Seasonal and Spatial Analysis of Populations of *Phytophthora parasitica* in Citrus Orchards in Florida

L. W. TIMMER, Professor, S. E. ZITKO, Biological Scientist II, H. A. SANDLER, Assistant in Plant Pathology, and J. H. GRAHAM, Associate Professor, University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred 33850

ABSTRACT

Timmer, L. W., Zitko, S. E., Sandler, H. A., and Graham, J. H. 1989. Seasonal and spatial analysis of populations of *Phytophthora parasitica* in citrus orchards in Florida. Plant Disease 73:810-813.

Populations of *Phytophthora parasitica* were determined five times per year for a 3-yr period in four citrus orchards. Season of the year, soil temperature, and soil moisture at sampling did not greatly affect measured propagule densities. Horizontal spatial pattern of propagules of *P. parasitica* in each of eight orchards was determined in 49 three × three tree quadrats. Means varied from 0.6 to 16.3 propagules/cm^3, and frequency counts data for populations were best described by a negative binomial probability distribution. Variance-to-mean ratios varied from 2.5 to 12.5, *k* values from the negative binomial distribution varied from 0.11 to 6.97, and Lloyd's index of patchiness varied from 1.12 to 20.17. All indices of dispersion indicated varying degrees of aggregation of propagules. The greatest aggregation was observed in orchards where mean propagule densities were lowest. However, spatial lag correlation analysis did not detect clusters with similar populations in most of the orchards. Sampling of citrus orchards to measure propagule densities can be conducted without regard to time of year or within-orchard location of sample collection, but populations may change from year to year.

Most rootstocks on which citrus is grown are resistant or tolerant to bark infection by *Phytophthora parasitica* Dast. (*P. nicotianae* Breda de Haan var. *parasitica* (Dast.) Waterhouse) (4,16). Nevertheless, feeder roots of most rootstock species and cultivars are susceptible to infection by *P. parasitica* (2,16; J. H. Graham, *unpublished*). Feeder root loss is most severe in nurseries but has occurred in mature orchards in California (9) and Florida (13,15). In California, application of metalaxyl or fosetyl-Al through the drip irrigation system has improved feeder root condition and, in some cases, increased yields (8). Application of four foliar sprays of fosetyl-Al or three soil-surface sprays of metalaxyl per year for 3 yr has increased feeder root densities by 25–100% above untreated controls and has increased yields in some cases (13,15). Thus, feeder root rot appears to be a chronic, often endemic problem whose severity probably depends on rootstock susceptibility, environmental conditions, and populations of the pathogen.

Although threshold values for soil populations of *P. parasitica* have not been firmly established, it has been suggested that orchards with mean densities of less than 10 propagules/cm^3 probably cannot be treated with fosetyl-Al or metalaxyl economically (9,15). Populations of the fungus are highest in the top 23 cm of soil under the drip line of the tree (17). Frequency count data for populations of *P. parasitica* in citrus orchards are best described by a negative binomial distribution, suggesting that the fungus in orchards is aggregated.

In a previous study, approximately 30–40 cores per 4 ha of orchard were needed to reliably estimate populations of *P. parasitica* (17). However, the most appropriate time of year to sample citrus orchards in Florida and the importance of the location in the orchard where samples are taken have not been determined. Thus, the purpose of this study was: 1) to determine the effect of the time of sample collection and temperature and moisture conditions at sampling time on measured propagule densities and 2) to provide additional information on the distribution and the horizontal spatial pattern of *P. parasitica* within orchards.

Florida Agricultural Experiment Station Journal Series No. 9457.

Accepted for publication 3 May 1989 (submitted for electronic processing).

MATERIALS AND METHODS

Seasonal studies. Four orchards in Florida were selected for seasonal studies: I) a Pineapple sweet orange (*Citrus sinensis* (L.) Osb.) orchard on Cleopatra mandarin (*C. reticulata* Blanco) rootstock near Immokalee planted on Oldsmar fine sand, II) a Ruby Red grapefruit (*C. paradisi* Macf.) orchard on sweet orange rootstock near Fort Pierce planted on a Winder sand, III) a Hamlin sweet orange orchard on sour orange (*C. aurantium* L.) rootstock near Fort Pierce planted on Pineda sand, and IV) a Hamlin sweet orange orchard on sweet orange rootstock near Lakeland planted on Tavares fine sand. Sample collection began in March 1985 and continued through February 1988. Five samples were collected per year in each orchard in January-February, March-April, May-June, July-August, and September-October.

Samples were collected from five four-tree plots selected at random in a 1- to 2-ha portion of each orchard. Six soil cores, each 18.5 cm deep and 8.0 cm in diameter (930 cm^3 total volume), were collected at random under the drip line within each plot. Soil was passed through a screen with 3-mm openings, and a 250-cm^3 subsample was withdrawn and taken to the laboratory. Propagule density was determined using a selective medium (6) and procedures described previously (17). A single determination was made for each plot, and propagule densities for the four plots were averaged to obtain the population for that date in the orchard.

Soil moisture was determined gravimetrically for each sample collected and averaged across plots within an orchard on each date to give percent soil moisture. Soil temperature data were obtained from nearby University of Florida Experiment Station records at Lake Alfred for the Lakeland orchard and at Immokalee for the Immokalee orchard. Soil temperatures were measured at the 10-cm depth beneath bare soil similar to conditions at the orchard sites. No soil temperature records were available for the Fort Pierce orchards. Regression analyses were conducted to assess the influence of soil moisture and temperature at the time of sample collection on propagule density of *P. parasitica.*

Spatial patterns of propagule densities of *P. parasitica*. Eight orchards were selected for spatial studies: A) a Valencia sweet orange orchard on sour orange rootstock near Fort Pierce planted on Riviera fine sand, B) a Valencia sweet orange orchard on rough lemon (*C. jambhiri* Lush.) rootstock near Fort Pierce planted on Riviera fine sand, C) a Hamlin sweet orange orchard on sour orange rootstock near Fort Pierce on Pineda sand, D) a Hamlin sweet orange orchard on sour orange rootstock near Bowling Green planted on Zolfo fine sand, E) a mixed Valencia sweet orange and Marsh grapefruit orchard on rough lemon rootstock near Dundee planted on

Candler fine sand, F) a Valencia sweet orange orchard on sweet orange rootstock near Lake Alfred planted on Candler fine sand, G) a Hamlin sweet orange orchard on rough lemon rootstock near Frostproof planted on Adamsville fine sand, and H) a Marsh grapefruit orchard on Carrizo citrange (*Poncirus trifoliata* (L.) Raf. × *C. sinensis*) rootstock near Frostproof planted on Candler fine sand.

A block of mature trees, 21 trees long × 21 rows wide, was selected within each orchard except orchard C, where the block was 24 trees long × 18 rows wide. Each block was divided into 49 three × three tree quadrats (48 in the case of orchard C) consisting of a single, centrally located tree. Soil around the central tree in each quadrat was sampled to determine the propagule density of *P. parasitica*. Eight cores (18.5 cm deep × 8.0 cm in diameter) were collected at the drip line of the tree at the cardinal compass directions. Cores were passed through a screen with 3-mm openings and composited, and a 250-cm^3 subsample was withdrawn for propagule density determinations. Propagule density was determined using a selective medium (6) and procedures developed previously (17). A single determination was made for each quadrat using 10 plates per sample, which gave a detection limit of one propagule per cubic centimeter of soil.

The Poisson, negative binomial, Thomas double Poisson, Neyman type A, Poisson with zeros, and logarithm with zeros probability distributions were examined for goodness-of-fit to propagule density, frequency-count data using a FORTRAN program (3). The *k* value of the negative binomial distribution was calculated (3) when appropriate. Values for Lloyd's (7) index of patchiness and the variance-to-mean ratio were also calculated for each orchard. Spatial auto correlation (lag correlation) analysis was conducted for each orchard as described by Modjeska and Rawlings (10).

RESULTS

Seasonal studies. Propagule densities of *P. parasitica* varied among orchards, years, and sampling dates (Fig. 1). The overall orchard means ranged from 23 to 25 propagules/cm^3 for orchards I, III, and IV; the mean for orchard II was 11.8 propagules/cm^3. Means for each year averaged across orchards, and sampling dates were 23.4, 10.5, and 29.1 propagules/cm^3 for 1985, 1986, and 1987, respectively. The means for sampling date did not follow a consistent pattern; when averaged across orchards and years, the means were 24.4, 15.1, 23.7, 25.4, and 16.6, respectively, in the January-February, March-April, May-June, July-August, and September-October sampling periods. Peaks in population occurred during the spring and summer in orchards I and IV but not in orchards II and III (Fig. 1). When orchard, year, and sampling date were considered as independent factors in an analysis of variance, orchard and year had significant effects on propagule densities, as might be expected. In the analysis of variance, sample date was not a significant factor ($P \leq 0.05$). All of the first-order interactions were significant, indicating that orchard, year, and sampling date did not have clearly separable effects on populations.

Throughout the 3-yr study, soil moisture ranged from 2.9 to 5.6% in one Fort Pierce orchard (III), from 2.9 to 11.7% in the other Fort Pierce orchard (II), from 1.0 to 3.9% in the Immokalee orchard (I), and from 2.7 to 8.2% in the Lakeland orchard (IV). Soil temperature ranged from 16.7 to 32.2 C in south Florida (orchard I, Immokalee) and from 15.2 to 33.3 C in central Florida (orchard IV, Lakeland). For the regression analysis of propagule densities of *P. parasitica* with the soil moisture or soil temperature measured at the time of sample collection, the slopes of the regression lines did not differ significantly from zero. Coefficients of determination (R^2) for soil moisture were 0.0 for the Lakeland orchard (IV) and one Fort Pierce orchard (III), 0.05 for the Immokalee orchard (I), and 0.13 for the other Fort Pierce orchard (II). Coefficients of determination for soil temperature were 0.06 and 0.17, respectively, for the Immokalee orchard (I) and the Lakeland orchard (IV).

Spatial pattern analysis. Mean propagule density varied from a high of 16.3 propagules/cm^3 to a low of 0.6 propagules/cm^3 in the eight orchards studied (Table 1). The negative binomial distribution provided the best fit of the propagule frequency count data from all

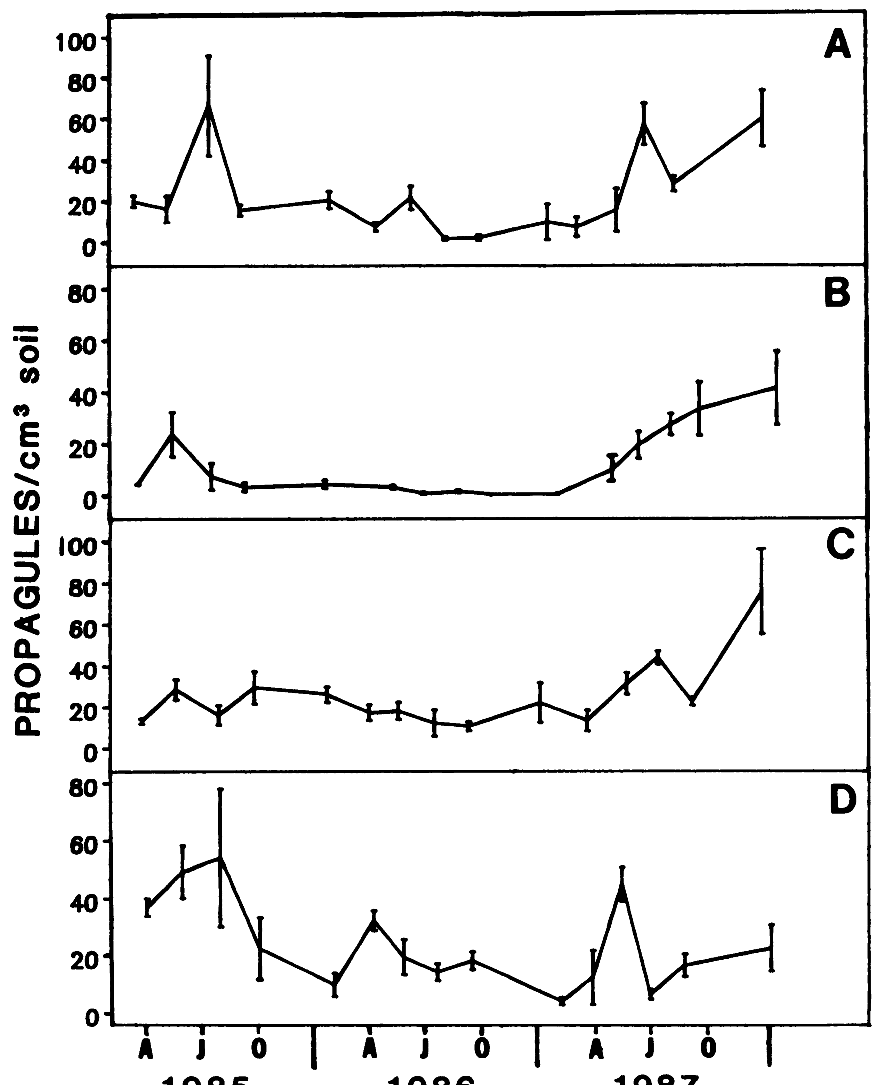

Fig. 1. Seasonal populations of *Phytophthora parasitica* in four Florida citrus orchards over 3 yr. Propagule density at each date is an average of single determinations for each of five replicate blocks of four trees selected at random in the orchard. Bars indicate the standard error of the mean. **(A)** Orchard I: Pineapple sweet orange on Cleopatra mandarin rootstock, Immokalee. **(B)** Orchard II: Ruby Red grapefruit on sweet orange rootstock, Fort Pierce. **(C)** Orchard III: Hamlin sweet orange on sour orange rootstock, Fort Pierce. **(D)** Orchard IV: Hamlin sweet orange on sweet orange rootstock, Lakeland.

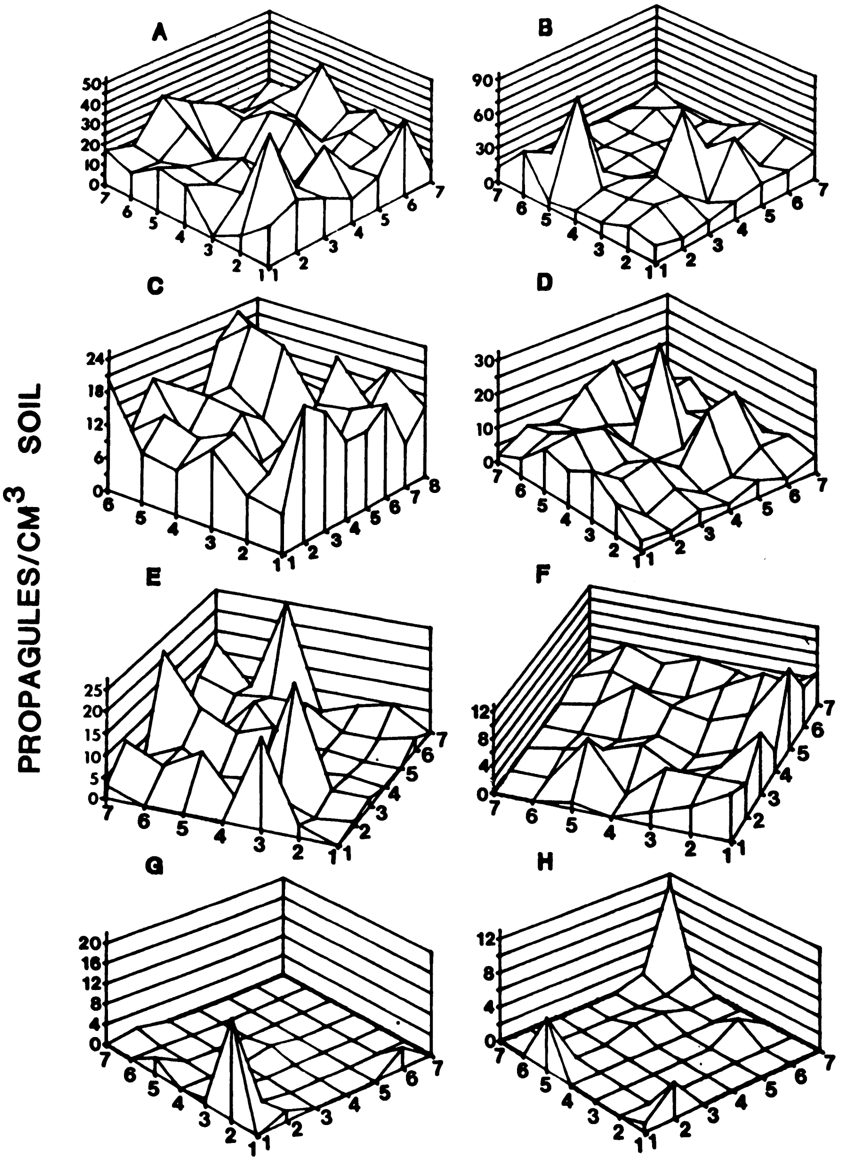

Fig. 2. Three-dimensional response surfaces of the spatial distribution of populations of *Phytophthora parasitica* in eight Florida citrus orchards. Propagule densities at each point were the result of a single determination from a composite sample of eight cores taken around the central tree in a three × three tree quadrat. Orchard designations A through H correspond to those in Table 1.

Table 1. Mean propagule densities and indices of dispersion of *Phytophthora parasitica* in eight Florida citrus orchards

Orchard	Location	Cultivar/rootstock	Mean propagule density/cm^3	Variance-to-mean ratio	k^y	Index of patchiness^z
A	Fort Pierce	Valencia sweet orange/ sour orange	16.3	5.4	3.55	1.27
B	Fort Pierce	Valencia sweet orange/ rough lemon	16.2	9.4	2.84	1.52
C	Fort Pierce	Hamlin sweet orange/ sour orange	13.0	2.5	6.97	1.12
D	Bowling Green	Hamlin sweet orange/ sour orange	6.6	4.6	2.42	1.54
E	Dundee	Valencia sweet orange + Marsh grapefruit/rough lemon	4.6	9.3	0.49	2.80
F	Lake Alfred	Valencia sweet orange/ sweet orange	2.7	2.7	1.28	1.63
G	Frostproof	Hamlin sweet orange/ rough lemon	0.6	5.8	0.11	9.00
H	Frostproof	Marsh grapefruit/ Carrizo citrange	0.6	12.5	0.82	20.17

^{y}Value of negative binomial distribution.
^{z}From Lloyd (7).

eight orchards, with chi-squared values ranging from 32.2 in orchard A to 2.88 in orchard H; all were significant at $P \geq 0.05$. Frequency count data from most orchards also were described by a Neyman type A distribution. Chi-squared values were always higher with the Neyman type A distribution than with the negative binomial distribution.

As indicated by the indices of dispersion, propagules of *P. parasitica* were aggregated to varying degrees in all eight orchards studied (Table 1). The three orchards with the highest populations tended to have more nearly random patterns, as indicated by the low values for the patchiness index. Orchard A had the highest populations in one corner, with locally high populations elsewhere (Fig. 2). Orchards C and D had no obvious pattern of distribution of propagules. The two orchards with the lowest propagule densities had highly aggregated populations with low *k* values and high indices of patchiness (Table 1). In these orchards, *P. parasitica* was not detected at most sample sites, but moderate densities were found at a few locations (Fig. 2). The other orchards tended to be intermediate in propagule densities and in the degree of aggregation (Table 1, Fig. 2).

Although the indices of dispersion indicated varying degrees of clumping in all eight orchards, lag correlation analysis showed very little evidence of spatial autocorrelation among quadrats for population density. No significant correlation was obtained at any spatial lag in six of the eight orchards. Correlations were positive and significant at lag orders one and two within the rows in orchard E and at lag orders two and four within the rows in orchard F. No other significant lag correlations were found.

DISCUSSION

Propagule densities of *P. parasitica* did not appear to be related to the time of year of sampling or to soil temperature or moisture at sampling time. This lack of relationship is probably due to the fact that propagule numbers most likely depend on the amount of root rot that occurred at some unknown time in the past. Most propagules are found in partially decayed root fragments (S. E. Zitko et al, *unpublished*). Probably, root rot is more prevalent under high temperature and moisture conditions, which are most likely to occur in Florida during August and September but can occur at any time of year. Little is known about the exact conditions that result in root rot and propagule production or the longevity of propagules in the soil.

Because the soil temperatures at all sample collection times were greater than 15 C, winter dormancy of propagules as previously described (6,15) was not a factor in this study. In accordance with our previously described procedure (17), samples were preincubated routinely for sufficient time before sampling to have broken dormancy. In citrus areas with cold winters, however, soil temperature could have a significant effect on measured propagule densities.

Most studies of spatial distributions of soilborne organisms have been conducted in annual crops (1,11,14). A few studies have dealt with nematode distribution in permanent pasture or in alfalfa (1,12,18). We previously demonstrated that frequency count data for populations of *P. parasitica* were best fit by the negative binomial distribution (17). Again, in this study, population counts were best fit by a negative binomial distribution, and the indices of dispersion indicated various degrees of clumping. In spite of this, spatial lag autocorrelation failed to reveal any pattern of clustering within the orchards. With *Verticillium dahliae* in potato fields, Johnson et al (5) also found that indices of dispersion indicated clumping, whereas autocorrelation analysis indicated a random spatial pattern of propagules. Propagules of *P. parasitica* may be clustered in citrus orchards, but smaller or larger quadrat sizes would be needed to determine the size and the shape of the clumps.

There are practical implications of spatial and seasonal patterns observed with regard to sampling orchards as well as to initiating fungicide treatment programs. Since there was no consistent effect of season of the year on propagule densities, sampling can be conducted at any time of year. However, there is variation from one sample date to the next and from one year to the next. Thus, caution should be exercised in interpreting the results of a single sampling. We would suggest that, for the present, fungicide treatment programs be applied only to orchards with consistently high populations. Those with low populations should be monitored every year or two to be sure that conditions have not changed.

The spatial patterns indicate that although some aggregation occurs even where population densities are high, differences are not sufficient to warrant treatment of specific rows or sectors of an orchard. Where mean populations in an orchard are low, there may be a few trees or small areas with high populations, but locating these for treatment is not economically feasible. A decision to initiate a long-term fungicide treatment program should be based on orchard condition and history, the susceptibility of the rootstock, and the propagule densities in the orchard, as we suggested previously (17).

In mature citrus orchards infested with *P. parasitica*, we are dealing with an endemic situation on a perennial crop whose root system has been present in the same soil for many years. This may contrast sharply with epidemics of soilborne pathogens that develop from overwintering foci of inoculum in annual crops. Conditions are not static in an endemic situation, however. Changes in nutrient status, soil moisture, temperature, and microbial populations affect citrus root growth and may directly or indirectly affect the propagule densities of *P. parasitica*. More research is needed to determine the relationship of feeder root production and death, environmental conditions, and population dynamics of *P. parasitica* to the health and yield of citrus trees in order to provide a more sound basis for management decisions.

ACKNOWLEDGMENTS

We thank N. L. Berger for technical assistance in conducting this study.

LITERATURE CITED

1. Campbell, C. L., and Noe, J. P. 1985. The spatial analysis of soilborne pathogens and root diseases. Annu. Rev. Phytopathol. 23:129-148.
2. Carpenter, J. B., and Furr, J. R. 1962. Evaluation of tolerance to root rot caused by *Phytophthora parasitica* in seedlings of citrus and related genera. Phytopathology 52:1277-1285.
3. Gates, C. E., and Ethridge, F. G. 1972. A generalized set of discrete frequency distributions with FORTRAN program. Math. Geol. 4:1-24.
4. Grimm, G. R., and Timmer, L. W. 1981. Control of Phytophthora foot rot and root rot. Citrus Ind. 62(10):34-39.
5. Johnson, K. B., Apple, J. D., and Powelson, M. L. 1988. Spatial patterns of *Verticillium dahliae* propagules in potato field soils in Oregon's Columbia Basin. Plant Dis. 72:484-488.
6. Kannwischer, M. E., and Mitchell, D. J. 1978. The influence of a fungicide on the epidemiology of black shank of tobacco. Phytopathology 68:1760-1765.
7. Lloyd, M. 1967. Mean crowding. J. Anim. Ecol. 36:1-30.
8. Lutz, A., and Menge, J. A. 1986. Breaking winter dormancy of *Phytophthora parasitica* propagules using heat shock. Mycologia 78:148-150.
9. Menge, J. A. 1986. Use of systemic fungicides on citrus. Citrograph 71(12):245-252.
10. Modjeska, J. S., and Rawlings, J. O. 1983. Spatial correlation analysis of uniformity data. Biometrics 39:373-384.
11. Nicot, P. C., Rouse, D. I., and Yandall, B. S. 1984. Comparison of statistical methods for studying spatial patterns of soilborne plant pathogens. Phytopathology 74:1399-1402.
12. Noe, J. P., and Campbell, C. L. 1985. Spatial pattern analysis of plant parasitic nematodes. J. Nematol. 17:86-93.
13. Sandler, H. A., Timmer, L. W., and Graham, J. H. 1987. Effect of systemic fungicides on *Phytophthora* populatio..., feeder root densities, and yield of citrus. (Abstr.) Phytopathology 77:1691.
14. Shew, B. B., Beute, M. K., and Campbell, C. L. 1984. Spatial pattern of southern stem rot caused by *Sclerotium rolfsii* in six North Carolina peanut fields. Phytopathology 74:730-735.
15. Timmer, L. W., Graham, J. H., Sandler, H. A., and Zitko, S. E. 1988. Populations of *Phytophthora parasitica* in citrus orchards and tree response to fungicide applications. Citrus Ind. 69(11):40-44, 54.
16. Timmer, L. W., and Menge, J. A. 1988. *Phytophthora*-induced diseases. Pages 22-24 in: Compendium of Citrus Diseases. J. O. Whiteside, S. M. Garnsey, and L. W. Timmer, eds. American Phytopathological Society, St. Paul.
17. Timmer, L. W., Sandler, H. A., Graham, J. H., and Zitko, S. E. 1988. Sampling citrus orchards for estimation of populations of *Phytophthora parasitica*. Phytopathology 78:940-944.
18. Wheeler, T. A., Kennerley, C. M., Jeger, M. J., and Starr, J. L. 1987. Effect of quadrat and core sizes on determining the spatial patterns of *Criconemella spaerocephalus*. J. Nematol. 19:413-419.