Seasonal Patterns Associated with *Tylenchulus semipenetrans* and *Phytophthora parasitica* in the Citrus Rhizosphere

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Florida Agricultural Experiment Station Journal Series R-02611.

We thank Denise Dunn, Nancy Timmer, H. Mauricio Rubio, Balla Traore, and Scott Zitko for technical assistance and W. Garvie Hall and Imre Nyiro for their kind cooperation.

Accepted for publication 3 February 1993.

ABSTRACT

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

Populations of *Tylenchulus semipenetrans* and *Phytophthora parasitica* were measured weekly during 27 mo in an orchard of mature grapefruit trees on rough lemon rootstock in the central ridge region of Florida. The study was conducted to identify potential key climatic and host factors affecting population changes in both parasites. Patterns of change in root mass density and concentrations of root lignin and nonstructural carbohydrate suggested annual as well as seasonal variation in the age structure and nutritional value of the fibrous root system. Numbers of nematode females on roots and juveniles and males in soil were related positively ($P = 0.01$) to root mass density and root concentration of reducing sugars, starch, and total nonstructural carbohydrates. Numbers of nematodes were related inversely ($P = 0.01$) to soil moisture and root lignin content. Numbers of fungal propagules in the soil were related inversely ($P = 0.01$) to root starch concentration, and the amount of fungal protein in roots (as measured by ELISA) correlated positively ($P = 0.01$) with soil temperature. Multiple linear regression models with three independent variables (soil moisture, root starch concentration, and an in vitro index of nematode preference for root exudates) explained 86 and 84% of the variation in mean monthly population densities of female nematodes on roots and juveniles and males in soil, respectively. The average monthly levels of *P. parasitica* protein in roots were best fit ($R^2 = 0.76$) by linear models incorporating soil moisture, temperature, and concentrations of ketone sugars in roots. Root mass density and concentration of ketone sugars explained 86% of the monthly variation in *P. parasitica* propagule densities in soil. Experimental verification of causality in these relationships would help explain seasonal and annual variation in the parasite burden posed by these two pathogens.

Additional keywords: nutrition, sampling.

The citrus nematode *Tylenchulus semipenetrans* Cobb and the fungus *Phytophthora parasitica* Dastur are common parasites of citrus worldwide. The nematode causes citrus slow decline characterized by a reduction in fibrous root biomass and symptoms of root dysfunction, such as small, sparse leaves and reduced yield often resulting from smaller-than-normal fruit (13,14). Recent fungicide trials in Florida demonstrated that *P. parasitica* reduces fibrous root density of mature trees, referred to as fibrous root rot, sufficiently to affect crop yield (41). *P. parasitica* also causes damping-off of seedlings, foot rot or gummosis on tree trunks, and brown rot of fruit.

Some edaphic and host factors that influence the epidemiology of slow decline and fibrous root rot have been characterized. Population size of *T. semipenetrans* is affected by soil texture and organic-matter content (34,50); soil moisture (51,52), temperature (36), acidity (50), oxygen content (52), salinity (32), and host genotype (25,28); developmental stage (9); and chemical composition (10) of citrus fibrous roots. Development of fibrous root rot is favored by warm soil temperature and cycles of wetting and drying sufficient to stress citrus roots. At the same time, free water must be available in well-aerated soil to produce sporangia and release zoospores (22).

Seasonal population-density patterns of *T. semipenetrans* on citrus and, to a lesser degree, *P. parasitica* have been reported (4,7,23,35,38,40,53). Nematode population densities often are highest during late autumn and spring, decline during winter, and reach their lowest levels during midsummer. Population growth generally is thought to coincide with flushes of new fibrous roots (23). The causes of low summer-population densities of *T. semipenetrans* are unclear.

Soil temperature and rainfall usually are most favorable for growth of *P. parasitica* during summer. Until recently, however, assays of fungal inoculum densities primarily measured chlamydospores from rhizosphere soil and decaying roots (45). Thus, studies of seasonal patterns of *P. parasitica* propagule densities primarily have measured a survival stage of the fungus. Recently, a commercial enzyme-linked immunosorbent assay (ELISA) technique for measurement of *P. parasitica* protein levels in root tissue became available, enabling study of the root-infection process (45).

The present investigation was undertaken to study seasonal population development of *T. semipenetrans* and *P. parasitica* under identical edaphic and meteorological conditions, as a benchmark for optimizing control strategies (sampling and treatment) when both parasites are present in an orchard. We also measured selected edaphic, climatic, and host variables to ascertain key factors that might limit population development of either parasite. The relative importance of root quality compared to root abundance and environmental factors as a reflection of parasitic activity was of particular interest.

MATERIALS AND METHODS

Site description. The study site was a 0.9-ha block in a commercial orchard of mature (>40 yr) grapefruit (*Citrus paradisi* Macfady) trees on rough lemon (*C. jambhiri* Lush.) rootstock planted (4.5 × 8.7 m) near Bartow, FL. The soil was a deep Astatula sand (hyperthermic uncoated Typic Quartzipsamments; 92% sand, 2% silt, and 6% clay). Each tree was irrigated with a low-volume microsprinkler (≃2-m wetting radius) located in-row beneath the canopy. Weeds were chemically managed in-row and disked between rows. Normal commercial fertilization and foliar pest control for juice production were practiced. Neither nematicides nor soil-applied fungicides had been used in the orchard in the previous 5 yr. Soil moisture (15-cm depth) was

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measured daily with two tensiometers. Ambient and soil (15 cm depth) temperatures were measured continuously with a mechanical two-point thermograph.

Sampling methods. Two soil samples were obtained from the site weekly for 27 mo. Fifteen soil cores (30- × 2.5-cm diameter) were combined in each sample. Cores for each sample were systematically (five cores per pair of rows) obtained from 15 trees across the entire site. Samples were taken from the irrigated zone beneath the tree canopy. Each sample was hand-mixed, and two 60-cm^3 subsamples were extracted for 48 h on modified Baermann funnels. The remainder of the soil was passed through a 2-mm sieve, and 50–100 cm^3 of soil was processed using a selective medium (27) and published procedures (46) to determine the inoculum density of *P. parasitica*. Material retained on the sieve was rinsed with water, and citrus fibrous roots (<2-mm diameter) were separated from debris. Fresh roots were weighed, and a 1-g subsample was removed and dried for 24 h at 70 C. The remaining roots were stored at 4 C until they were processed to recover root-inhabiting stages of *T. semipenetrans* (6).

At the end of the survey period, oven-dried root samples were weighed and ground to pass through a screen with a 40-mesh pore size. Aliquots of the tissue were analyzed for carbohydrate, lignin, and nitrogen content. Other aliquots were processed by ELISA (Agri-diagnostics, Cinnamonson, NJ) to measure levels of *P. parasitica* protein (45) and were used in two bioassays.

The precision of the sample nematode and fungus population measurements were estimated. Taylor's Power Law (44) was fit to the data by regressing the natural logarithms of the variances against those of the means of the 103 pairs of weekly samples. The resulting regression formulae were used to estimate the variances associated with mean densities of either organism. Sample precision was expressed as the ratio of the estimated standard error ($n = 2$) to the mean.

Root chemical analyses. Root carbohydrates were extracted by boiling 50 mg of tissue in 15 ml of water for 2 min, followed by centrifugation (8,000 × *g*) for 2 min. Glucose oxidase (Sigma Chemical Company, St. Louis, MO) was used to analyze glucose in the supernatant. Soluble starch (amylose) in the supernatant and insoluble starch in the pellet were analyzed with amyloglucosidase (Sigma Chemical Company) and corrected for free glucose (43). Glucose was used as the standard in these measurements. The arsenomolybdate method was used to analyze reducing sugars (33), and resorcinol reagent (3,39) was used to analyze ketone sugars (fructans, fructose, and sucrose, among others). Fructose was used as a standard to measure reducing sugars because previous studies with HPLC methods demonstrated that fructose exceeded free glucose in rough lemon roots from trees comparable to those in this study (19).

Lignin was measured by acid hydrolysis of the pellet after a series of ethanol washes. The remaining lignin residue plus minerals was weighed, ashed, and reweighed to determine the separate lignin and mineral weights (18). The ethanol washes were evaporated (50 C) and resuspended in 15 ml of boiling ethanol. Phenolic compounds in the ethanol were quantified with Folin-Ciocalteu reagent (42). Gallic acid (crystalline, Sigma Chemical Company) was used as a standard acid, and activity was expressed in units of gallic acid equivalents.

Bioassays. Juvenile and male *T. semipenetrans* used in the bioassays were extracted from rough lemon roots obtained from the survey orchard. Roots were vigorously shaken in plastic bags filled with water that then was poured through 25-μm sieves. The contents of the sieves were placed on Baermann funnels for 20 h to obtain active nematodes.

From tissue samples pooled by month ($n = 27$), 50-mg aliquots of dried, ground root tissue were soaked in 5 ml of distilled water for 1 h, filtered, (0.45 μm), and frozen. The preference of *T. semipenetrans* for these leachates was measured. Two wells (1.0-cm diameter × 0.5-cm deep) were cut in 2.0% water agar (Difco Labs, Detroit, MI) plus streptomycin sulfate (1,000 mg/L) 0.25 cm from opposite edges of 15-cm-diameter petri dishes. One well was filled with 150 μl of root leachate and the other with 150 μl of tap water. The liquid in the wells was permitted to evaporate and diffuse into the agar for 2 h in uncovered dishes, after which the wells were filled with molten agar. Approximately 500 nematodes in 50 μl of water were pipetted onto the center of the agar surface. When the water drops containing nematodes had nearly evaporated, the lids were placed on the dishes, which were then stacked and wrapped in black plastic to exclude light. To avoid temperature gradients across the agar, the stacks of plates were rotated (1 rpm) on small turntables for 24 h at 25 C and were stored at 4 C to stop nematode movement. Four plates were prepared for each monthly sample. The bioassay was repeated once.

To evaluate patterns of nematode migration, the dishes were mounted on a template made from an inverted petri dish on which lines were scribed in six equal wedge-shaped sectors. A 1.5-cm-diameter circle, circumscribing the point at which nematodes were originally located, was also scribed on the center of the template. Dishes mounted on the template with the wells centered in opposite sectors were viewed through a dissecting microscope. Nematodes that moved beyond the circle and into the sectors that contained wells were counted. A preference index, $I_p = y/(x + y)$, was calculated, in which y = number of nematodes in the sector containing the leachate well and x = nematodes in the sector containing the water well.

Two bioassays were conducted to investigate the ability of fibrous roots collected during each survey month to support *P. parasitica* growth. In one bioassay, 2 ml of supernatant (pooled by month) from the carbohydrate extraction procedure was used; in the other, 1 ml of root leachate, as in the nematode bioassay, was used. Equal quantities of roots or supernatant from each weekly sample were pooled by month. In each bioassay, 1 ml of test solution was added to 9 ml of mineral salts medium consisting per liter of 0.5 g of KH_2PO_4, 0.5 g of $(NH_4)_2H_2PO_4$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 100 mg of thiamine-HCl, 0.1 g of $CaCl_2 \cdot 2H_2O$, and 16.7 mg of 138 Fe sequestrene (Ciba-Geigy Corp., Agric. Div., Greensboro, NC). Two-millimeter-diameter plugs from the margin of actively growing colonies of *P. parasitica* (Hall isolate) on corn meal agar (Difco Labs, Detroit, MI) were placed in each 25-ml flask. Flasks were placed on an orbital shaker at 60 rpm and were incubated for 7 days at 23–25 C. Mycelia were filtered onto preweighed 47-mm nylon filters with 0.45-μm openings, air-dried at 23–25 C, and weighed. Mycelial biomass was determined from five replicate flasks per sample date. Growth responses to the extracts from each monthly sample were compared to fungal biomass measured with ELISA in corresponding aliquots of root tissue. The assay with leachate was repeated and produced similar results. The assay with extract was not repeated because ELISA and bioassay measurements were unrelated.

Nematode energetics. Calorie requirements for female nematode respiration were estimated to compare a portion of the energy requirements of the nematode with the energy available in the measured nonstructural carbohydrate in fibrous roots. Nematode fresh weight was estimated (2) using published morphometric measurements (26). Total monthly oxygen consumption of female nematodes per gram of roots (dry weight) was estimated (29) based on a fresh weight of 1.79 μg per female and was corrected for soil temperature (12). A calorie equivalent (C_F) for the monthly oxygen consumption by females was 4.78 calories per milliliter of oxygen (54). The monthly average calorie value (C_R) of available carbohydrate in roots was defined as 3,625 per gram (dry weight) of mean total nonstructural carbohydrate. Monthly estimates of the caloric energy (E) in the fibrous roots available to nematodes were calculated as $E = C_R + C_F$.

Data analyses. Average monthly measurements of all variables were used for most data analyses. Because of the large number of factors involved, pooling monthly measurements served to dampen the effects of unknown response-time lags. A linear correlation coefficient ($n = 27$) matrix between all variables was generated. Nematode and fungal population measurements were plotted against all other measured variables to ascertain obvious linear or nonlinear relationships. Multiple linear regression models were fit to data collected during the entire survey period and to data collected during April–November 1988 and 1989, periods

of active population change. Natural logarithms of measurements of *T. semipenetrans* (juveniles and males per 100 cm^3 of soil or females per 100 cm^3 of soil) or *P. parasitica* (propagules per 1 cm^3 or nanogram of fungal protein per gram of root) were regressed against the other measured root quality and environmental variables using a stepwise procedure (Minitab Statistical Software). Two-tailed *t* tests were used to make a priori comparisons of the levels of variables measured at different times.

RESULTS

Sample precision. Two 15-core samples resulted in estimated standard errors ranging from 8 to 12% of the mean numbers of *T. semipenetrans* juveniles and males recovered from soil and in standard errors ranging from 9 to 46% of the mean numbers of females recovered from roots during the course of the survey (Table 1). Estimates of the standard error/mean ratio for fungal propagules in soil during the study ranged from 0.10 when population densities were high to 0.59 when they were low (Table 1). This trend was reversed for measurements of *P. parasitica* from roots, for which estimated ratios increased from 0.23 when fungal protein concentrations were low to >0.39 when concentrations were greatest. Coefficients of determination of the linear fit of the logarithms of the variances to those of the means were 26–28% for parasites recovered directly from soil and were 12–73% for measurements of parasites from roots.

Root abundance and chemistry. Root mass density increased by 75% (2.26 to 3.97 mg/cm^3 of soil) during two distinct periods between May and November 1988 (Fig. 1A). After a gradual winter decline, the density increased during late April–May 1989 to its highest level (5.39 mg/cm^3 of soil) during the survey. Density declined during June and September 1989 and increased only slightly during the remainder of autumn. After a freeze on 24 December 1989, which killed most of the above-ground tree tissue, root mass density declined continually until it reached an average level of 0.61 mg/cm^3 of soil during June 1990, when the study was terminated. The average weekly root mass density during summer (June–August) was 30% higher ($P = 0.01$) in 1989 than in 1988.

Root lignin content increased ($P = 0.01$) between May and July during both years of the study (Fig. 1B). From July to November 1988, lignin concentration declined 20%. A 10% decline ($P = 0.05$) in lignin concentration occurred from July to September 1989, after which levels remained constant until the December freeze. An inverse relationship occurred between lignin content and percent moisture in fibrous roots ($r = -0.68$, n = 103, and $P = 0.01$). Gallic acid units were correlated ($r = 0.41$ and $P = 0.05$) with lignin concentration (Fig. 1C). The correlation was improved ($r = 0.56$ and $P = 0.01$) when gallic acid units were compared to lignin concentration in the succeeding month. Lignin also was negatively correlated with glucose ($r = -0.40$ and $P = 0.05$), starch ($r = -0.61$ and $P = 0.01$), and reducing sugars ($r = -0.56$).

Starch concentration increased in fibrous roots during autumn in 1988 and 1989 (Fig. 1D). Highest levels (1.5–2.0%) were generally attained during January or February each year. Lowest

TABLE 1. Parameter estimates for Taylor's Power Law[a] (TPL) and of sample precision for pairs of soil samples. Each sample was a composite of 15 mixed and subsampled soil cores. Pairs of samples were collected weekly (n = 103) to estimate population density of *Tylenchulus semipenetrans* and *Phytophthora parasitica* in soil or *P. parasitica* protein in roots

	Coefficients of TPL			Estimated SE/mean ratio at density			
Organism[b]	a	b	r^2	N[c]	10N	100N	1,000N
Nematode juveniles and males	0.07	1.83	0.28	0.15	0.12	0.10	0.08
Nematode females	10.70	1.30	0.12	1.03	0.46	0.21	0.09
Fungal propagules	0.68	1.21	0.26	0.59	0.24	0.10	0.04
Fungal protein	0.07	2.22	0.73	0.23	0.30	0.39	0.50

[a] $Y = aX^b$, in which Y = sample variance and X = sample mean.
[b] Juveniles and males per 100 cm^3 of soil; females per gram of roots; propagules per cm^3 of soil; and nanogram of fungal protein per gram of roots.
[c] For nematodes in soil and roots and fungi in roots, N = 10; for fungi in soil, N = 1.

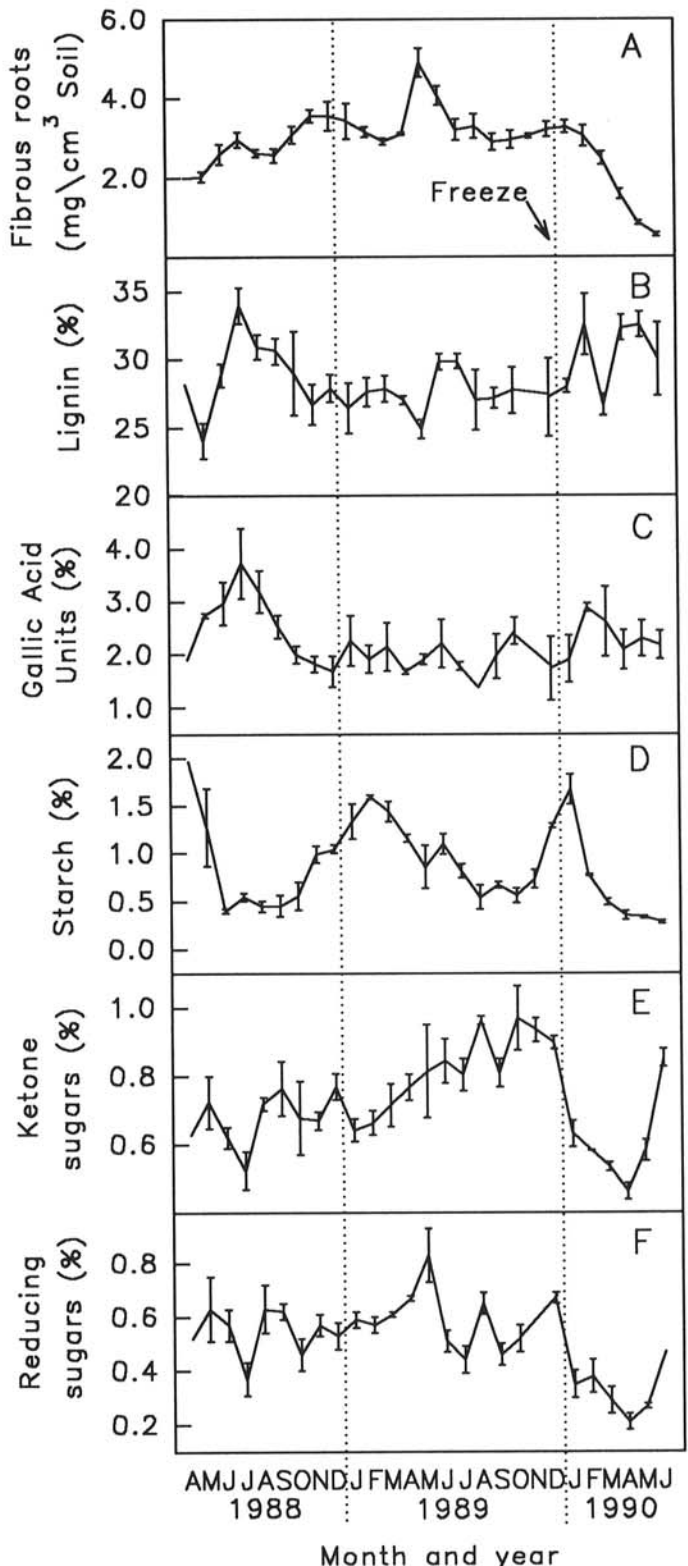

Fig. 1. A, Seasonal patterns in rough lemon fibrous root mass density; **B,** concentrations of fibrous root lignin; **C,** phenolic compounds; **D,** starch; **E,** ketone sugars; and **F,** reducing sugars. Monthly means and standard errors for root measurements are from pairs of weekly samples collected from depths of 0–30 cm.

levels of starch were measured during June–October in 1988 and August–October in 1989. During the summer months (June–August), starch concentration was 30% lower in 1988 than in 1989 (0.57 vs. 0.86%, $P = 0.01$). Prewinter starch accumulation apparently was earlier in 1988 (November) than in 1989 (December). Starch levels declined sharply after the December 1989 freeze.

Concentrations of ketone and reducing sugars exhibited fewer seasonal trends than did other root constituents (Fig. 1E and F). Levels of ketone sugars decreased during the summer of 1988 but increased steadily until December during 1989. After the freeze of 1989, the concentration declined sharply and did not increase until the next summer. The average concentration of ketone sugars during the period of greatest fungal activity in roots (June–September) was higher ($P = 0.01$) in 1989 (0.85%) than in 1988 (0.66%) (Fig. 1E).

Bioassays. No measure of nematode population density was significantly correlated with the bioassay preference index (Table 2). When soil moisture and root starch concentration were considered in multiple linear regression models, however, the preference index explained an additional 13% of the variability in female nematode density when entered last in the equation (Table 3). The preference index explained an additional 14% of the variability in juvenile and male density.

Growth of *P. parasitica* in the bioassay with root leachate was related to the concentration of lignin in roots ($r = -0.49$ and $P = 0.05$), reducing sugars ($r = 0.56$ and $P = 0.01$), and ketone sugars ($r = 0.39$ and $P = 0.05$). No relationships between the measured root variables and fungal growth were significant when supernatant from the carbohydrate extraction process was used as a nutrient source. The concentration of *P. parasitica* in roots was related to fungal growth in the root leachate (Table 2) but not in the hot-water extract. Propagule densities in soil were inversely related to growth ($P = 0.05$) in the hot-water extract (data not shown).

Nematode populations. The seasonal and annual trends of population densities were essentially the same for *T. semipenetrans* juveniles and males in soil and for females on roots (Fig. 2A and B). Annually, population density was bimodal. Maximum levels occurred during late autumn and spring, and minimum densities occurred during midwinter and summer. During the period from summer to autumn, female density increased earlier than did juvenile and male densities. Population densities of nema-

TABLE 2. Linear correlation coefficients (r) between average monthly population measurements of *Tylenchulus semipenetrans* or *Phytophthora parasitica* and selected environmental variables

Environmental variables	*T. semipenetrans* Juveniles and males/sample	*T. semipenetrans* Females/sample	*P. parasitica* Propagules/g soil	*P. parasitica* ng protein/g root
Soil temperature (C)	−0.18	−0.35	0.06	0.51** [a]
Soil moisture (−J/kg)	−0.59**	−0.54**	−0.17	0.25
Root mass density (mg/cm^3 soil)	0.71**	0.77**	0.07	0.26
Nonstructural carbohydrate [b]	0.53**	0.58**	−0.36	0.10
Starch [c]	0.48**	0.56**	−0.49**	−0.21
Ketone sugars [c]	0.34	0.11	0.30	0.34
Reducing sugars [c]	0.61**	0.48*	−0.17	0.03
Free glucose [c]	0.11	0.15	0.23	−0.30
Lignin [c]	−0.54**	−0.55**	0.15	0.09
Nematode I_p (proportion) [d]	0.35	0.21	...	...
Fungal assay (mg mycelium/dish) [e]	...	...	−0.20	0.44*

[a] r significantly greater than zero at $P \leq 0.05$ (*) or $P \leq 0.01$ (**).
[b] Estimated by adding the percent dry weights of starch, ketone sugars, and free glucose.
[c] Percentage of dry weight.
[d] In vitro preference index.
[e] In vitro growth assay.

TABLE 3. Best fit predictive variables from stepwise multiple regression of measurements of *Tylenchulus semipenetrans* or *Phytophthora parasitica* on environmental factors [a]

Pathogen	Soil moisture (%)	Insoluble starch (% dry wt)	Attraction index	Soil temp.	Ketone sugars (% dry wt)	Root mass density	R^2
	T-ratio of independent variables [b]						
Log$_e$ *T. semipenetrans* females/100 cm^3 soil							
1 [c]	−4.30						0.62
2	−4.74	2.49					0.73
3	−5.37	3.00 (<0.02)	2.43 (<0.03)				0.86
Log$_e$ *T. semipenetrans* juveniles and males/100 cm^3 soil							
1	−2.90						0.43
2	−3.28	3.02					0.70
3	−3.78	3.85	2.77 (<0.02)				0.84
Log$_e$ *P. parasitica* (ng protein/g root)							
1				4.64			0.51
2				4.83	2.75		0.63
3	3.05			5.33	3.78		0.76
Log$_e$ *P. parasitica* propagules/cm^3 soil							
1					6.31		0.75
2					7.05	3.12	0.86

[a] Listed in Table 2.
[b] $P \leq 0.01$ unless indicated in parens.
[c] Number of independent variables in model.

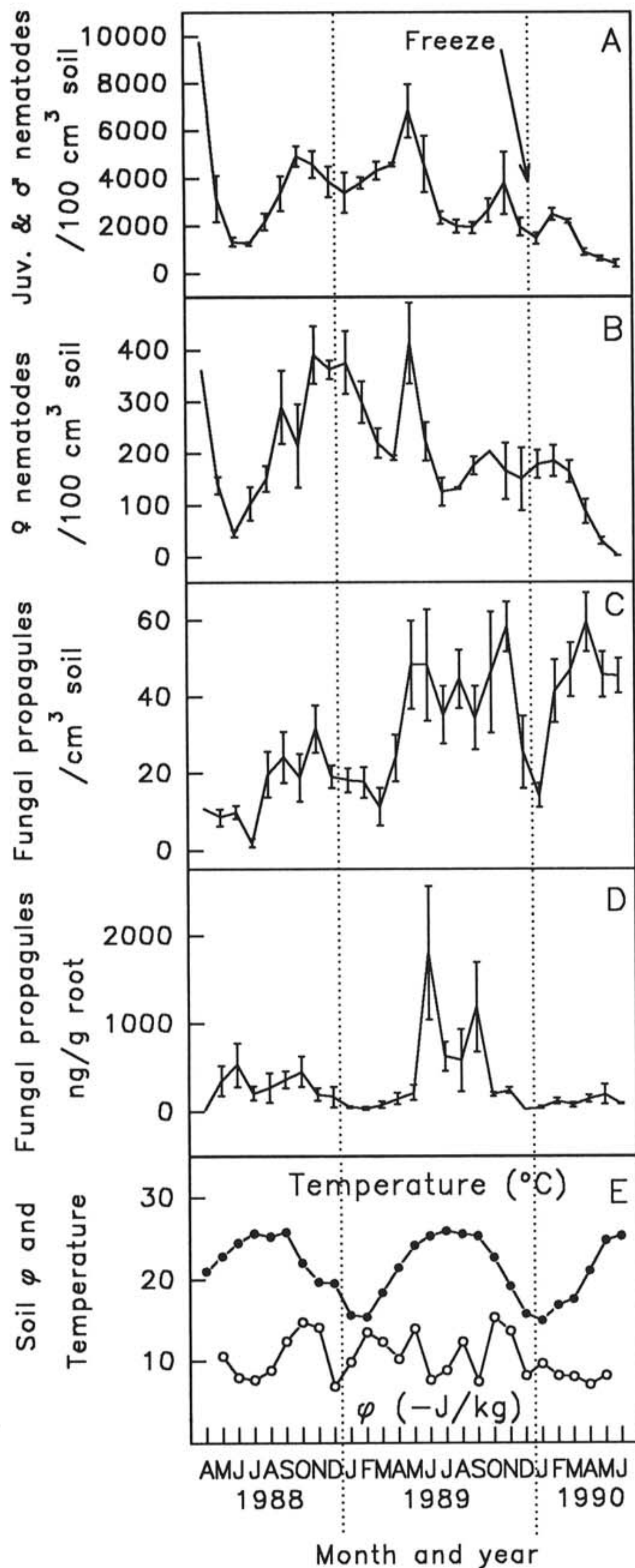

Fig. 2. A, Seasonal patterns in the population densities of *Tylenchulus semipenetrans* juveniles and males from soil; **B,** females from fibrous roots; **C,** *Phytophthora parasitica* propagules from soil; **D,** fungal protein concentrations in fibrous roots of mature grapefruit trees on rough lemon rootstock; and **E,** soil temperature and moisture. Monthly means and standard errors are from pairs of weekly samples collected from depths of 0–30 cm. Soil temperature (15-cm depth) is the average of daily minimum-maximum values, and soil moisture (25-cm depth) is the average of daily tensiometer readings.

todes recovered from soil increased gradually during February–May in 1989; female population density declined during most of the same period, until it increased during May, similar to the trend for root mass density. Levels of juveniles increased by 390%, and females increased by 890% during autumn 1988. The increase in nematode density during autumn 1989 was less than the previous year: 99% for juveniles and 23% for females. The average weekly population density of juveniles and males during summer (June–August) was higher ($P = 0.01$) in 1989 (3,012/100 cm^3 of soil) than in 1988 (1,696/100 cm^3 of soil), as was that of females per 100 cm^3 of soil (155 vs. 100, $P = 0.05$). The average female density per gram of root was also higher ($P = 0.01$) during summer in 1989 (457) compared to 1988 (269). When all nematode life stages were expressed per unit of available carbohydrate (root weight by percent total nonstructural carbohydrate), however, no measurable differences occurred during summer in 1988 and 1989 (Fig. 3).

Numbers of soil and root-inhabiting *T. semipenetrans* correlated positively with root mass density and with most measures of root carbohydrate (Table 2). *T. semipenetrans* was inversely related to soil moisture content (Fig. 2A, B, and E) and root lignin concentration (Fig. 1B). Soil moisture, root starch concentration, and the bioassay preference index were the best predictors of numbers of juveniles and males in soil ($R^2 = 0.84$) and females on roots ($R^2 = 0.86$) in multiple regression models incorporating data from April through November during both 1988 and 1989 (n = 16) (Table 3).

Nematode energetics. Average monthly nonstructural carbohydrate in fibrous roots represented 9.6–81.4 calorie equivalents per gram of root (Fig. 4). The average monthly respiration energy required by *T. semipenetrans* was estimated to be 31.8 calories per gram of root. When estimates of the energy used in nematode respiration each month were added to monthly measurements of carbohydrate energy available in roots, a shift occurred in the time at which available energy appeared to increase in root tissue (Fig. 4). Although carbohydrate concentrations increased measurably in roots beginning during November each year, the estimates of total energy (calories) availability suggest carbohydrate in roots began to increase as early as July.

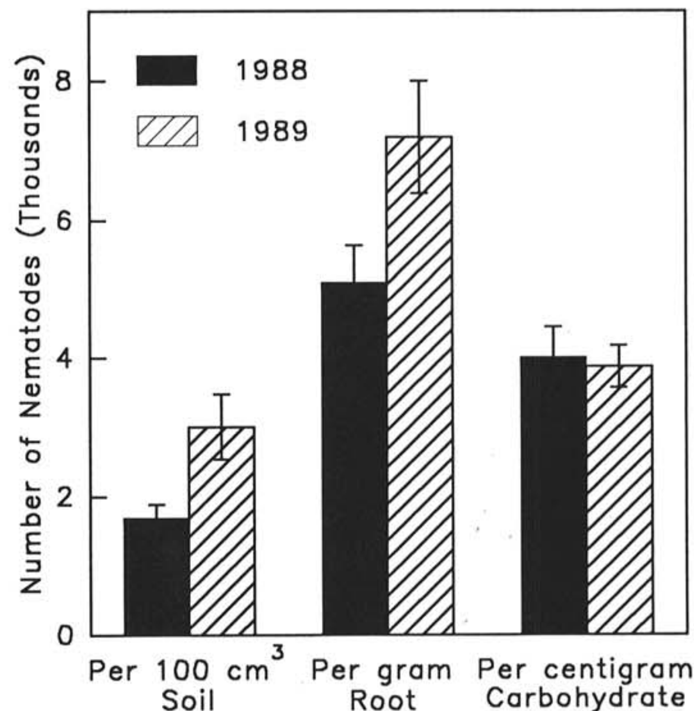

Fig. 3. Populations (mean and standard error) of *Tylenchulus semipenetrans* juveniles and males in samples collected during June–August 1988 and 1989 and expressed per unit of soil, per unit of root per unit of soil, and per unit of nonstructural carbohydrate per unit of soil. Samples were taken from depths of 0–30 cm.

Fungal populations. *P. parasitica* propagules in soil exhibited few consistent seasonal trends other than a tendency to decline during winter in 1988 and 1989 (Fig. 2C). Propagule densities differed between years. Average weekly density of the fungus during April–December 1989 was 2.5 times ($P = 0.01$) the density during the same period of the previous year. Two months after the freeze of December 1989, coincident with the beginning of a sharp decline in root mass density, fungal propagules increased and remained at high levels until the study was terminated. The amount of fungal protein in roots was not correlated with propagule density in soil. Protein concentrations were higher during the summer and early autumn than during the winter (Fig. 2D). The average weekly concentration of fungal protein in roots during the summer (June–August) of 1989 (931 ng/g of root) was nearly threefold ($P = 0.01$) that of summer 1988 (334 ng/g of root).

P. parasitica levels in soil and roots were not correlated with most other factors (Table 2). Fungal propagules in soil were related inversely to the concentration of starch in roots. The level of fungal protein in roots was related directly to soil temperature. Soil temperature, concentration of ketone sugars, and soil moisture explained 76% of the annual variation of fungal protein levels in a multiple linear regression model (Table 3). Multiple regression on root mass density and concentration of ketone sugars as measures of the amount and quality of available energy sources explained 86% of the variation in fungal propagule counts from soil.

DISCUSSION

Seasonal patterns of root quality and annual differences in root abundance and quality were related to populations of both the nematode and the fungus. In this and a previous survey of citrus (17), root mass density increased and was then maintained during alternate years, much as fruit load varies biennially in many citrus cultivars. Root mass density increased by 75% during summer and autumn of 1988 and by a further 56% immediately prior to summer of 1989. Root mass density did not increase significantly during summer and autumn of 1989. Although mass-density measurements do not describe the dynamics of root turnover, changes in root-chemical composition supported the possibility that more root growth occurred during summer and autumn in 1988 than in 1989. A steady decrease in average lignin concentration during summer and autumn of 1988 was consistent with increased root growth and was much less pronounced the next year. Higher concentrations of starch and sugars in fibrous roots during summer and autumn of 1989 compared to the previous year also are consistent with decreased root growth.

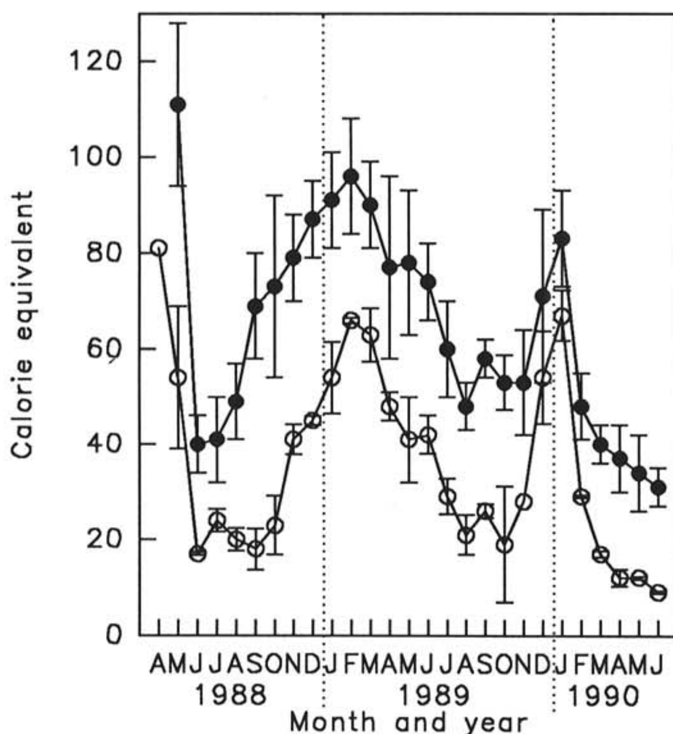

Fig. 4. Calorie equivalent of the concentration of nonstructural carbohydrates in fibrous roots (open circle), and the estimated calorie equivalent (solid circle) obtained by adding estimates of calories utilized for nematode respiration to the nonstructural carbohydrate-calorie equivalents measured in roots.

Growth and maintenance of roots in alternate years should affect parasites in several ways. Young roots are infected more readily by *T. semipenetrans* and *P. parasitica* than are old roots (9,22); nevertheless, carbohydrate (nutrient) levels increased in roots during the year when root growth appeared to be low. Increased root mass density reduces soil moisture, influencing parasites directly and indirectly through changes in the rhizosphere community. Thus, annual as well as seasonal patterns of change in roots may occur and influence parasite populations.

Nematode population density was related more closely to fibrous root mass density than to any other single factor measured during the study. Nevertheless, root availability did not adequately explain population trends, such as the summer decline in nematode levels. Variables, such as carbohydrate concentration, soil moisture, and preference of migrating *T. semipenetrans* for root extracts, however, were revealed by the multiple linear regression model as possible key factors.

The quality of roots as food for *T. semipenetrans* may decline during summer because the concentration of total nonstructural carbohydrate and starch, in particular, were lowest annually during summer. Histological stains indicate lower levels of starch in the nematode feeding or nurse cells than in normal cortical cells (10). Pronounced amylytic activity of macerated *T. semipenetrans* (10) suggests that starch is digested by nematode-produced enzymes in the nurse cells prior to ingestion. The possibility that nematodes are limited by carbohydrates was supported by annual differences in population density. All nematode life stages expressed per unit of soil or per root mass density were more numerous during summer 1989 than during 1988, but nematodes per unit of starch or per unit of total nonstructural (starch plus glucose plus ketone sugars) carbohydrate were not. *T. semipenetrans* egg production increased proportionately with starch levels in roots of trees from which fruits were removed for 14 mo (15). Although nematode populations increased independently of starch concentration during both autumn seasons, estimates of energy flux in roots suggest that carbohydrate availability increased in roots by midsummer. Such a pattern is consistent with the possibility that during early development, citrus fruits are a competitive carbohydrate sink (15). Dry-matter accumulation by fruit is nearly complete by midsummer (5), coincident with the beginning of nematode population growth.

Roots may be less susceptible to infection by *T. semipenetrans* during summer than during other seasons. Lignin concentrations in fibrous roots increased between May and June during the first 2 yr of the study and declined after July each year. A major decrease in lignin throughout late summer–autumn 1988 corresponded with large increases in root mass density and nematode population density. The corresponding changes in lignin concentration, root mass density, and nematode density in late summer–autumn 1989 were much reduced. Infection of citrus roots by *T. semipenetrans* declines with development of root secondary characteristics (9). If the age structure of the fibrous root system is reflected by lignin concentration, increased lignin suggests a decline in root susceptibility. These assumptions were supported by a negative correlation between lignin and root succulence as measured by percent water in roots and by the negative correlation between lignin and *T. semipenetrans*.

A positive association between *T. semipenetrans* and the root-leachate preference index suggests that roots may also be less attractive to the nematode during summer than during other seasons. Juice pressed from citrus roots inhibited the motility of *T. semipenetrans*, but the effect was least pronounced during summer (49). *T. semipenetrans* never preferred root leachates used

in this study to water controls. The positive association was derived from a stronger repulsion by leachates from roots collected during summer than by those collected during spring or autumn. The effect of leachates from dried root tissue in this study is similar to that noted for other nematodes exposed to exudates from living host roots (8,11), and leachates from live citrus roots have the same qualitative effect on *T. semipenetrans* (L. W. Duncan, *unpublished data*). Dissociation of certain chemicals in leachates from volatile root exudates that serve as nematode attractants (8) may be an artifact of the bioassay technique that favors expression of repellent compounds. Seasonal trends in the production of such compounds that coincide with favorable conditions for parasite development could be a selective advantage for the host. For example, when the preference index was low during summer, average soil temperature was 25 C, which is optimum for development of *T. semipenetrans* (36).

Soil moisture was the strongest predictive factor of nematode density in multiple regression. The negative correlation may be spurious because rainfall in Florida is highest in summer and decreases during autumn–spring. If irrigation had been inadequate during this study, the linear correlation coefficient probably would have been less than it was because *T. semipenetrans* is a poor anhydrobiote (48), and populations decline in substantially dry soil. Nevertheless, there is evidence that soil moisture within the normal range for crop production affects *T. semipenetrans* significantly. In pot studies, *T. semipenetrans* populations increased in fine-textured soil proportionately with water deficits that ranged from −10 to −60 J/kg (52). The trend across the same moisture deficit was reversed in coarse-textured soil. Fluctuations in water deficit in sandy soils tended to be rapid and extreme, so nematodes frequently experienced either drought or oxygen deficits. Soil texture in the present study was very sandy; however, because of irrigation, daily water tensions rarely were less than −20 J/kg and generally exceeded −15 J/kg. Within this range, we assume that the nematode did not experience stress from moisture deficit but may have experienced episodes of limited oxygen availability during periods of high rainfall. The occurrence of high-moisture content coincident with high soil temperature may also favor nematode antagonists.

Fibrous root infection by *P. parasitica* was highest during summer and was related directly to soil moisture during those months. Optimum temperature for fungal development is 30–32 C, and periodic flooding of soil favors fungal infection (22). In Mediterranean climates, the fungus is most easily isolated from rhizosphere soil during summer months (24). In the subtropical climate of central Florida, seasonal trends in propagule density are not consistent (47), although an over-winter decline occurred during both survey years and in another study (1). In general, the numbers of fungal propagules in soil appeared to be influenced by root mass density, carbohydrate characteristics of the roots, and root mortality. After the freeze in December 1989, numbers of propagules but not the nanograms of fungal protein per gram of root increased sharply, apparently in response to the high level of root mortality and decay.

Although temperature was the factor most closely related to seasonal changes of *P. parasitica* in roots, as measured by ELISA, annual differences in levels of the fungus were associated with patterns of growth and carbohydrate allocation in the host. The average annual level of root infection generally reflected the level of propagules in soil. A significant, prolonged increase in propagule numbers beginning during May 1989 coincided with a corresponding increase in fungal protein per unit of soil (root mass density by fungal protein concentration in roots; data not shown) and was followed by the highest levels of root infection (fungal protein by root weight) measured during the study. During the maintenance phase of the root-growth cycle, the concentration of nonstructural carbohydrates in fibrous roots increased. The combination of many roots with high-carbohydrate content may have provided a substrate that increased the fungal mass and fungal concentration in roots when temperature and moisture conditions were favorable. Ketone sugars, but not the other carbohydrates, were closely related to the level of fungus in soil and roots. Sucrose (a ketone sugar) is superior to many carbohydrates as a nutrient source for *P. parasitica* growth in culture (24), and *P. parasitica* does not produce amylytic enzymes (37). When the environmental effects of soil temperature and moisture were controlled in the root-leachate bioassay, *P. parasitica* growth increased in response to root levels of ketone and reducing sugars. Phenolic compounds and lignin were correlated in this study, and thus, specific phenolic precursors of lignin (31) could have contributed to the negative correlation between lignin concentration and fungal growth in vitro. Lack of correlation between total phenolics and in vitro fungal growth suggests that increased lignin simply reflected a reduction in nutrient availability, however.

Root age also influences infection by the fungus (21). In the field, root infection by *P. parasitica* requires a root-growth flush (30). Zoospores of *P. parasitica* are attracted by root exudates to the zone of elongation of growing roots, where they encyst and infect (22). In our study, however, the concentration of *P. parasitica* protein in roots was greatest in 1989 when the change in root mass density and lignin were least, suggesting that nutrient allocation within the tree is important in regulating parasitism. Fungal growth after fibrous-root infection during summer 1988 may have been limited by nutrient concentration despite an abundance of growing roots. High-infection rates during 1989 may reflect the high summer concentration of sugars in root tissue available after a major root flush in May.

Both parasites measured in this survey are commonly detected in Florida citrus orchards. The occurrence of *T. semipenetrans* in commercial orchards has been estimated at 50–90% (20). The nematodes do not inhabit nurseries because of the site-certification program of the Division of Plant Industry (Department of Agriculture and Consumer Services). Approximately half the field nurseries and nearly all the orchards in Florida are estimated to be infested with *P. parasitica* (55). Because both parasites infect the cortex of the fibrous roots and cause mild to moderate tree decline, the integration of methods to detect, quantify, and manage both organisms is desirable.

The sampling protocol was adequate to detect differences in most of the variables examined in the study. The coefficient of variation for Taylor's Power Law was as low as 12% for female *T. semipenetrans* because each mean and variance estimate was based on only two samples. The large numbers ($n = 103$) of observations in the regression helped compensate for the lack of precision in the estimates, however. At most densities, the standard error/mean ratios were higher for root-inhabiting stages in the life cycle of either parasite than for soil-inhabiting stages. When parasite densities on roots were high, fungal measurements were more variable than were nematode counts. The trend measured by Taylor's Power Law suggests that conditions favoring fungal activity are highly localized throughout the root zone because the sample precision decreased with increased fungal infection. Based on the mean-variance relationships measured in this study, seven samples would be required to measure fungal protein in roots (assuming 500 ng of fungal protein per gram of root) with precision similar to that obtained for the nematode (assuming 1,000 females per gram of root) numbers from two samples (16). In this site, two samples were adequate (standard error/mean ratio = 0.20) to measure soil stages of *P. parasitica* at the damage-threshold level of 15 propagules/cm^3 of soil (41). The same number of samples would measure *T. semipenetrans* with at least as much precision at any mean density.

Fungal density in soil was high (>10 propagules/cm^3 of soil) throughout most of the study. Prior to the freeze during December 1989, however, *P. parasitica* density in soil continually increased during three distinct phases that corresponded roughly to changes in root mass density and concentration of nonstructural carbohydrate. That trend supports the observation (46) that, for management purposes, a time profile of sample measurements is required in orchards in which propagule density is low. Sample data are more likely to accurately reflect population trends when collected between rather than within annual growing seasons. Soil population levels in this and other studies (46) did not exhibit

seasonality to a degree that would preclude sampling during most of the year, with the possible exception of winter.

Current recommendations that samples of *T. semipenetrans* be collected in late autumn or mid-to-late spring were supported by these data, which show highest population densities during these times. As a result, soil samples collected during autumn or spring would be appropriate to measure both organisms. To date, however, ELISA techniques have been used infrequently to estimate *P. parasitica* levels in roots of mature trees. The trends in this study indicate that annual summer root sampling may provide the best indication of potential *P. parasitica* damage in an orchard. This appears to preclude sampling roots for both parasites simultaneously as an optimum strategy.

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