, each consisting of a composite of 12 cores, were required for the mean estimates to be within 20% of the true mean. No fewer soil cores can be collected (to keep the cost of sampling at a minimum) and still maintain the desired precision. This information should assist in developing methods for predicting crop loss, establishing economic soil nematode population thresholds, and studying the characteristics of soil populations of *T. semipenetrans* on citrus that may result in the development of improved control measures for this nematode.

LITERATURE CITED

1. Bliss, C. I., and R. A. Fisher. 1952. Fitting the negative binomial to biological data and note on the efficient fitting of the negative binomial. Biometrics 8:176–200.

2. Christie, J. R., and V. G. Perry. 1951. Removing nematodes from soil. Proceedings of the Helminthological Society of Washington 18:106–108.

3. Davis, R. M., C. M. Heald, and L. W. Timmer. 1982. Chemical control of the citrus nematode on grapefruit. Journal of the Rio Grande Valley Horticultural Society 35:59–63.

4. Goodell, P., and H. Ferris. 1980. Plant-parasitic nematode distributions in an alfalfa field. Journal of Nematology 12:136–141.

5. Goodell, P., and H. Ferris. 1981. Sample optimization for five plant-parasitic nematodes in an alfalfa field. Journal of Nematology 13:304–313.

6. Heald, C. M. 1970. Distribution and control of the citrus nematode in the lower Rio GrandeValley of Texas. Journal of the Rio Grande Valley Horticultural Society 24:32–35.

7. Proctor, J. R., and C. F. Marks. 1975. The determination of normalizing transformations for nematode count data from soil samples and of efficient sampling schemes. Nematologica 20:395–406.

8. Snedecor, G. W., and W. G. Cochran. 1976. Statistical methods. Ames: Iowa State University Press.

9. Timmer, L. W. 1977. Control of citrus nematode *Tylenchulus semipenetrans* on fine-textured soil with DBCP and oxamyl. Journal of Nematology 9:45–50.

10. Timmer, L. W., and R. M. Davis. 1982. Estimate of yield loss from the citrus nematode in Texas grapefruit. Journal of Nematology 14:582–585.

11. Timmer, L. W., and J. V. French. 1979. Control of *Tylenchulus semipenetrans* on citrus with aldicarb, oxamyl, and DBCP. Journal of Nematology 11: 387–394.