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Responses of *Tylenchulus semipenetrans* to Citrus Fruit Removal: Implications for Carbohydrate Competition[1]

LARRY W. DUNCAN[2] AND DAVID M. EISSENSTAT[2]

Abstract: Sixteen mature Valencia orange trees on rough lemon rootstock were selected on the basis of approximately equal, naturally occurring populations of *Tylenchulus semipenetrans* in soil. In March, fruit 1 cm in diameter or less were removed from eight of the trees, which were kept free of fruit for 15 months. In July, 4 months after fruit removal, fibrous root (<2 mm d) mass density of defruited trees was 51% greater and insoluble starch in fibrous roots was 24% less than on control trees with fruit. Female *T. semipenetrans* per gram of root were 64% more numerous on roots of control trees than on defruited trees at this time. Numbers of female nematodes per tree and of juveniles and males in soil did not differ between treatments 4 months after fruit removal. Root mass density remained higher on defruited than control trees for the remaining 13 months that the trees were studied, while nematode density in soil beneath defruited trees rapidly increased to levels proportionate to the additional root mass density. Nine months after fruit removal (December), starch concentration was 84% higher in roots of defruited trees compared to controls and remained 28% higher than in controls 15 months (May) following fruit removal. Between months 9 and 15 following fruit removal, nematode density in soil beneath defruited trees increased at a rate five times that of nematode density beneath control trees. In May, female fecundity (eggs/female) on defruited trees was 41% greater than on control trees. The data were consistent with the hypothesis that carbohydrate competition between developing citrus fruit and *T. semipenetrans* influences seasonal fluctuations in nematode population densities.

Key words: carbohydrate, citrus, competition, host–parasite relations, nematode, nutrition, starch, sugar, *Tylenchulus semipenetrans.*

The seasonal population development of *Tylenchulus semipenetrans* on citrus is well-defined (2,5,11,15,20,22). Population densities generally increase in autumn and spring, presumably in response to flushes of new fibrous roots, which are the nutritional substrate of the nematode (10,15). Low soil temperatures in winter reduce developmental and reproductive rates and result in population declines. However, nematode numbers are often lowest during summer, when average temperatures in most soil horizons approach the optimum (25 C) for nematode development (15,19).

The reason for the summer decline of populations of *T. semipenetrans* is not understood. In addition to temperature, soil moisture in many citrus growing regions is often seasonal. Moisture deficits can result in significant mortality of *T. semipenetrans,* which is a poor anhydrobiote (25). However, in subtropical and tropical zones, moisture deficits are least likely to occur during summer. Seasonal development of antagonists of the nematode has not been studied, but natural control of *T. semipenetrans* is probably significant (28). Although seasonal differences have been reported in the attractiveness to *T. semipenetrans* of citrus root extracts (11) and of root constituents that affect nematode motility (26), the root chemicals responsible for these activities have not been identified. In this study, we examine seasonal changes in the quality of the citrus root as a food source, which could also conceivably affect nematode population densities.

The nutritional quality of roots is likely affected by their age and by temporal demands from other plant organs. As citrus roots age, they become less susceptible to infection by *T. semipenetrans* (8). Amylytic activity in homogenates of *T. semipenetrans* juveniles and reduced starch levels in the nurse (food) cells compared to surrounding cortical cells indicate that starch is a nutrient source for the nematode (9). The

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[2] Associate and Assistant Professors, University of Florida, IFAS, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850.

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starch concentration in citrus fibrous roots begins to increase in autumn, before winter dormancy. Starch concentrations decrease from spring to summer concurrent with increasing carbohydrate demand by the developing fruit (3,6,11). If carbohydrates in fibrous roots are at levels that limit the population development of *T. semipenetrans,* seasonal changes in carbohydrate flux could be a factor in nematode population dynamics.

In this study, we attempt to examine whether carbohydrate limits *T. semipenetrans* development on citrus roots by increasing carbohydrate flux to the roots by defruiting. We tested the hypothesis that increasing the carbohydrate concentration in citrus fibrous roots increases the fecundity of female nematodes, produces higher numbers of nematodes per unit root, and mitigates the summer decline in nematode density.

Materials and Methods

Sixteen Valencia orange (*Citrus sinensis* (L.) Osbeck) trees on rough lemon (*Citrus jambhiri* Lush) rootstock were selected based on preliminary samples (March 1988) of naturally occurring nematode population density in soil from beneath trees in a 0.08-ha area near Lake Alfred, Florida. The 18-year-old trees were spaced 4.6 × 4.6 m in a Candler fine sand (Typic quartzipsamment) soil. Trees were irrigated in spring and autumn with portable impact sprinklers and fertilized with 260, 27, and 215 $kg \cdot ha^{-1} \cdot year^{-1}$ of N, P, and K.

All fruit were removed from eight trees in March 1988 and again in March 1989. The remaining eight trees served as controls. Treatments were assigned randomly. Two pooled soil samples, each representing a composite of 16 subsamples extracted with augers (2.0 cm i.d. × 30 cm long), were collected from each tree on 27 July, 12 October, and 23 December 1988 and on 5 May 1989. The 16 cores in each sample were taken systematically around the trunk from a circular area with a 2.3-m radius that was divided into four quadrants. Four equally spaced cores were extracted in each quadrant along imaginary lines extending radially from the trunk, the end points of which were marked with stakes to avoid future resampling. One composite sample from each tree on each date was processed to estimate nematode population density and fibrous root (<2 mm d) mass density. Fibrous roots in the other sample were processed to determine concentrations of carbohydrates. Lignin concentration in roots was measured only on the first and final sample dates.

Nematodes were extracted for 48 hours from a 60-cm^3 subsample of soil and root fragments with Baermann funnels. In each sample, the number of second-stage juveniles and males was expressed on the basis of both 100 cm^3 of soil and total root weight in the sample. The remaining fibrous roots in samples were collected by gently washing the soil through a screen and hand separating roots from debris. Roots were weighed and processed to extract all nematode life stages with a modified method of Baines et al. (4), in which a 500-mesh (25-μm opening) sieve was substituted for a 325-mesh (44-μm opening) sieve. The various life stages were expressed per gram fibrous root fresh weight and per gram fibrous root starch.

Fibrous roots in the second sample were recovered as described above. Roots were weighed, dried at 70 C for 48 hours, reweighed, and ground (<420 μm) in a mill. Samples were stored at room temperature until the termination of the experiment, when all chemical analyses were conducted.

Root carbohydrates were extracted by boiling 50 mg of dried tissue in 15 ml of water for 2 minutes followed by centrifugation (8,000*g*) for 2 minutes. Glucose oxidase (from *Aspergillus niger,* type V, Sigma Chemical Co., 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 (from *Aspergillus niger* in $(NH_4)_2SO_4$ suspension, Sigma) and

corrected for free glucose (16,24). Glucose was the standard in these measurements. Arsenomolybdate was used to analyze reducing sugars (18) and resorcinol reagent (1,21) to analyze ketone sugars (fructans, fructose, and sucrose, among others). Fructose was the reducing sugar standard because, in previous HPLC studies, it exceeded free glucose in rough lemon roots similar to those in this study (13). Lignin concentration was measured by acid hydrolysis of the pellet followed by a series of ethanol washes. The remaining residue of lignin plus minerals was weighed, ashed, and reweighed to determine separately the lignin and mineral weights (12). Total N and C content as percentages of root dry matter from the May samples were measured by mass spectroscopy (11).

The effect of treatments on whole-plant biomass partitioning was measured destructively. The fibrous root biomass was estimated in early May 1989 by extracting a 1-m-deep × 4-cm-d soil core. The sampling pattern was as described for the initial 16-core samples with cores taken 0.5, 1.0, 1.5, and 2.0 m from the trunk. Fibrous roots were separated from coarse roots (2.0–15.0 mm). Roots of diameter >15.0 mm were not measured. Root mass was estimated as the product of the average root mass at a given radial distance from the trunk and the area of the annulus for which that distance was a midpoint.

Total leaf mass was estimated in mid-June to mid-July by harvesting 25–50% of the leaves on a tree in a vertical section. Leaves were dried (70 C) and weighed. Mature fruit were harvested on 6 April 1989 and weighed fresh. Dry weight was calculated from the dry-weight fraction (0.17) derived from a sample of 20 fruit.

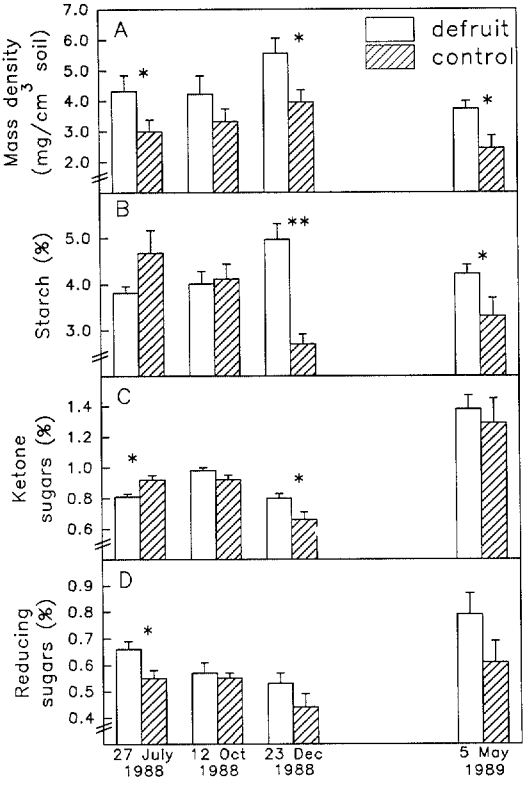

FIG. 1. The effect of fruit removal in March 1988 and 1989 on the fibrous root mass density (A) and the concentration (% dry weight) of starch (B), ketone sugars (C), and reducing sugars (D) in mature rough lemon rootstock naturally infested with *Tylenchulus semipenetrans* with Valencia sweet orange scions. Error bars represent standard errors, and asterisks represent differences at $P = 0.05$ (*) and $P = 0.01$ (**) according to a two-tailed *t*-test.

RESULTS

Plant responses: Four months following March fruit removal, fibrous root mass density was 51% greater on defruited trees than controls (Fig. 1A). A similar proportional difference (27–53%) continued throughout the experiment. In both treatments, mass density increased ($P = 0.05$) between 34–40% from July to December and declined ($P = 0.01$) between 30–39% from December to May.

Amylopectin was lower ($P = 0.05$) in roots of defruited trees than in control roots in July (1.8% vs. 2.3%), but there was no treatment difference in amylose (2.0% vs. 2.3%). Thus, total starch (i.e., amylopectin plus amylose) tended to be lower in roots of defruited than control trees (Fig. 1B). Although defruiting apparently diminished starch concentrations in the first 4 months of the experiment, in the following 5 months from July to December, starch concentrations increased in the roots of defruited trees, whereas it de-

clined in the roots of controls (Fig. 1B). Starch did not differ between treatments in October 1988; but by December, starch was 84% higher in roots from defruited trees than those from controls. Starch concentration remained 28% higher in fibrous roots of defruited trees than those of controls when the experiment was terminated in May 1989.

The quantity of ketone and reducing sugars in fibrous roots exhibited less pronounced but generally similar treatment effects as starch (Fig. 1C,D). In July 1988, the effects of prior defruiting on the concentrations of root ketone sugars and starch were similar, but reducing sugars exhibited an opposite response with a 20% higher concentration in defruited than in control trees. On other sampling dates, the effects of defruiting were consistent for the different forms of nonstructural carbohydrates.

Mean lignin concentrations in fibrous roots collected in July 1988 and May 1989 ranged from 16.9 to 18.7% (data not shown). No treatment effects were detected. Similarly, defruiting had no effect in May 1989 on the percentage (dw) of total root N (1.53% in defruited trees vs. 1.5% in controls), total C (39.6 vs. 39.6), or the wt:wt ratio of N:C (0.039 vs. 0.038).

Defruiting affected the biomass partitioning in the tree 14–16 months following treatment (Table 1). Although there were no differences in the weights of leaves and coarse roots, fibrous root mass to a depth of 1 m was 39% greater in defruited trees. Consequently, the mass ratio of fibrous roots to leaves for control trees was 0.72, whereas it was slightly greater than one on defruited trees.

Nematode responses: Average nematode densities per 100 cm^3 soil beneath control and defruited trees in March 1988 were 3,208 and 3,896 juveniles and males (s_{pooled} = 2,987), respectively. In July 1988, the density of female *T. semipenetrans* was 39% less on roots of March defruited trees than on control roots (Fig. 2A). Defruiting also tended to lower female fecundity (Fig. 2B). There was no treatment difference when fecundity was expressed as a proportion of the concentration of starch in the roots (1.03 vs. 0.97 eggs/female/mg starch). In July, the population densities of juveniles and males in soil did not differ between the two treatments (Fig. 2C). Because the root mass density was highest on defruited trees, the density of nematodes

TABLE 1. Biomass† (mean and standard error) partitioning among leaves, fruit, and roots of Valencia orange trees on rough lemon rootstock naturally infested by *Tylenchulus semipenetrans*, 14–16 months following fruit removal.

	Defruited trees	Control trees
Fine roots‡	8.74 (0.81)*	6.31 (0.71)
Coarse roots§	11.89 (0.98)	11.85 (1.70)
Leaves	8.25 (0.91)	8.82 (0.91)
Fruit	0.00	15.85 (0.93)
Fine roots/leaves	1.06 (0.13)*	0.72 (0.09)

† Expressed as kg dry weight per tree.
‡ Diameter < 2.0 mm.
§ Diameter = 2.0–15.0 mm.
* Different (P = 0.05) according to a two-tailed t-test.

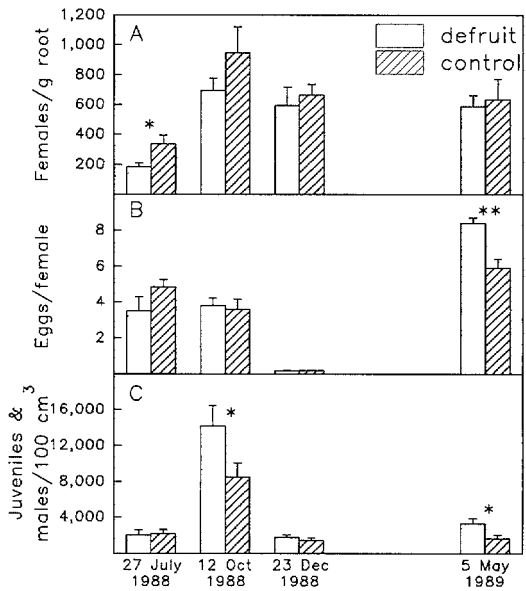

FIG. 2. The effect of fruit removal in March 1988 and 1989 from Valencia sweet orange trees on rough lemon rootstock on the density of female *Tylenchulus semipenetrans* (A), nematode fecundity (B), and the number of juvenile and male nematodes in soil (C). Error bars represent standard errors and asterisks represent differences at P = 0.05 (*) and P = 0.01 (**) according to a two-tailed t-test.

per gram of root was lower on defruited trees (5,803 vs. 8,164, $P = 0.05$).

Nematode densities on defruited trees increased to levels comparable to those of controls during October and December 1988. There were no treatment differences in female number per gram of root or in female fecundity during either month. Juveniles and males in soil beneath defruited trees were 66 and 21% more numerous than controls in October and December, respectively. When juveniles and males were adjusted for root density differences (number of nematodes per gram of root), there were no treatment differences in either month.

In May 1989, there were no differences between treatments in the number of females per gram of root, but fecundity was 41% greater for females on roots of defruited trees. There was no difference between treatments when fecundity was expressed per gram starch (1.78 vs. 1.98 eggs/female/g starch in control vs. defruited trees). There tended ($P < 0.10$) to be more eggs, juveniles, and males per gram of roots from defruited trees (4,825) than from controls (3,475). The mean number of nematodes in soil beneath defruited trees was twice that beneath controls. Between December and May, numbers of juveniles and males in soil beneath defruited trees increased at a greater rate (87 vs. 17%, $P = 0.05$) than those of control trees. However, the mean number of nematodes in soil after adjusting for root density (nematodes per gram root) under defruited trees (9,933) was not significantly ($P = 0.27$) greater than that for controls (7,718).

Discussion

Populations of *Tylenchulus semipenetrans* responded to qualitative and quantitative differences in roots caused by fruit removal. Root age and carbohydrate concentration were two aspects of fibrous root quality affected by the experimental treatment. Initially, fruit removal increased citrus fibrous root growth. Reduced concentrations of starch and some sugars in the roots of defruited trees in July was likely a result of recent root growth. Carbohydrate concentration did not increase in these roots until sometime after October 1988. However, during December 1988 and May 1989, both quality (starch concentration) and quantity (root mass density) of roots were increased by defruiting.

Both numbers of females and numbers of juveniles and males per unit soil were nearly identical between treatments during July. Therefore, lower nematode density on roots of defruited trees in July was likely due to a lag between root growth and infection. The tendency for defruited trees in July to have lower female fecundity could have resulted from nutrient deficiency or from a change in the age structure of the female population. The apparent increase in root mass in response to fruit removal preceded increases in nematode numbers. Thus, the citrus nematode may be able to acquire excess carbohydrates only after plant demands have been satisfied.

By October 1988, the nematode–host relationships in both treatments appeared to have stabilized. Carbohydrates were not different in the two treatments in October, and nematode densities on roots and in soil of both treatments were proportional to root mass density.

If soil conditions are optimal for the plant, *T. semipenetrans* population densities in Florida increase or decline only slightly between October and December (10). In this study, however, nematode levels decreased by as much as seven-fold between autumn and early winter, presumably in response to the soil water deficit that developed when irrigation was not supplied during a drought (cumulative precipitation from 5 October to 4 November was 2.5 mm). Nematode populations in soil and on roots of the two treatments were in similar proportion to root mass density. Fecundity was not different between treatments, despite higher levels of starch in

roots of defruited trees. Lack of response to starch in December may have resulted from the obvious soil moisture and temperature limitations to nematode growth at this time.

By May 1989, the most evident effect of fruit removal was increased female fecundity. As in the previous July, in both treatments fecundity was proportional to the concentration of starch in roots. The rate of root infection was unaffected by fruit removal, but higher fecundity appears to have resulted in more eggs, juveniles, and males on roots of the defruited trees. In May, the concentration of total nonstructural carbohydrate (starch + ketone sugars + glucose) was greater in defruited trees primarily because of higher starch. These higher starch levels in defruited trees coincided with increased growth of soil population densities beneath defruited trees compared to controls.

If *T. semipenetrans* responds to available carbohydrate, then citrus fruit represents a major carbohydrate sink with which the nematode must compete. Indeed, fruit biomass on control trees was nearly twice that of leaves or fibrous roots. Moreover, the average life-span of fruit (14 months) is less than that of leaves (1.5–3 years) and likely less than roots (27). Consequently, annual fruit production represents a major sink for plant carbohydrates. Because the dry weight accumulation in Valencia fruit (3) is greatest 2–4 months after bloom, the competition for available plant carbohydrates likely occurs during this period (April–July in Florida). Also, during spring and summer, available carbohydrate in older leaves (23) and in roots (6) is relatively low. Thus, nematode population decline (11) can be linked to high carbohydrate demand by fruit and reduced carbohydrate concentrations in the remaining tissues of the plant. A similar phenomenon occurs with an obligate biotrophic mycorrhizal fungus, which infects *Chrysanthemum morifolium* at lower rates during early flower bud development, when levels of sugars and amino acids in root exudates are relatively low (17).

Despite a close correlation between nonstructural carbohydrate in roots and nematode density during spring and summer, *T. semipenetrans* numbers often increase in autumn before carbohydrate concentration increases (11). This again may relate to a weaker sink strength of fruit in autumn than during early development.

An inconsistency between these results and those from a 27-month study of seasonal changes of *T. semipenetrans* and citrus root carbohydrates (11) was the lack of response to defruiting by the nematode infection rate. In our present study, the number of females per gram of root on defruited trees never exceeded that on controls. However, numbers of female *T. semipenetrans* per unit soil and per gram root in the seasonal study were well-correlated with either starch or nonstructural carbohydrate. Both studies were on rough lemon rootstock in very similar soils and climates, whereas the scion cultivars and tree ages differed. The discrepancy in infection response may result from the lower concentration of starch in roots in the long-term study (range = 0.4–1.8%) compared to the present one (range = 2.9–4.9%). If these nematodes are carbohydrate limited, threshold starch levels at which female density is affected may be lower than thresholds at which fecundity is reduced. Higher reserves of carbohydrate may also result in the production of higher levels of phenolic compounds or other secondary metabolites or defensive structures that could attenuate an infection response to change in carbohydrate.

The hypothesis that decreasing carbohydrate concentration contributes to summer nematode population decline was not tested, because carbohydrate levels did not increase in response to fruit removal during the spring and summer of 1988. However, the correlation of carbohydrate with nematode population density during spring and summer in previous work (11) and the nematode response to defruiting in the present work support the possibility of a causative relationship. Nevertheless,

because plant-parasitic nematodes are obligate parasites, a definitive answer to whether *T. semipenetrans* is limited by carbon availability requires the ability to manipulate carbohydrate to the exclusion of other factors in the plant. It is unlikely that treatment differences in plant defensive compounds resulted in the nematode population differences observed, because defruited trees had higher level of storage carbohydrate and presumably more resources to devote to defense. However, nitrogen levels cycle in root tissues in a manner similar to carbohydrate reserves (7), and phytophagous insects are frequently limited by protein rather than carbohydrate availability (14). Although we detected no differences in total root N in May when treatment effects on nematodes were greatest, we did not measure levels of amino acids, proteins, or other classes of specific nutrients that may be required by the nematode.

In conclusion, we successfully manipulated carbohydrates in roots by defruiting. During periods of the year when abiotic factors were not limiting, *T. semipenetrans* responded to defruiting with increased fecundity and population growth in the soil.

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