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Citrus Nematode Alters Carbohydrate Partitioning in the 'Washington' Navel Orange

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Abstract. **Root infection by the citrus nematode (*Tylenchulus semipenetrans* Cobb) at population densities above the economic threshold caused altered patterns of root and shoot growth of 20-year-old 'Washington' Navel orange trees (*Citrus sinensis* L. Osbeck) budded on Troyer citrange rootstock (*Poncirus trifoliata* L. Raf. x *C. sinensis*), compared with control trees protected from nematode infection by oxamyl (1.1 kg/ha/3 weeks) and permitted to recover from prior nematode infection. Nematode-infected trees initiated new roots every 4–6 weeks, beginning as early as mid-March through as late as mid-January. Each root flush was equal to or greater in magnitude (number of new roots per gram roots) than those observed from oxamyl-treated trees. Oxamyl significantly reduced the population of female nematodes. Oxamyl-treated trees produced 4 relatively small root flushes, typical of healthy trees and exhibited a net increase in root biomass; nematode-infected trees failed to increase root biomass despite repeated root initiation. Oxamyl-treated trees also produced more new shoots and exhibited a greater increase in length per shoot and mean number of leaves per unit shoot length than untreated trees. Measurements of the levels of available glucose, sucrose, and starch provided evidence that nematode-infected trees were carbohydrate depleted. Starch levels were typically 70% higher in the canopy and 96% higher in the roots of oxamyl-treated trees compared to untreated. Root initiation appears to be the first response of the trees penetrated by citrus nematode larvae; repeated root initiation in response to the many generations of larvae that hatch each year is probably a key factor contributing to the depletion of carbohydrate in nematode-infected trees.**

Citrus nematode (*Tylenchulus semipenetrans* Cobb.) is a serious root pest of citrus trees and is found in all major citrus-growing regions of the world (22). Previous research investigating the influence of the citrus nematode on citrus productivity has established that the response of all commercial citrus varieties is the same; there is a general reduction in the mass of soil-invading roots, accompanied by a slow decline in tree vigor that manifests itself as a reduction in both the size and number of fruit at harvest (1, 3, 14, 19, 22). Little is known about the physiological response of the citrus tree to root infection by the citrus nematode, or the mechanism by which the citrus nematode stresses the tree.

In this report, we provide evidence demonstrating that root infection by the citrus nematode at population densities significantly above the economic threshold [currently, the economic threshold is set at 700 females/g roots during May to July (10)] alters root and shoot growth and reduces carbohydrate availability of 20-year-old 'Washington' navel orange trees budded to Troyer citrange rootstocks. In addition, we provide strong evidence suggesting that the repeated initiation of new root growth every 4–6 weeks brought about by root infection by the citrus nematode is the initial stress that leads to the loss in carbohydrate availability and sets in motion the series of events contributing to the slow, continuous decline in tree vigor and the reduction in fruit size and number.

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Materials and Methods

Research was conducted at the Citrus Research Center and Agricultural Experiment Station, Univ. of California, Riverside, on 20-year-old 'Washington' navel orange trees on the rootstock 'Troyer' citrange. All trees were infected by citrus nematode (*T. semipenetrans*); half of the trees were protected from subsequent nematode infection by soil application of oxamyl (methyl-N,N-dimethyl-N-[(methyl-carbamyl)oxy]-1-thiooxmimidate) at the rate of 1.1 kg/ha/3 weeks from April through the end of October (applications were timed to coincide with regular irrigations) (19).

Nematode population densities and root growth were determined every 2 weeks for 2 full years, beginning in March of 1982, by taking soil samples from 2 different depths, 0 to 30 cm and 30 to 60 cm, at the drip line of the south side of the tree, using a 7.5-cm bucket auger. The trees ran east-west and were furrow-irrigated. Most of the feeder roots and nematodes were concentrated at the dripline which paralleled the furrow ridge in these trees. The number of new root tips and the total fresh weight of feeder roots in each sample were recorded. An aliquot of feeder roots was stained with acid-fuchsin and extracted for nematode females (2). About 1000 g of the soil sample were extracted for citrus nematode larvae using the elutriator technique (5).

Shoot length was measured once a month on tagged shoots located at heights ranging from 0.5 to 2 m from the ground on the north and south sides of the tree. The number of new shoots produced on each tagged shoot also was determined. The number of nodes and persisting leaves per unit shoot length was determined for a random sample of 5 tagged shoots on each of 10 trees per treatment at the end of the growing season.

Starch, sucrose, and free glucose content of leaves and roots were determined biweekly. Twenty mature leaves were harvested from the south side of the tree. Leaves were rinsed with cool distilled H_2O, the basal 1 cm of the leaf was cut off to remove the petiole, and the leaves were cut into small pieces (about 0.25–0.5 cm^2). Feeder roots from the soil samples taken

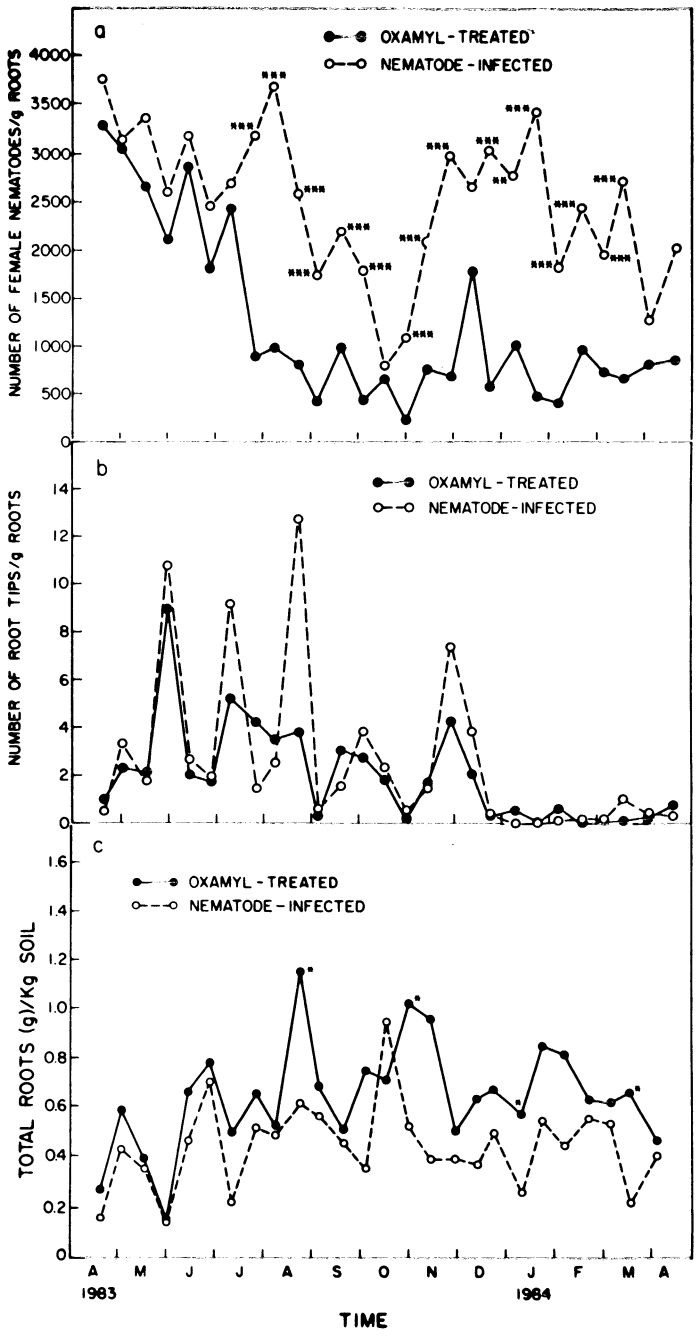

Fig. 1. **A.** Population densities of *Tylenchulus semipenetrans*; nematode-infected (-○-) and oxamyl-treated (1.1 kg/ha/3 weeks (-●-), starting mid-April 1983) 20-year-old 'Washington' navel orange trees budded to Troyer citrange rootstocks. The ** and *** denote significant differences at the 1% and 0.1% level, respectively, by Student's *t* test. **B.** Influence of nematode infection (-○-) and oxamyl treatment (-●-) (1.1 kg/ha/3 weeks, starting mid-April 1983) on the number of new roots initiated throughout the year by 'Washington' navel orange budded on Troyer citrange. **C.** Influence of nematode infection (-○-) and oxamyl treatment (-●-) (1.1 kg/ha/3 weeks, starting mid-April 1983) on root biomass of 'Washington' navel orange budded on Troyer citrange. The * denotes significant differences at the 5% level by Student's *t* test.

at the 2 sampling depths were pooled and cut into small segments about 2–3 mm in length. Samples of leaf tissue (300 mg) and root segments (150 mg) were extracted by the method of Richmond et al. (16), and the glucose content of the extract and starch content of the insoluble fraction were determined by a modification of the glucose oxidase-peroxidase-*o*-dianisidine method (9, 13).

Leaf and root samples were homogenized in 4.0 and 1.5 ml 80% ethanol, respectively, using a Polytron tissue homogenizer (PCU-2, Brinkman Instruments) at speed 6. The Polytron probe was rinsed with 1 ml 80% ethanol, which was added to the homogenate. The homogenate was heated in a water bath at 80°C for 30 min, allowed to cool to room temperature, and centrifuged in a Dynac tabletop centrifuge for 7 min at 70 rpm. The supernatant liquid was transferred to a conical centrifuge tube; the insoluble fractions of leaves and roots were resuspended in 5.0 and 1.5 ml of 80% ethanol, respectively, and extracted an additional 30 min at 80°, cooled, and centrifuged. The supernatant fluids of the 2 extractions were combined, diluted with 1 ml H_2O, and boiled until no alcohol remained. Samples were adjusted to a final volume of 1 ml with H_2O and used to determine the level of available glucose and sucrose. The insoluble fractions of leaf and root samples were resuspended in 1 ml H_2O and boiled until no alcohol remained. The suspension of the insoluble fraction and standards of amylose and amylopectin were adjusted to a final volume of 0.5 ml with H_2O, diluted with 0.5 ml 10 mM acetate buffer, pH 4.5, and

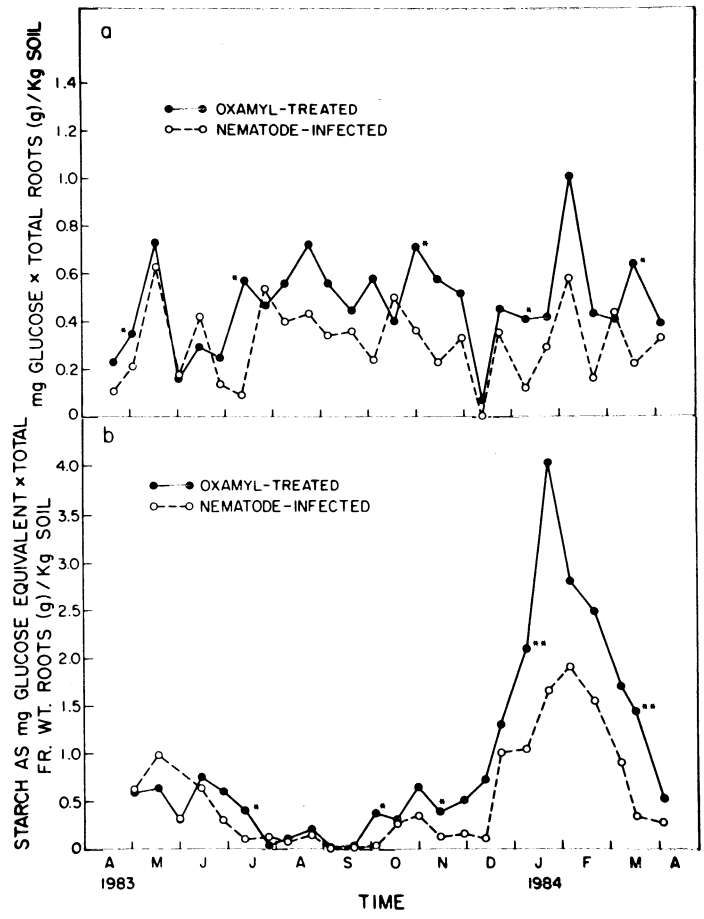

Fig. 2. **A.** Glucose content of roots from nematode-infected (-○-) and oxamyl-treated (-●-) 'Washington' navel orange budded on Troyer citrange. The * denotes significant differences at the 5% level by Student's *t* test. **B.** Starch content of roots from nematode-infected (-○-) and oxamyl-treated (-●-) 'Washington' navel orange budded on Troyer citrange. The * and ** denote significant differences at the 5% and 1% level, respectively, by Student's *t* test.

placed in a boiling water bath for 60 min. The cooled sample and standards were incubated with 1 ml of aqueous amyloglucosidase, 14 units (*Aspergillus niger*, Boehringer Mannehim; 1 unit will liberate 1.0 mg of glucose from starch in 3 min at 55° at pH 4.5), at 45° for 20 hr. The incubations were terminated in a boiling water bath for 2 min, cooled, and centrifuged in a Dynac tabletop centrifuge for 7 min at 70 rpm. The supernatant of each sample was transferred to a conical centrifuge tube and analyzed for glucose as follows.

A 0.5-ml sample of the leaf and root extracts or of the hydrolyzed insoluble fraction and starch standards was incubated with 0.2 mg *o*-dianisidine diHCl, 25 units glucose oxidase. (*A. niger*; 1 unit oxidizes 1 μmol of β-D-glucose to D-gluconic acid and H_2O_2 per min at pH 5.1 at 35°C), and 5 units peroxidase (horseradish; 1 unit will form 1 mg purpurogallin in 20 sec from pyrogallol at pH 6.0 at 20°) (Sigma PGO enzymes 510-6, containing buffer salts) in a final volume of 5.5 ml at 37° for 30 min. Absorbance at 450 nm was determined for all samples within 30 min. The assay was linear for glucose concentrations from 10 to 100 μg/ml. Samples were diluted before the enzyme treatment to give concentrations in this range. Four concentrations of amylose, amylopectin, and glucose standards were run with each assay.

Aliquots (20 μ l) of leaf and root extracts filtered through a 0.45 μM Metricel membrane (Gelman Filtration Products, Ann Arbor, Mich.) were analyzed for sucrose by high-performance liquid chromatography using a Whatman Partisil PXS 10/25PAC column (4.6 mm × 25 cm) connected in tandem with a Waters μ Bondapack/Carbohydrate (Waters Associates, Milford, Mass.) column (4.2 mm i.d. × 30 cm) as the solid phase, and acetonitrile-water-ethanol (80/15/5, by volume) at a flow rate of 1.8 ml/min as the mobile phase (4). The assay was linear for sucrose concentrations from 2.5 to 40 mg/ml.

Results

Soil applications of oxamyl became effective in reducing the population of female nematodes 3 months after the initial application (Fig. 1a). Thereafter, the population of female nematodes was maintained effectively at 756 ± 72 ($\bar{x}$ ± SE, N = 20)/g roots throughout the year. This level was slightly above the economic threshold (10) but was always significantly less than that of the untreated trees ($P < 0.01$ by Student's t test), with the exception of those times of the year when the female nematode population decreased due to natural causes. This occurred during 1983 in October and again in December. Despite these natural drops in the number of female nematodes, the population for untreated trees averaged 2493 ± 148 ($\bar{x}$ ± SE, N = 27) female nematodes/g roots throughout the year.

Root infection by citrus nematode at population densities well above the economic threshold altered both root and shoot growth. Nematode-infected trees initiated new root growth every 4–6 weeks throughout both growing seasons of the 2-year study. To facilitate comparison of a number of parameters, only data from the 2nd year of the study are shown (Fig. 1b). During the period July through December, when oxamyl effectively held root infection by the citrus nematode at the threshold level, the number of new roots initiated per gram roots by oxamyl-protected trees averaged 4.0 ± 0.5 (± SE, N = 4 flushes), whereas nematode-

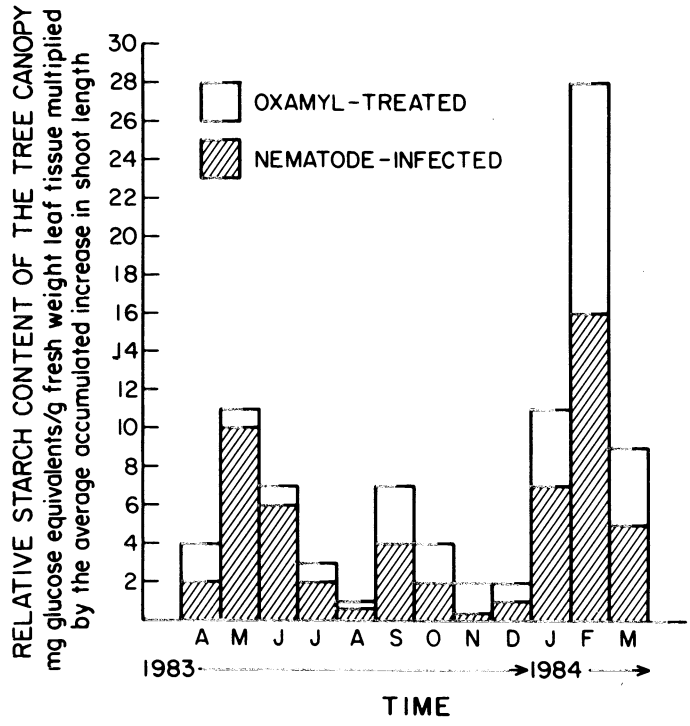

Fig. 3. Relative amount of starch available in the canopy of nematode-infected (▨) and oxamyl-treated (□) 'Washington' navel orange on Troyer citrange; based on mg glucose equivalents/g fresh weight leaf tissue multiplied by the average accumulated increase in shoot length of 40 tagged shoots for each sampling date.

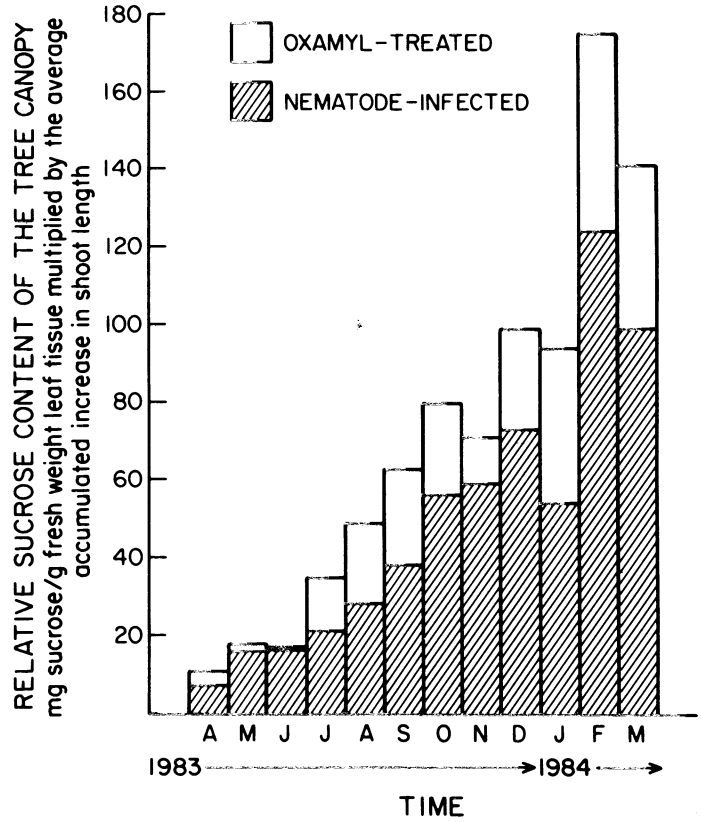

Fig. 4. Relative amount of sucrose available in the canopy of nematode-infected (▨) and oxamyl-treated (□) 'Washington' navel orange on Troyer citrange; based on mg sucrose/g fresh weight leaf tissue multiplied by the average accumulated increase in shoot length of 40 tagged shoots for each sampling date.

infected trees initiated 8.3 ± 1.9 new roots per gram roots ($\bar{x}$ ± SE, N = 4 flushes) (Fig. 1b). However, despite the increased frequency and initiation of roots by nematode-infected trees, these trees failed to increase root biomass (Fig. 1c). Concomitant with the reduction in the population of female nematodes effected after 3 months of oxamyl treatment (July), the oxamyl-protected trees began to exhibit a reduction in the number of new roots initiated (Fig. 1b) and an increase in root biomass (Fig. 1c). On the average, oxamyl-treated trees produced more roots during the growing season (the first year of recovery from nematode infection) than untreated trees, a mean (± SE) 716 ± 162 mg fresh weight roots/kg dry soil/sampling date compared to 482 ± 95, ($P < 0.01$ by Student's t test).

Sucrose was either absent or too low in concentration to be detected in roots. The glucose content of roots of oxamyl-treated trees increased during the first year of recovery (Fig. 2a) and accumulation of starch increased during the period of root dormancy following the first year of recovery (Fig. 2b).

When expressed, per gram fresh weight leaf tissue, starch, sucrose, and glucose levels during the first year of recovery from nematode infection were about the same or occasionally significantly higher for oxamyl-treated trees relative to untreated. Shoot elongation and leaf production for a number of sampling periods were significantly greater in the healthy, oxamyl-treated trees than in nematode-infected trees. Shoots borne on oxamyl-treated trees were not only longer than those on nematode-infected trees, but also had significantly more leaves per unit shoot length during development, ($P < 0.001$ by Student's t test), 9.5 ± 0.9 leaves per cm shoot length compared to 7.5 ± 0.6 ($\bar{x}$ ± SD, N = 10). In addition, oxamyl-treated trees retained a significantly greater number of leaves by the end of the growing season, 7.2 ± 0.6 vs. 5.7 ± 1.0 leaves per cm shoot length ($\bar{x}$ ± SD, N = 10), respectively ($P < 0.01$ by Student's t test).

Even though the levels of carbohydrate in leaves of both treatments were similar, the fact that shoots were longer and that there were more leaves on the oxamyl-treated trees indicates an increase in starch, sucrose, and glucose available in the canopy of the oxamyl-treated trees (Fig. 3, 4, 5). In addition, the number of new shoots initiated by oxamyl-treated trees also increased with successive oxamyl treatments (Table 1), making the differences in the carbohydrate content of the leaves even more important. Thus, the reduced canopy size of the nematode-infected trees means the total amount of carbohydrate available to these trees is also reduced. Starch accumulated before each shoot flush and disappeared at the time of their initiation (Fig. 3), emphasizing the importance of starch reserves in the canopy. Sucrose and glucose continued to accumulate throughout the year (Fig. 4, 5).

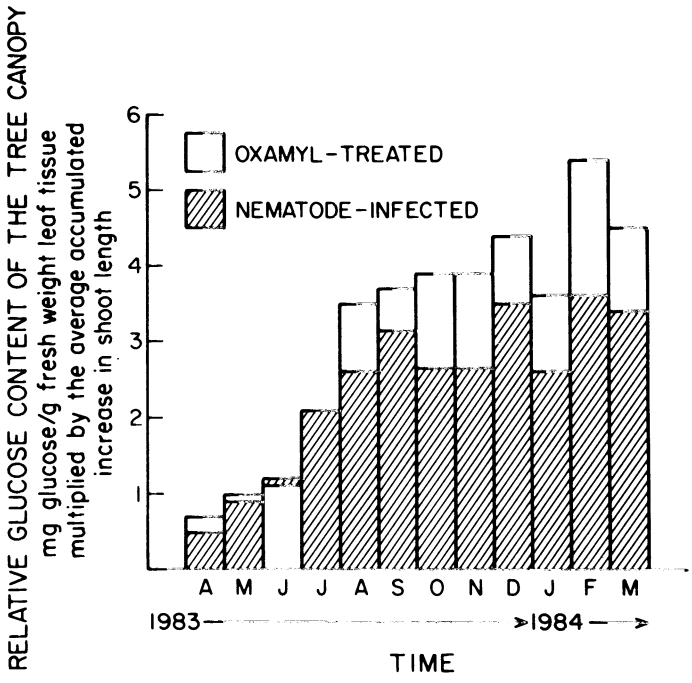

Fig. 5. Relative amount of glucose available in the canopy of nematode-infected (▨) and oxamyl-treated (□) 'Washington' navel orange on Troyer citrange; based on mg glucose/g fresh weight leaf tissue multiplied by the average accumulated increase in shoot length of 40 tagged shoots for each sampling date.

Discussion

Research to determine the actual manner by which root infection by the citrus nematode stressed citrus trees has been minimal. Van Gundy and Kirkpatrick (20) demonstrated that the citrus nematode caused direct injury to the root cortex, which resulted in a reduction in the number and quality of metabolically active roots (3, 23). It has been assumed that the loss in metabolically active roots caused poor water relations and mineral nutrient deficiencies in the scion which led to the slow decline in tree vigor and productivity. However, several researchers (7, 12, 21) subsequently demonstrated that the adverse effects of root infection by citrus nematodes usually were not caused by changes in the mineral nutrient status of the tree when the trees were grown under an optimal fertilizer program as determined by leaf analysis. The only work on tree water status failed to demonstrate that a water deficit occurred in nematode-infected trees during periods of high transpiration rate (3).

Failure of previous research to identify the etiology of the

Table 1. Mean cumulative number of shoots initiated on 40 tagged branches (7 mm in diameter) by nematode-infected trees with and without oxamyl applied at a rate of 1.1 kg/ha/3 weeks April through October of 1983

Sampling date	Nematode-infected trees (2493 ± 148 females/g roots)[z]	Oxamyl-treated trees (756 ± 72 females/g roots)[z]
1983		
22 Mar.	0	0
1 May	0	0
9 June	0	0
8 July	2.8	1.8
12 Aug.	4.8	8.5
15 Sept.	6.7	8.8
23 Oct.	7.5	10.0
15 Nov.	7.7	10.3
16 Dec.	8.3	10.3
1984		
1 Jan.	8.3	10.3
8 Feb.	10.0	16.5
9 Mar.	13.0	17.8
30 Mar.	15.0	17.8
30 Apr.	20.2	29.8

[z] $\bar{x}$ ± SE, N = 20 to 27 observations.

stress caused by root infection by citrus nematode led us to compare the phenology and carbohydrate status of nematode-infected trees and those protected from infection and permitted to recover from prior infection.

The results of the present study provide evidence demonstrating that citrus trees infected with nematodes at levels well above the economic threshold initiate new roots more often than healthy trees (6,11) or trees recovering from root infection by *T. semipenetrans* and protected from subsequent infection. This response may be common in trees infected with root nematode. Ritchie (17) recently demonstrated that peach trees initiated extra root growth in response to nematode infection. In addition, our results show a strong correlation between the successful reduction of the population of female nematodes (by treating nematode-infected trees with oxamyl 1.1 kg/ha/3 weeks) and a decrease in the number of new roots initiated. Concomitantly, the root and shoot biomass of the trees recovering from nematode infection increased as oxamyl treatments continued. These observations are consistent with increased availability of carbohydrates to support such growth.

We suggest that the utilization of energy for repeated initiation of new roots lowers the level of carbohydrate available to nematode-infected trees. The low glucose content observed for roots from nematode-infected trees and the failure of starch to accumulate in these roots to the same degree it did in the roots of trees protected from heavy nematode infection is cited as evidence to support this hypothesis. In addition, the reduced rate of shoot elongation and number of new shoots initiated by nematode-infected trees are consistent with a lack of an available energy source to support new growth.

We also propose that the reduction in both shoot elongation and number of leaves per unit shoot length, in addition to the decrease in number of new shoots initiated by nematode-infected trees, aggravates further the low carbohydrate status of the tree by reducing the total photosynthetic area of the tree and by shifting the age structure of the canopy to one dominated by old leaves, those predominantly from spring flushes of previous years. Leaves of *C. sinensis* remain on the tree for up to 3 years; old leaves exhibit a lower capacity to fix CO_2 (8, 15). The importance of adequate leaf area per fruit to insure good size and fruit quality is well established for the 'Washington' navel orange variety (18). Shamel and coworkers (18) demonstrated that fruit size increased as the number of leaves per fruit increased; optimal fruit size required 60 leaves per fruit.

Taken together, our data strongly suggest that the overall loss in photosynthate results in altered patterns of carbohydrate allocation and availability in specific organs of the tree. The failure of nematode-infected trees to accumulate adequate carbohydrate reserves, in turn, reduces vegetative growth, flower numbers, and/or fruit set, and slows rate of fruit growth. This interpretation is consistent with the slow, subtle decline in tree vigor and the reduction in both the size and number of fruit at harvest characteristic of nematode-infected trees.

Literature Cited

1. Baines, R.C. and O.F. Clarke. 1952. Citrus-root nematode effects on young lemon and orange trees studied in inoculation tests under controlled conditions. Calif. Agr. 6:9–13.
2. Baines, R.C., T. Miyakawa, J.W. Cameron, and R.H. Small. 1969. Infectivity of two biotypes of the citrus nematode on citrus and some other hosts. J. Nematol. 1:150–159.
3. Baines, R.C., S.D. Van Gundy, and E.P. DuCharme. 1978. Nematodes attacking citrus, p. 321–345. In: W. Reuther, E.C. Calvan, and G.E. Carman (eds.). The citrus industry, vol. 4. Univ. of California, Berkeley.
4. Brandao, S.C.C., M.L. Richmond, J.I. Gray, I.D. Morton, and C.M. Stine. 1980. Separation of mono- and disaccharides and sorbitol by high performance liquid chromatography. J. Food Sci. 45:1492–1493.
5. Byrd, D.W., K.R. Barker, H. Ferris, C.J. Nusbaum, W.E. Griffin, R.H. Small, and C.A. Stone. 1976. Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. J. Nematol. 8:206–212.
6. Crider, F.J. 1927. Root studies of citrus trees with practical applications. Citrus Leaves. 7:1–3, 27–30.
7. Embleton, T.W., W.W. Jones, and C.K. Labanauskas. 1962. Sampling orange leaves-leaf position important. Calif. Agr. 47:382–396.
8. Erickson, L.C. 1968. The general physiology of citrus, p. 86–126. In: W. Reuther, L.D. Batchelor, and H.J. Webber (eds.). The citrus industry, vol. 2. Univ. of California, Berkeley.
9. Hassig, B.E. and R.E. Dickson. 1979. Starch measurement in plant tissue using enzymatic hydrolysis. Physiol. Plant. 47:151–157.
10. Integrated Pest Management Group of the Statewide Integrated Pest Management Project. 1984. Integrated pest management for citrus. Div. of Agr. and Natural Res. Pub. 3303. Univ. of California, Berkeley.
11. Krishnamurthi, S., G.S. Randhawa, and P.C. Sivaraman Nair. 1960. Growth studies in some citrus species under subtropical conditions. Indian J. Hort. 17:171–184.
12. Labanauskas, C.K., R.C. Baines, and L.H. Stolzy. 1965. Effect of citrus nematode (*Tylenchulus semipenetrans*) and two levels of water suction on nutrient concentration in navel orange leaves and roots. Soil Sci. 99:367–374.
13. MacRae, J.C. 1971. Quantitative measurement of starch in very small amounts of leaf tissue. Planta 68:1389–1394.
14. O'Bannon, J.H. and A.C. Tarjan. 1969. Increasing yield of Florida citrus through chemical control of citrus nematode, *Tylenchulus semipenetrans*. Proc. 1st Intern. Citrus Symp. 2:991–998.
15. Rhodes, W. and R.T. Wedding. 1953. The photosynthetic and respiratory rates of citrus leaves of four different ages. Citrus Leaves 33(9):10–11.
16. Richmond, M.L., S.C.C. Brandao, J.I. Gray, P. Markakis, and C.M. Stine. 1981. Analysis of simple sugars and sorbitol in fruit by high-performance liquid chromatography. J. Agr. Food Chem. 29:4–7.
17. Ritchie, D. 1984. Why peach trees go into sudden death. Agrichemical Age 28(2):12, 42.
18. Shamel, A.D. and C.S. Pomeroy. 1933. Relation of foliage to fruit size in Navel oranges. Calif. Citrog. 18:296–297.
19. Van Gundy, S.D., S. Garabedian, and E.L. Night. 1981. Alternatives to DBCP for citrus nematode control. Proc. Intl. Soc. Citriculture 1:387–390.
20. Van Gundy, S.D. and J.O. Kirkpatrick. 1964. The nature of resistance in citrus rootstocks to the citrus nematode. Phytopathology 54:419–427.
21. Van Gundy, S.D. and J.P. Martin. 1961. Influence of *Tylenchulus semipenetrans* on growth and chemical composition of sweet orange seedlings in soils of various exchangeable cation ratios. Phytopathology 51:146–151.
22. Van Gundy, S.D. and J.W. Meagher. 1977. Citrus nematode (*Tylenchulus semipenetrans*) problems worldwide. Proc. Intl. Soc. Citriculture 3:823–826.
23. Van Gundy, S.D. and P.H. Tsao. 1963. Infesting citrus seedling with citrus nematode, *Tylenchulus semipenetrans*. Phytopathology 53:228–229.