The spatial distribution of citrus feeder roots and of the citrus nematode, *Tylenchulus semipenetrans*

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SUMMARY

Three surveys were conducted in mature citrus groves to describe the spatial distribution of citrus feeder roots and of *Tylenchulus semipenetrans*. Feeder root and nematode population density declined with distance from the trunk on both 20-year-old " sour orange " and 30-year-old " rough lemon " rootstocks. Qualitative tree differences were reflected by fewer feeder roots recovered from trees with decline symptoms than from healthy trees. Covariation of roots and nematodes with distance from the trunk was pronounced so that the number of *T. semipenetrans*/g root did not vary with location even under canopies and in bare row middles. In linear regression analyses, roots and nematodes collected from the first 30 cm soil depth explained 75 and 73 %, respectively, of the variability in densities from 0 to 60 cm soil depth. When nematode population levels in shallow (0 to 30 cm) samples were used to predict levels from 0 to 60 cm in a simulation model, forecasting error declined exponentially with increased numbers of soil cores/sample. A sample strategy for nematode related crop loss assessment in mature groves was developed, based on these survey results. An essential aspect of the strategy is random procurement of samples within a standard unit-area around each tree, rather than utilizing tree architecture to define the sample space.

RÉSUMÉ

Répartition spatiale des racines nourricières de citrus et du nématode Tylenchulus semipenetrans

Trois prospections ont été effectuées dans des vergers de citrus en production afin de préciser la répartition spatiale des racines nourricières et du nématode *Tylenchulus semipenetrans*. La densité de ces racines et celle des populations du nématode diminuent lorsqu'on s'éloigne du tronc, aussi bien chez des porte-greffe « sour orange » âgés de 20 ans que « rough lemon » âgés de 30 ans. Les différences observées dans l'aspect des arbres étaient reflétées par le nombre de racines nutritives, plus faible chez ceux présentant des symptômes de faiblesse que chez les arbres sains. La covariation des quantités de racines et de nématodes avec la distance au tronc était très nette, si bien que le nombre de *T. semipenetrans* par gramme de racines ne varie pas avec la localisation, même en comparant la zone sous frondaison et les interlignes sans végétation. Dans les analyses de régression linéaire, les racines et les nématodes prélevés dans les 30 cm supérieurs du sol participent pour, respectivement, 75 et 73 % à la variabilité de ces valeurs pour les 60 cm supérieurs du sol. Lorsque les niveaux de population observés dans les échantillons de surface (0-30 cm) sont utilisés, dans un modèle de simulation, pour prédire ces niveaux, pour l'horizon 0-60 cm, l'erreur de prévision diminue exponentiellement avec l'augmentation du nombre des échantillons. Grâce aux données de cette étude, il a pu être mis au point une stratégie d'échantillonnage visant à permettre de connaître la part prise par les nématodes dans les pertes de récolte causées aux vergers de citrus âgés. Le point essentiel de cette stratégie est la répartition au hasard des points de prélèvements à l'intérieur d'une surface standard autour de chaque arbre plutôt que la détermination de ces points en fonction du port des arbres.

The quantitative measurement of host-nematode relationships requires knowledge of nematode distribution in the soil profile in order to optimize sample procurement (Goodell & Ferris, 1980, 1981; McSorley, 1982). In perennial crops such as citrus, soil samples may also provide root density estimates which reflect tree vigor (Cahoon, Harding & Miller, 1956) and can be used to measure the severity of nematode infestations. For example, in soil samples containing a given nematode population level, the infestations should be expected to have greatest effect on trees with the fewest feeder roots per sample.

Nematode distribution in the rhizosphere of individual trees can influence research sample strategies when individual trees represent the experimental unit. Knowledge of the nematode distribution about single trees can also influence sample strategies for estimating grove infestation levels by directing attention to locations with highest population densities which require the least effort for adequate sample procurement (Ferris & McHenry, 1976).

The distribution of *Tylenchulus semipenetrans* did not vary by location beneath the canopy of 4-year-old lime trees (McSorley & Parrado, 1982 *a*) and within-tree variation was greater than that between trees (McSorley & Parrado, 1982 *b*). Although the vertical distribution of *T. semipenetrans* in mature citrus groves has been described (O'Bannon & Stokes, 1978; Hamid, Van Gundy & Lovatt, 1985), less has been written about its horizontal distribution. Similarly, the horizontal distribution of citrus feeder roots has been documented more adequately in young (Castle & Youtsey, 1977;

McNamee, 1955) than in mature (Cahoon, Harding & Miller, 1956) trees.

The surveys described herein were conducted to ascertain the spatial distribution in mature groves of citrus feeder roots and associated populations of the parasite *T. semipenetrans*. Vertical and horizontal distributions were considered with respect to questions about optimum sampling location and depth and sources of variability in sample data.

Materials and methods

All samples were taken with 2.5 cm i.d. steel soil probes and consisted of single soil cores from either 0 to 30 or 0 to 60 cm soil depth. Samples were processed by passing soil through a 2 mm mesh screen after which the soil was hand mixed and juvenile and male *T. semipenetrans* in 60 cm^3 subsamples were extracted during 48 h on Baermann funnels. Feeder roots less than 2.5 mm dia remaining on the sieves were rinsed and oven-dry (100°; 24 h) weights were measured.

Samples were obtained on three occasions in 1985. Individual trees were intensively sampled on two sample dates in order to collect data to design sample schemes for estimating *T. semipenetrans* population levels and feeder root densities in small plot studies. On the third occasion, samples were collected from random trees in a 4 ha grove to obtain a data base to optimize sampling to determine general grove infestation levels. Irrigation of groves in these surveys was by means of overhead sprinklers.

One 8 May, 99 samples were obtained from a 9 × 11, 0.9 M grid pattern (Fig. 2) around a single tree in a grove in Bowling Green, Florida. The tree was a 20-year-old " Hamlin " orange growing on a sour orange rootstock in a non-bedded grove on loamy sand. Sample depth was 30 cm. On 23 May, two 30-year-old trees in a Babson Park, Florida grove were sampled as described above. Trees were " Valencia " orange growing on rough lemon rootstocks in fine sand in a non-bedded grove. Trunk circumferences and canopy branch architecture of the two adjacent trees were similar; however, one tree was vigorous while the other indicated a condition of citrus blight. To confirm this diagnosis, trunk water uptake was measured on both trees and cambium samples were analyzed for zinc levels (Graham, Timmer & Lee, 1983).

One 60 cm deep sample per tree was collected at a distance of either 0.76, 1.52, 2.28, 3.05 or 3.81 m from the trunk or at the drip line of 60 trees on 23 August, in the Bowling Green grove described previously. Feeder roots and nematodes from 0 to 30 cm depth and from 30 to 60 cm depth were extracted separately. Ten samples per distance from the trunk were collected from randomly selected trees within a 4 ha section of the grove. The precision from using 0 to 30 cm nematode population levels to predict population levels in the zone 0 to 60 cm was tested by a combination of linear regression and simulation techniques. The number of *T. semipenetrans* from soil cores 0 to 60 cm deep were regressed against population levels in the first 30 cm of each core. A Fortran program was also used to randomly select data to simulate the effect of increasing the number of cores in a sample on the precision with which 0 to 30 cm population levels could be used to predict levels from 0 to 60 cm. For a given number of soil cores per sample, 0 to 30 cm populations from randomly selected locations were averaged and used in the linear regression formula described above to predict the mean 0 to 60 cm population level from the same locations. Percent deviation was calculated as the absolute value of (observed-predicted)/observed values (Goodell & Ferris, 1981). The mean percent deviation for samples of different core numbers was estimated from 1 000 simulations for each sample size.

Results

Feeder root density and nematode population levels declined with increasing distance from the tree trunk (Figs 1-5). Samples taken from individual healthy trees in Bowling Green and Babson Park (Fig. 1) reflect

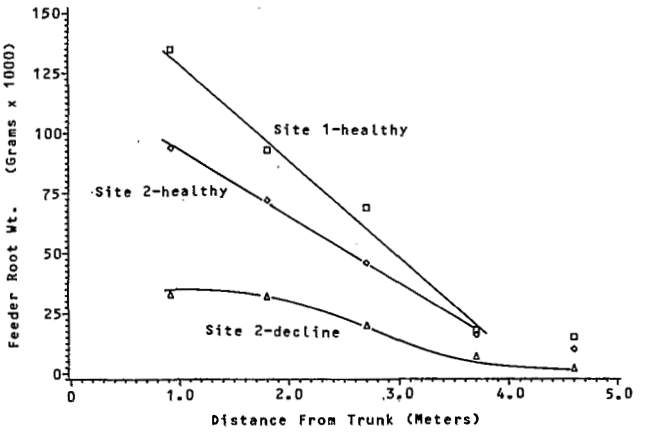

Fig. 1. The horizontal distribution of sour orange (square) and rough lemon (diamond and triangle) feeder roots, in the top 30 cm soil depth. Symbols are mean root weights obtained from 8 to 22, 2.5 cm dia soil cores. Data are plotted at a maximum distance of the arbitrary ranges, 0 to 0.9 m, 0.91 to 1.85 m, 1.86 to 2.7 m, 2.71 to 3.7 m and 3.71 to 4.6 m.

similar patterns of feeder root growth with average densities in cores taken from outside the canopy drip line 12 and 14 % of the densities in cores taken nearest the trunk. Linear regression of standardized root data from the two healthy trees resulted in similar slope values of — 0.29 and — 0.30, indicating very similar relative

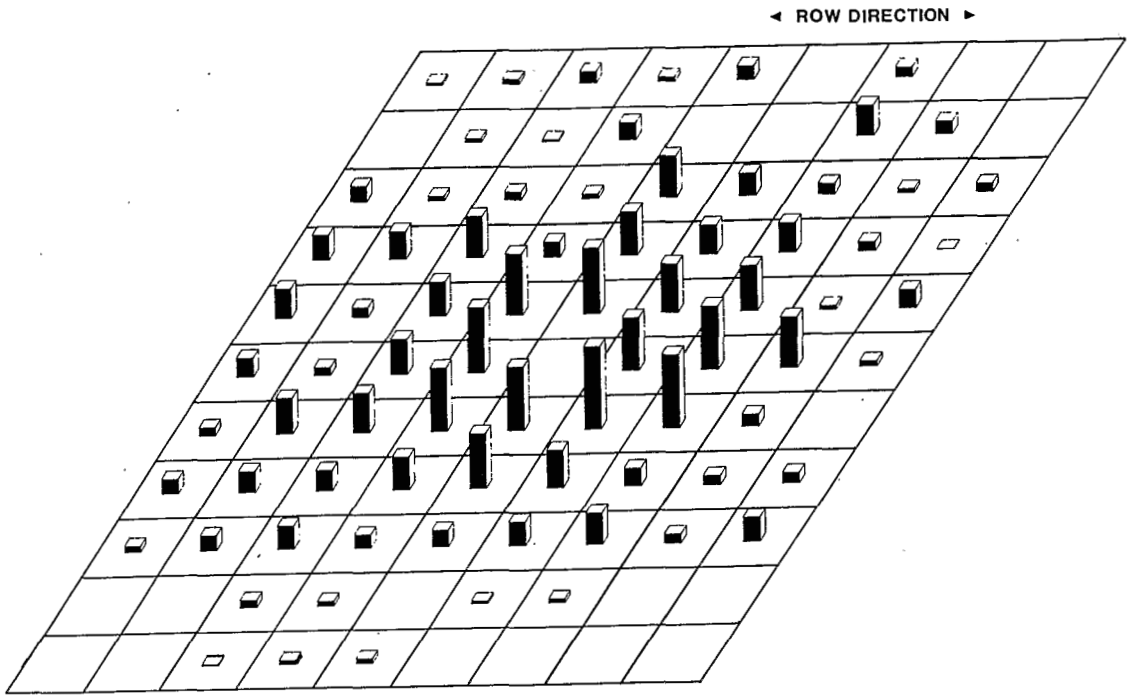

Fig. 2. The horizontal distribution of sour orange feeder roots in the first 30 cm soil depth. Height of vertical bars correspond to feeder root weights at 0.9 m intervals from the tree trunk which is represented at the centermost square section.

distributions in trees from two separate groves. Root density at the drip line within rows where canopies abut one another were higher than at the drip line between rows (Fig. 2). The root distribution from the diseased tree at Babson Park was more sigmoid in shape (Fig. 1). Mean root density at different distances from the trunk of the diseased tree ranged from 56 to 80 % less than corresponding densities under the healthy tree.

Samples taken from the 60 trees in Bowling Green indicated a sigmoid horizontal distribution of feeder roots in the first 30 cm depth and a more uniform distribution in the second 30 cm (Fig. 3). Within the first 1.5 m from the trunk, approximately 2.4 times greater ($p = 0.001$) feeder root density was recovered from the first 30 cm soil depth than from the second. There was no significant difference in mean feeder root density at 0 to 30 or 30 to 60 cm depths when samples were procured at or beyond the drip line. This trend resulted in high autocorrelation between root density from 0 to 30 and 0 to 60 depths, so that the linear regression model $y = 25.8 + 1.1\,x$ predicted 75 % of the variability in the 0 to 60 cm root densities from the corresponding 0 to 30 cm densities.

Nematode population density paralleled feeder root density in all experiments. Standardized data show the relationships between feeder root and nematode

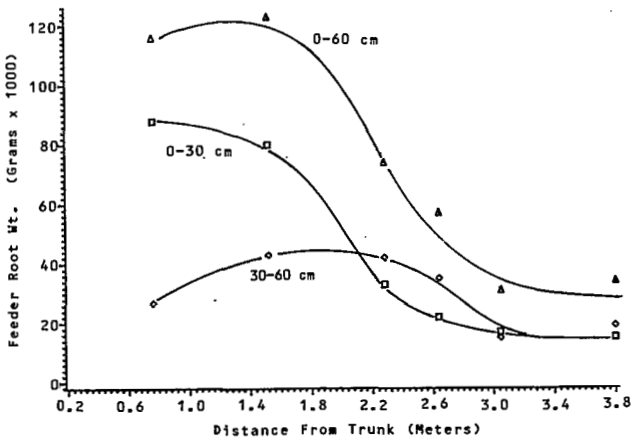

Fig. 3. The horizontal distribution of sour orange feeder roots in the first (square), and second (diamond) 30 cm soil depth and cumulative distribution in the first 60 cm soil depth (triangle). Each point represents the mean root weight obtained from 10, 2.5 cm dia soil cores collected from 10 different trees.

population densities at 0 to 30 cm depth from the single tree experiment at Bowling Green (Fig. 4) and at 0 to 60 cm depth from the multiple tree experiment at the same site (Fig. 5). However, unlike feeder root density,

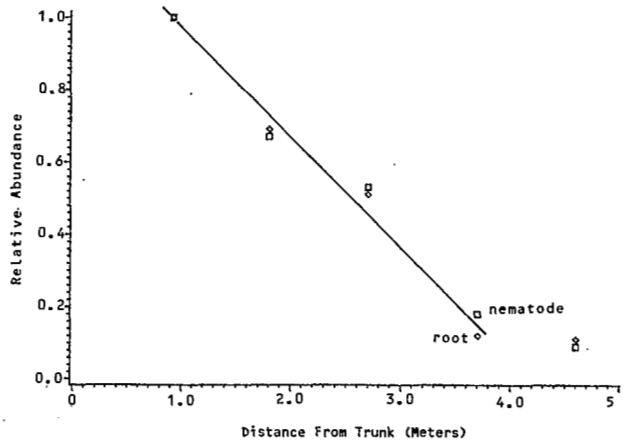

Fig. 4. The relative abundance in the first 30 cm soil depth of sour orange feeder roots and *Tylenchulus semipenetrans* juveniles and males as a function of the distance from the trunk. Relative abundance was calculated by dividing each observation by the highest root weight or population level in the survey. Symbols represent mean root weights or population levels obtained from 8 to 22, 2.5 cm dia soil cores. Data are plotted at maximum distance of the arbitrary ranges, 0 to 0.9 m, 0.91 to 1.85 m, 1.86 to 2.7 m, 2.71 to 3.7 m and 3.71 to 4.6 m.

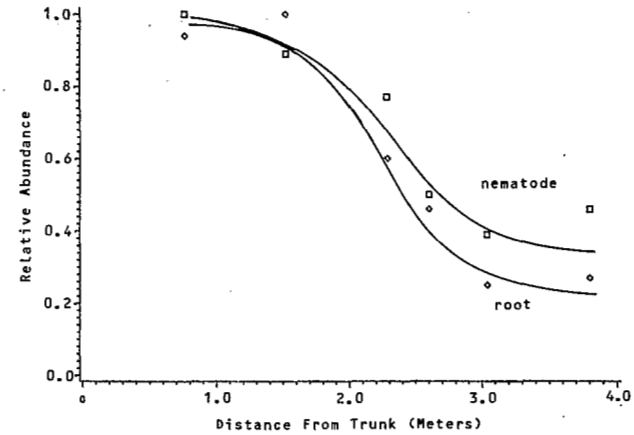

Fig. 5. The relative abundance in the first 60 cm soil depth of sour orange feeder roots and *Tylenchulus semipenetrans* juveniles and males as a function of the distance from the trunk. Symbols represent mean root weights or population levels obtained from 10, 2.5 cm dia soil cores.

T. semipenetrans population levels were not significantly higher in the first than the second 30 cm soil depth. Thus, there was a non-significant increase in nematodes/g feeder roots at the lower soil depths. Within a given soil strata, there were no differences in *T. semipenetrans*/g root by horizontal location. For example, in the single tree survey at Bowling Green, the mean population level/100 cm^3 soil beneath the canopy was 4.3 times higher (2 051 *vs.* 476, $p = 0.001$) than the population level in row middles, while there was no

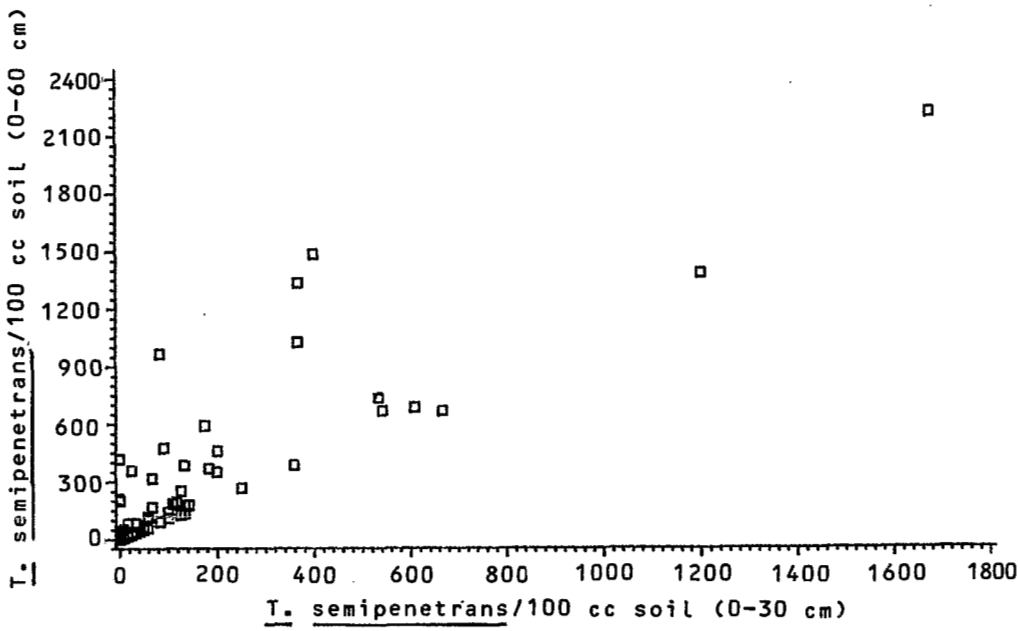

Fig. 6. Relationship between soil populations of *Tylenchulus semipenetrans* juveniles and males extracted from the first 30 cm soil depth to those extracted from the first 60 cm soil depth.

significant difference in nematodes/g feeder roots (64 425 *vs.* 57 174) at the two locations. Seventy-three percent of the variability in population levels (per unit soil) from 0 to 60 cm was described by the linear regression model $y = 12.1 + 1.28\ x$, where x is the population level recovered in the 0 to 30 cm depth (Fig. 6). Simulated use of multiple cores to predict mean

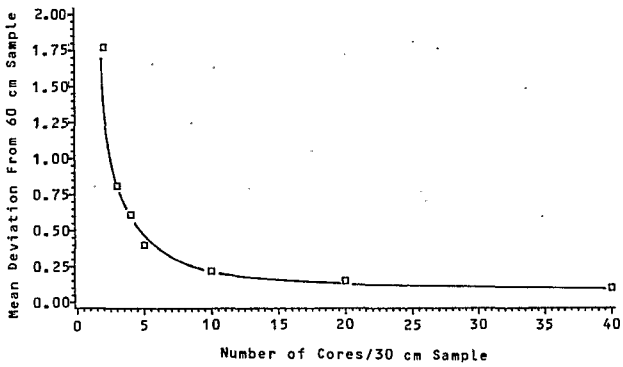

Fig. 7. Effect of increasing core number/sample on deviation of predicted from actual mean *Tylenchulus semipenetrans* population level in 0 to 60 cm soil depth. Points represent mean deviation of 1 000 simulations/core number. The appropriate number of data (illustrated in Fig. 6) were randomly selected and mean 0 to 60 cm population levels were estimated from the formula $y = 12.1 + 1.28\ x$ where x = mean population level from 0 to 30 cm portion of the samples.

population levels at 0 to 60 cm from 0 to 30 cm measurements indicated an exponential decline in prediction error as core number increased (Fig. 7). Increasing sample size from 1 to 2 cores reduced mean deviation of predicted from observed population levels by 85 %.

Discussion

The information needed from soil sampling related to agricultural nematology depends on the purpose of the exercise. Two different areas in which sampling is particularly important are crop loss assessment and damage forecasting. Sample estimates of the horizontal distribution of feeder roots and *T. semipenetrans* were similar in each of the three surveys reported above and reflect on aspects involved in both measuring and predicting losses. Since different information is required for each of those purposes, they are considered separately.

A regression approach is frequently used to assess crop losses associated with different levels of a pest. In regression studies, more information is required to assess nematode related crop losses in mature groves or orchards than in field crops or in grove or orchard replant situations. Within a grove, the experimental units are trees or blocks of trees which are less uniform at the outset of an experiment than newly sown annual plants or seedlings in replanted groves. Thus, tree condition must be ascertained initially so that an attempt can be made to project the different growth (yield)

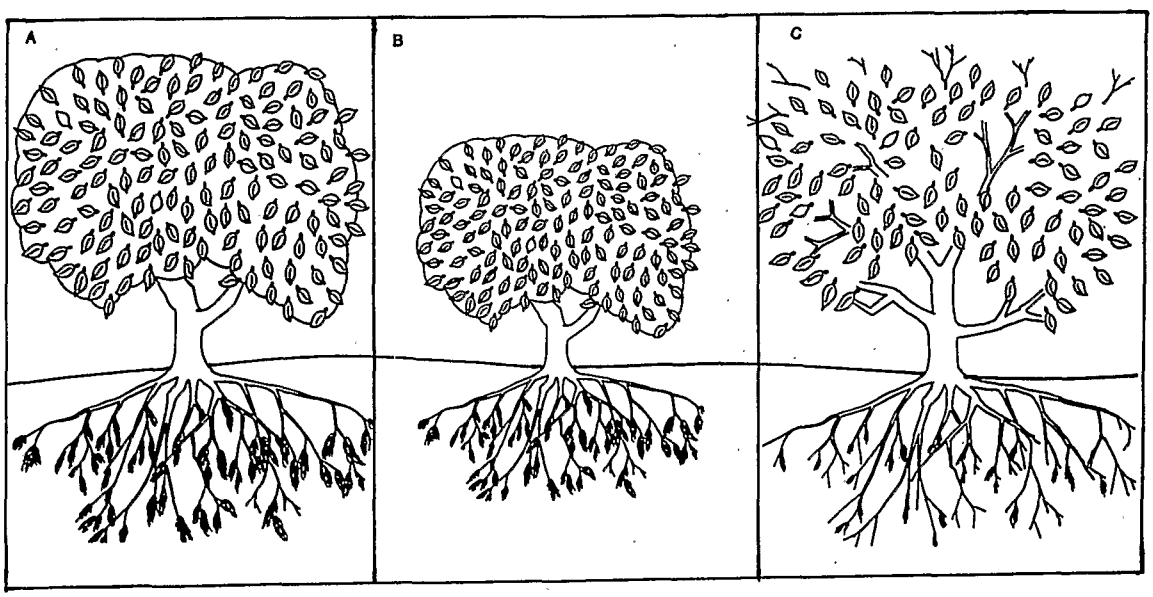

Fig. 8. Diagram illustrating major sources of experimental error where growth of mature perennial crops is regressed against nematode infestation level for crop loss assessment. Experimental units (trees) may be of different size (A *vs.* B), quality (A *vs.* C) or both (B *vs.* C).

potential of individual experimental units. Growth measurements, obtained later in an experiment, can then be standardized prior to evaluating the damage related to nematode parasitism. The diagram in Figure 8 illustrates some types of variability encountered in mature groves. Within a given planting site or area of ground, different tree size (A *vs.* B) or condition (A or B *vs.* C) is related to growth potential regardless of nematode infestation level. Feeder root density under a given soil surface area is one measure of tree size and condition which can be obtained during nematode sampling. Feeder root density may be more closely related to growth potential than other variables such as canopy volume or trunk diameter since feeder root density can change more rapidly than other variables in response to recent stress. Cahoon, Harding and Miller (1956) found linear relationships between feeder root densities and fruit yield of Navel and Valencia oranges when root samples were obtained under canopies or in row middles. In the illustration above, root samples procured from the drip lines or under the canopies of trees A and C would reflect the qualitative differences between them. Markedly different feeder root density was noted (Fig. 1) between two trees of similar canopy architecture but different states of decline in the grove at Babson Park. On the other hand, drip line or under canopy root samples may not reflect size differences between qualitatively similar (A & B) trees. However, random sampling around and under each tree within the equal areas represented by the solid vertical lines should reflect differences between all trees depicted in Figure 8. Reduction of feeder root densities with distance from the trunk noted in all surveys (Figs 1-3) will result in different quantities being obtained even from trees A and B above. Thus, some of the growth variation in research units which is unrelated to nematode parasitism can be controlled if samples are randomly procured within a constant, quantitatively defined area around each tree, rather than with reference to the tree architecture.

Similar consideration is necessary to measure nematode effects in a meaningful way. Random sampling within each of the equal areas depicted by the solid vertical lines in Figure 8, will permit estimation of the nematode population density in a given soil volume beneath each tree. However, a given number of nematodes/unit volume soil represents a different infestation level in each case since the feeder root density is variable. Expressing nematode population levels/g of feeder roots presents a more accurate reflection of the parasitic damage potential. This is fortunate, since the horizontal distribution of nematode population levels was similar to that of feeder root densities (Figs 4 & 5) so that data in all surveys was less variable when expressed as nematode infestation/g of feeder root tissue than per volume soil. This observation implies that fewer samples need be obtained to accurately estimate nematode infestations in relation to the root mass than to a volume of soil. Similarly, the fact that *T. semipenetrans*/g feeder root did not vary significantly by horizontal location suggests that this relationship can be ascertained by sampling without regard to the tree architecture.

Citrus yield reduction associated with *T. semipenetrans* parasitism is presently forecast based on juveniles/unit volume soil or females/g feeder root (Anon., 1984). Results of the surveys reported herein indicate that sampling done for the purpose of making management decisions based on damage forecasts may be facilitated by the latter type of data. Growers seek to determine an average infestation level in a grove of trees to estimate the proportionate reduction in yield from what is considered to be maximum potential yield of the grove in question. An economic analysis can then be made of the expected financial benefits to be gained from the cost of nematode management. Due to historical harvest information, a grower has no need to standardize innate yield differences between his particular sample units (groves) as is the case in the research plots described above. Thus, the sample area is usually defined with reference to the canopy architecture, generally at the canopy drip line. Since absolute population levels decline with distance from the trunk (Figs 4 & 5), it is important to define a fixed location with reference to the tree architecture if samples are expressed per unit soil when relating crop loss data to forecast samples. Fewer samples may be needed for a certain level of precision when samples are obtained within the canopy rather than at the drip line because population levels are higher near the trunk. In many groves, however, it is difficult to move beneath the dense canopy so that the canopy drip line is the most practical sample location even if more samples are required to predict nematodes/volume soil at a given level of precision. Since populations/g of feeder root do not vary significantly with location, expressing data in this manner permits sampling without regard to location. Within a grove, drip line samples could be combined with under canopy samples whenever possible to take advantage of greater root densities under the canopy.

Citrus root systems can penetrate deeply in sandy, well-drained soils; however, the results of the multiple tree survey at Bowling Green indicate that shallow sampling may adequately reflect the degree of parasitism by *T. semipenetrans* throughout the root system. Although the linear relationship between population levels from 0 to 30 cm and 0 to 60 cm in a 2.5 cm soil core was quite variable, combining multiple cores in a sample markedly improved the relationship in a simulation model (Fig. 7). There was an indication that conditions for parasitism were better at lower soil depths, possibly due to less variable temperatures and moisture. Further study of population levels and edaphic conditions at these and greater depths is needed. Variability of nematode and root distribution under different irrigation methods is another aspect of sample optimization requiring investigation.

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Accepté pour publication le 10 mars 1986.