Journal of Nematology 19(3):296-303. 1987.

Effect of Initial Population Density of *Criconemella xenoplax* on Reducing Sugars, Free Amino Acids, and Survival of Peach Seedlings over Time

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Abstract: Percentage of mortality, growth suppression, and changes in free amino acid and reducing sugar content in root and (or) stem tissue of Nemaguard peach seedlings were studied in the greenhouse in relation to time and eight different initial population densities (Pi) of *Criconemella xenoplax.* After 90 and 180 days, free amino acid content in root tissue significantly increased with increasing nematode numbers. Suppression of root volume, dry root and stem weight, height increase, plant survival, and content of reducing sugars in root tissue were detected at 180 and 270 days and following pruning. All criteria were negatively correlated with nematode Pi. Changes in growth, metabolic parameters, and survival percentage were attributed to Pi density of *C. xenoplax,* duration of the experiment, and nematode reproduction rate.
Key words: *Criconemella xenoplax,* ring nematode, *Prunus persica,* interaction, peach tree short life.

Host damage caused by plant-parasitic nematodes is dependent upon host tolerance, population density, and rate of reproduction (26). In initial studies, *C. xenoplax* (Raski) Luc & Raski caused no appreciable damage to peach (*Prunus persica* (L.) Batsch) after 4 months with an inoculum level of 600 *C. xenoplax* per 250 cm^3 soil (15). High initial numbers of *C. xenoplax,* however, caused poor growth of peach and predisposition to *Pseudomonas syringae* pv *syringae* van Hall in 15-liter containers after 2 years (1,16). Discrepancies in earlier studies with this nematode might have been related to inoculum density and duration of experiments because 50,000 *C. xenoplax* per seedling suppressed peach growth as early as 4 months after inoculation (1).

Quantitative associations between plant growth and nematode density are essential to understanding of host-parasitic relationships and control of a nematode pest. For example, under South Carolina conditions, a nematicide treatment threshold of >50 *C. xenoplax*/100 cm^3 soil is recommended in peach orchards for prolonging tree longevity and maintaining production in orchards. Because it is feasible to establish a threshold level for this nematode with regard to peach tree short life (PTSL), it is important to understand the effect that different population densities of *C. xenoplax* have on physiological parameters of peach as related to cold hardiness. The effect of *C. xenoplax* on free amino acids (6) and reducing sugars (4,27) may give us some clue to peach tree survival. Parasitism by ectoparasitic nematodes affects free amino acid levels in roots as measured by ninhydrin-reactive compounds. *Longidorus africanus* Merny feeding caused an increase in free amino acids in bur marigold (*Bidens tripartita* L.) roots (7) but not in grape (*Vitis vinifera* L.) roots. *Criconemella xenoplax* reduced the content of free amino acids in peach shoots and roots after 13 months (22), with specific root molar percentage increases occurring in proline, glycine, and alanine and a decrease in arginine. No information is available, however, regarding this nematode's effect on reducing sugars in peach.

Although environmental influence on *C. xenoplax*–peach relationship in the field is difficult to simulate, effects of different population densities of *C. xenoplax* over time may help to understand this parasitic relationship. We investigated effects of different population densities of *C. xenoplax*

Received for publication 12 June 1986.
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The authors thank D. Watts and C. Haralson for technical assistance, and B. Joyner for advice on the statistical analysis.

on reducing sugars, free amino acids, and growth of peach in the greenhouse.

Materials and Methods

Stratified 'Nemaguard' peach seed were planted directly into 15-cm-d plastic pots containing approximately 1,500 cm^3 steam-pasteurized loamy sand (86% sand, 10% silt, 4% clay) in November 1984. Twelve days later, pots with uniform seedlings were selected and inoculated with *C. xenoplax* in a geometric series of increasing concentration (0 and 7, 28, 112, . . . , 14,336 adults and juveniles/1,500 cm^3 soil). The isolate of *C. xenoplax,* from a PTSL orchard in Byron, Georgia, was cultured on Nemaguard peach seedlings grown in a sand: vermiculite medium. Nematodes were extracted from the medium using centrifugal flotation (12). Experimental seedlings were inoculated with a 50-ml aqueous suspension of nematodes which was poured onto the soil surface, previously tilled to a depth of 1.0 cm. Additional water was applied to wash the *C. xenoplax* into the soil. A 50-ml aliquot of nematode-free solution obtained from the extraction procedure was added in a similar manner to soil of control seedlings.

Plants were sampled after 3, 6, and 9 months. Seedlings that remained after each sampling date were pruned back to a height of 18.0 cm. Treatments, replicated seven times in the 3-month and 6-month studies and four times in the 9-month study, were arranged in a randomized complete block design on benches in an air-conditioned greenhouse (25 ± 5 C). Plants were watered daily and fertilized every 2 weeks (21). At each sampling date root volume, height increase (as determined on new teminal growth only), dry root weight, dry stem weight (minus leaves), content of reducing sugars in stem and root tissue, content of free amino acids (measured as ninhydrin-reactive compounds) in root tissue, and *C. xenoplax* population density (Pf) were measured. Nematodes were extracted from 100 cm^3 soil using a semi-automatic elutriator (2) combined with centrifugal flotation (12).

Sugar content in stem and root tissue was determined colorimetrically using Nelson's modification of Somogyi's method (11). Approximately 300 mg ground and dried plant material was extracted in triplicate with 70% ethanol for 24 hours. Sugar contents were determined by measuring absorbance at 490 nm and converted to micrograms glucose per milligram dry weight basis by using a standard curve.

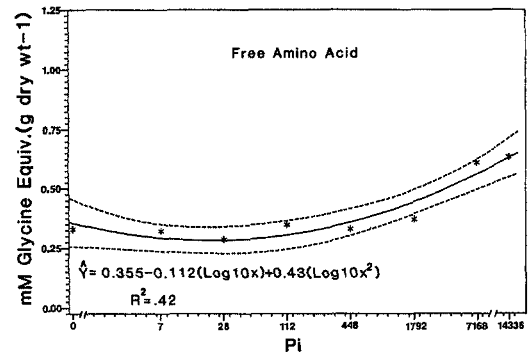

Fig. 1. Influence of initial inoculum density (Pi) of *Criconemella xenoplax* on free amino acid content in Nemaguard peach root after 90 days. Dotted lines = 95% confidence limits; * = treatment mean.

Free amino acid content in root tissue was determined after 0.05–0.10 g ground-dried tissue was extracted (18,22).

All data were subjected to general linear model and regression analysis, relating dependent variables (root weight, reducing sugars, etc.) to initial numbers (Pi) of nematodes (independent variable) in each sampling.

Results

Root volume, height increase, dry root and stem weight, content of reducing sugars in root and stem tissue, and seedling survival were not significantly affected by *C. xenoplax* after 90 days. Visible symptoms of stress or nutrient deficiencies also were not evident. Free amino acid content (FAA) in root tissue, however, increased ($P = 0.01$) in proportion to the Pi of *C. xenoplax* (Fig. 1). The nematode population density increased in all inoculated treatments, but the rate of increase was greatest in the treatment receiving the lowest Pi density (Table 1).

Following pruning at 90 days, all Ne-

TABLE 1. Influences of initial population density of *Criconemella xenoplax* upon reproductive rate, height increase, dry root weight, and survival of Nemaguard peach in the greenhouse 90, 180, and 270 days after inoculation.

	Reproductive rate‡			Height increase§ (cm)			Dry root wt (g)			Survival (%)		
Pi†	90	180	270	90	180	270	90	180	270	90	180	270
0	0.0	0.0	0.0	110	78	71	1.3	4.7	6.6	100	100	100
7	64.2	57,758.6	92,391.4	115	76	44	1.4	4.1	4.0	100	100	100
28	27.3	43,433.0	27,070.7	114	65	13	1.5	4.1	2.5	100	100	75
112	8.4	8,015.6	6,240.3	114	68	13	1.2	3.9	4.3	100	100	75
448	7.7	3,261.9	1,340.4	110	65	1	1.3	2.6	2.0	100	100	25
1,792	7.0	686.2	116.9	114	54	0	1.2	2.2	1.2	100	100	0
7,168	6.2	54.3	29.5	112	14	0	1.5	0.9	1.2	100	43	0
14,336	7.2	27.1	15.3	115	5	0	1.4	1.1	0.6	100	29	0

Data are means of seven replications for 90 and 180 days and four replications for 270 days.
† Pi = initial population density of *C. xenoplax* per 1,500 cm^3 soil.
‡ Reproductive rate = Pf/Pi, where Pf = final population density per 1,500 cm^3 soil.
§ Height increase = measurement of new terminal growth only.

maguard seedlings growing in the five lowest Pi densities and the control produced new top growth and grew normally until the 180-day sampling. Plants in the two highest Pi levels continue to grow normally, or initiated new growth and wilted and died after several days, or died without producing new top growth (Table 1). Root damage resulting from nematode feeding was obvious after 180 days (Fig. 2A). Plants in these treatments were not studied further because so few plants survived.

Root volume, dry stem and root weight, height increase (Fig. 3A–D), and content of reducing sugars in root tissue (Fig. 4A) all decreased ($P = 0.01$) as the Pi of *C. xenoplax* increased, whereas the content of FAA in root tissue increased ($P = 0.01$) (Fig. 4B). Free amino acid content was negatively correlated with root volume ($r = -0.54$), dry shoot ($r = -0.51$) and root ($r = -0.65$) weight, height increase ($r = -0.41$), and reducing sugar content in root tissue ($r = -0.47$). Reducing sugar content in stem tissue also increased ($P = 0.01$) in proportion to the Pi, but the coefficient of correlation was low ($r = 0.12$). Stem sugars were negatively correlated with height increase ($r = -0.51$) and positively correlated with sugar content in root tissue ($r = 0.87$).

Criconemella xenoplax increased in all

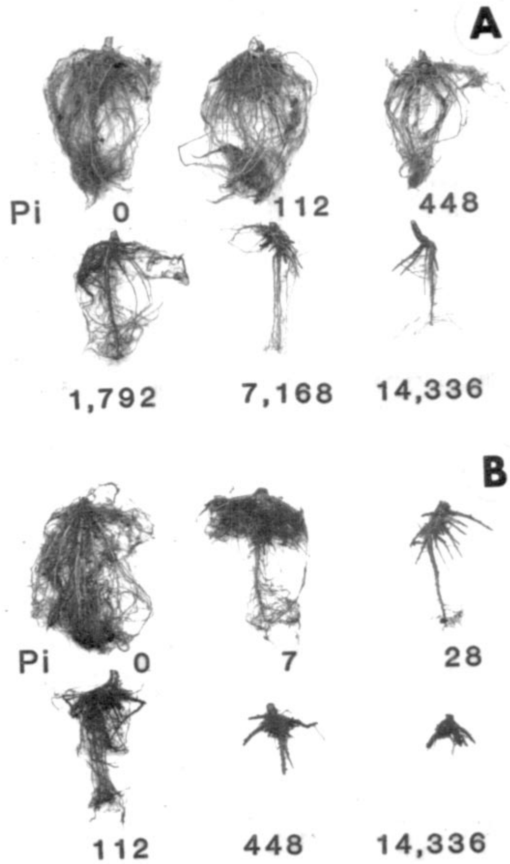

FIG. 2. Influence of six different initial inoculum densities of *Criconemellá xenoplax* per 1,500 cm^3 soil on Nemaguard peach root growth in the greenhouse. A) 180 days. B) 270 days.

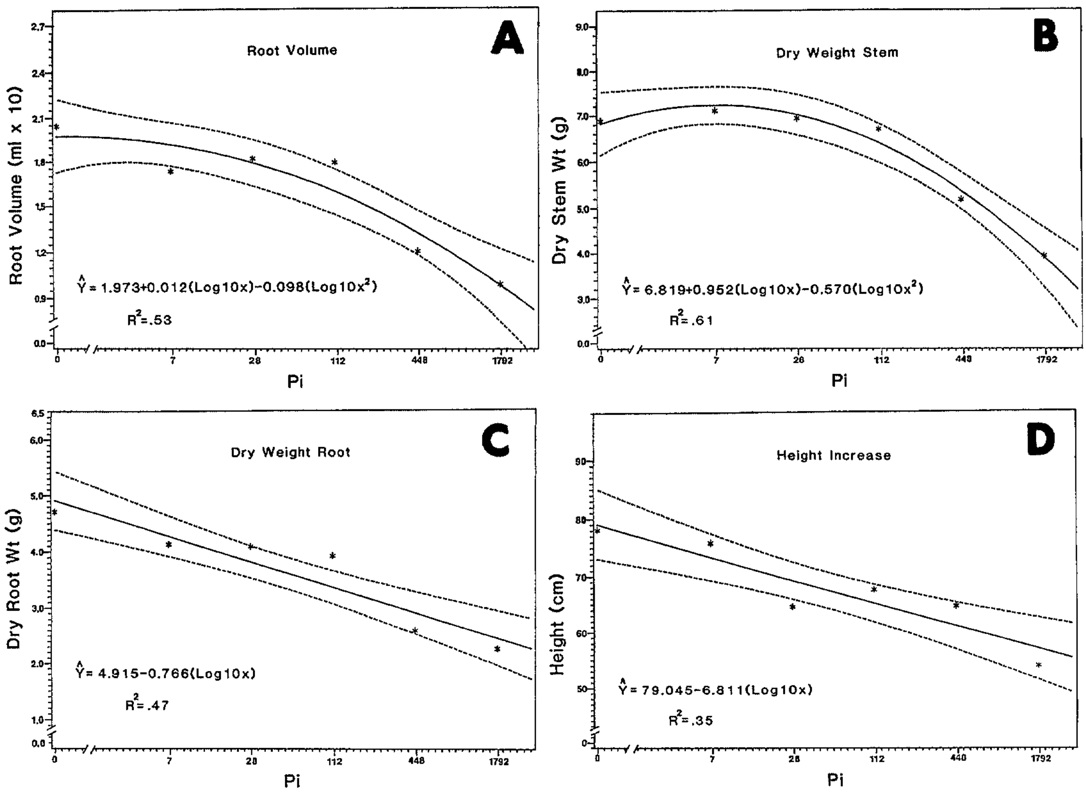

FIG. 3. Influence of initial inoculum density (Pi) of *Criconemella xenoplax* on Nemaguard peach after 180 days in the greenhouse. Dotted line = 95% confidence limit; * = treatment mean.

treatments (Table 1), but the greatest percentage increase occurred at the lowest Pi level. Rate of nematode increase was inversely proportional to the Pi density levels of the nematode. The relative influence on growth and physiological parameters of peach was more closely related to Pi than Pf (Table 2).

Percentage of survival at 270 days decreased considerably, compared with 90 and 180 days. Tree survival was 100% only in the lowest Pi treatment and the control (Table 1). All seedlings exposed to the three highest Pi levels were dead by 270 days. Only the first three Pi densities and the control were therefore analyzed. Root volume ($P = 0.01$), dry root ($P = 0.05$) and stem ($P = 0.01$) weight, height increase ($P = 0.01$) (Fig. 5A–D), root growth (Fig. 2B), and content of reducing sugars in root tissue ($P = 0.01$) (Fig. 6) decreased as the Pi density of *C. xenoplax* increased. The content of reducing sugars in root tissue was positively correlated with sugar content in stem tissue ($r = 0.75$), height increase ($r = 0.81$), dry stem weight ($r = 0.66$), and root volume ($r = 0.72$).

Population density of *C. xenoplax* was high only at the lowest Pi, and the population decreased in all other treatments when compared with population densities at 180 days (Table 1). As at the previous sampling date, rate of nematode increase was inversely proportional to the Pi density of *C. xenoplax* (Table 1). Again, the relative influence on peach tree growth and physiological variables was more closely related to Pi than Pf (Table 2). Survival and growth of Nemaguard peach at 180 and 270 days in the presence of *C. xenoplax* was dependent on Pi, duration of the experiment, and nematode reproduction rate (Table 1).

Discussion

Duration of the experiment and Pi have previously been implicated as a possible explanation for the earlier discrepancies in pot experiments involving *C. xenoplax* on peach (1). Our results substantiate this. This experiment also demonstrates for the first time that the presence of *C. xenoplax* was required to kill peach seedlings under greenhouse conditions without drastic changes in temperature (23). Presumably all seedlings were exposed to the same organisms other than *C. xenoplax,* because all check pots received an equal aliquot of nematode-free solution obtained from the extraction procedure as a means of introducing other micro-organisms. We conclude, therefore, that the presence of *C. xenoplax* was one contributing factor required for plant mortality to occur. Death did not occur, however, before 90 days or until plants were pruned back to a uniform height. Seedlings were initially pruned for maintenance purposes. Even though a nonpruned treatment was not included in this particular study, we believe pruning may have also contributed to tree death (13,14,25). In the southeastern United States, conventional pruning of peach trees reduces total dry weight of roots below that of lightly pruned trees (25). The alteration of the root/shoot ratio results in reduced root growth either by photosynthesis reduction or diversion of carbohydrates (14). According to Klingler (13), *C. xenoplax* significantly reduced top growth of grape seedlings (*Vitis vinifera* var. Blauburgunder) especially after pruning. It may be possible that what we are seeing is a diversion of seedling reserves and energy to the wound response and away from the influence of the nematode. In addition, popu-

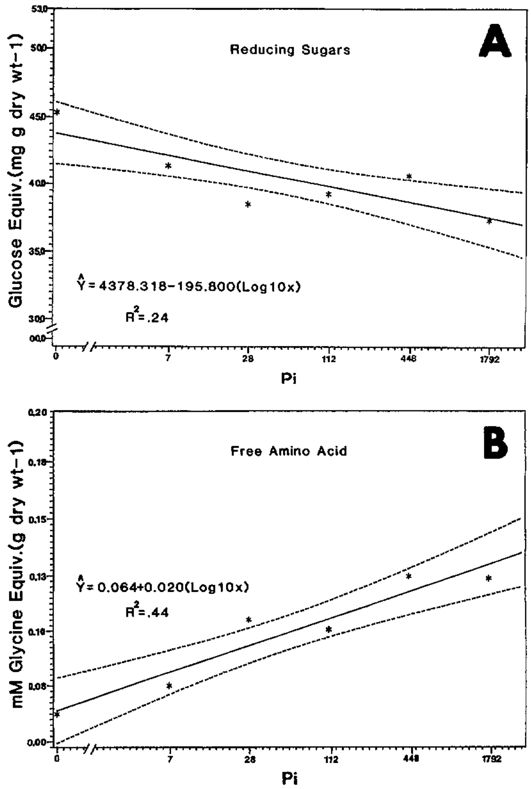

FIG. 4. Influence of initial inoculum density (Pi) of *Criconemella xenoplax* on A) reducing sugars and B) free amino acid content in peach roots after 180 days in the greenhouse. Dotted line = 95% confidence limit; * = treatment mean.

TABLE 2. Coefficient of determination (R^2) of initial (Pi) and final (Pf) population density of *Criconemella xenoplax* with physiological and growth variables of Nemaguard peach 90, 180, and 270 days after inoculation.

	90†		180‡		270§	
Variable	Pi	Pf	Pi	Pf	Pi	Pf
Root volume	ND	ND	0.69	0.33	0.75	0.41
Dry root weight	ND	ND	0.66	0.40	0.65	0.47
Dry stem weight	ND	ND	0.79	0.31	0.79	0.47
Height increase	ND	ND	0.71	0.55	0.80	0.39
Free amino acids	ND	ND	0.64	0.51	0.52	0.40
Reducing sugars (root)	ND	ND	0.55	0.38	0.75	0.34
Reducing sugars (stem)	ND	ND	0.41	0.35	0.40	0.15

† Based on eight Pi levels of *C. xenoplax* and seven treatment mean replications. ND = no detectable difference.
‡ Based on six Pi levels of *C. xenoplax* and seven treatment mean replications.
§ Based on four Pi levels of *C. xenoplax* and four treatment mean replications.

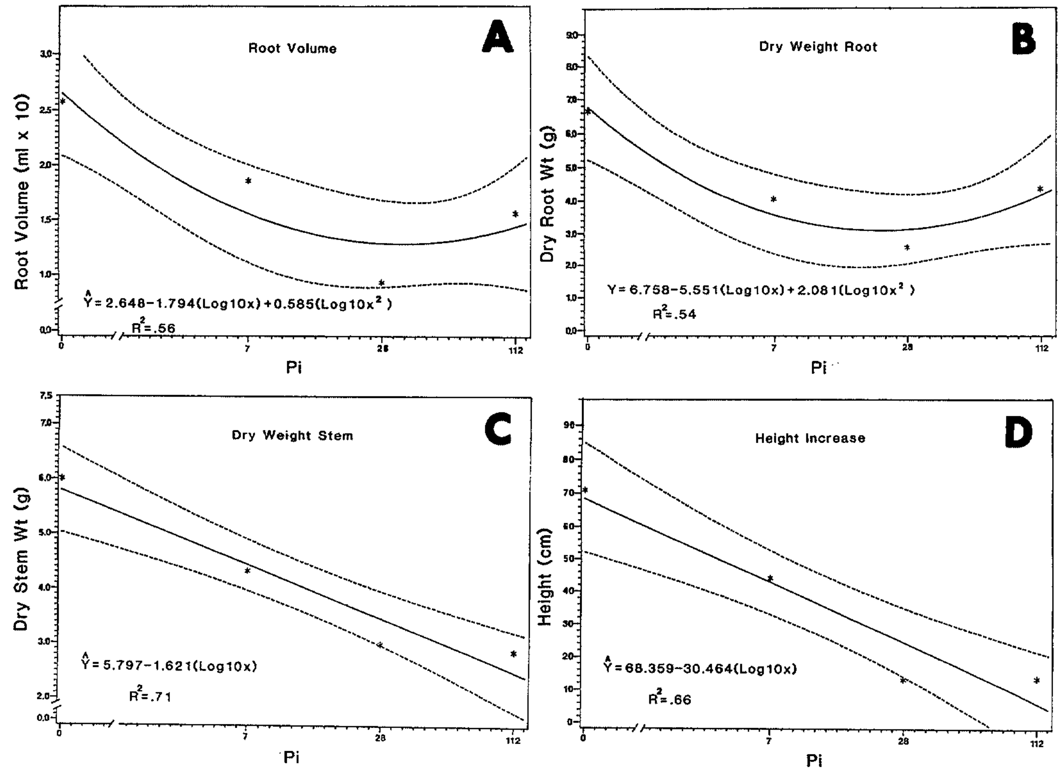

FIG. 5. Influence of initial inoculum density (Pi) of *Criconemella xenoplax* on Nemaguard peach on A) root volume, B) dry stem weight, C) dry root weight, and D) height increase after 270 days in the greenhouse. Dotted line = 95% confidence limit; * = treatment mean.

lation density of *C. xenoplax* increased after pruning Nemaguard peach seedlings (Nyczepir, unpubl.) and under fall pruned trees of an unknown rootstock in the field (19).

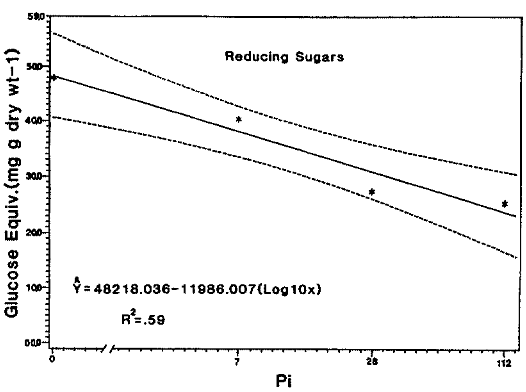

FIG. 6. Influence of initial inoculum density (Pi) of *Criconemella xenoplax* on reducing sugar content in root tissue of Nemaguard peach after 270 days in the greenhouse. Dotted line = 95% confidence limit; * = treatment mean.

Pruning apparently enhances nematode reproduction; however, the explanation as to how this happens or as to why the plants are dying is not known at the present time.

Altered metabolic processes were first observed in root systems at the 90-day sampling, even though seedlings exhibited no signs of stress. Free amino acid content in roots was elevated as Pi of *C. xenoplax* increased. Similar results occurred again at 180 days but not at 270 days. Increase in FAA content is commonly associated with pathogenesis (9). Nematodes with varied feeding habits, however, affect FAA levels in plants differently (7,9,17,22). In a previous study (22), *C. xenoplax* did not alter FAA in Nemaguard roots after 8 months, but significantly decreased them after 13 months. Our present study (270 days = 9 months) not only agrees with their 8-month FAA data, but also with the decrease in dry root weight for this period of time. It is not certain why a significant decrease in dry root weight occurred after 8 and 9 months without influencing root FAA content.

Reducing sugars in roots were significantly decreased as the Pi density of *C. xenoplax* increased at 180 and 270 days, but increased in stem tissue with increasing Pi at 180 days. This first report that *C. xenoplax* influences reducing sugars in roots and stems of peach indicates that parasitism by *C. xenoplax* can alter carbohydrate content of those sugars associated with plant growth. Since early deciduous tree growth is initiated before photosynthetic activity, it must depend on stored food reserves (14). Food reserves are believed to come primarily from roots which contain approximately twice the amount of sugar found in stem tissue (14). Therefore, the severely reduced root systems, occurring as a result of nematode feeding following pruning, may have contributed to tree death by limiting carbohydrate reserves and reducing sugars used in plant metabolism. Why the concentration of reducing sugars in stem tissue increased as the Pi of *C. xenoplax* increased at 180 days but not at 270 days is not known, but the average sugar content for treatments analyzed was lower in stem tissue than in root tissue at 180 days (40.44 mg glucose/g dry root vs. 28.52 mg glucose/g dry stem) and 270 days (35.21 mg glucose/g dry root vs. 21.34 mg glucose/g dry stem), indicating that reducing sugars were present in both root and stem tissue, but in greater content in roots.

Seasonal fluctuations in individual amino acids (6) and reducing sugars in stem tissue (4,6,27) have been associated with cold hardiness of different deciduous trees, including peach. Sugars are believed to induce cold hardiness in plants by protecting and stabilizing sensitive cell membranes against damage due to freezing, heating, and desiccation (10). Stabilization of cell membranes is dependent on both the content and molecular weight of sugars (24). The influence of *C. xenoplax* on FAA levels in roots was studied previously (22); however, data on the effect of *C. xenoplax* on winter injury and changes of sugar content in root tissue are lacking. Two migratory endoparasitic nematodes, *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven (5) and *Ditylenchus dipsaci* (Kuhn) Filipjev (3), and the ectoparasitic nematode *Xiphinema americanum* Cobb (8) are associated with reduced cold hardiness of Montmorency cherry (*Prunus cerasus* L.), alfalfa (*Medicago sativa* L.) and Colorado Blue spruce (*Picea pungens* Engelm.), respectively. *Ditylenchus dipsaci* induced a decrease in percentage of total nonstructural carbohydrates in alfalfa roots. The interesting aspect of this association is that the nematode parasitized stem tissue, but root sugar content essential for overwintering of alfalfa decreased (3). Our results demonstrate that reducing sugars in roots of potted peach trees decrease in proportion to the Pi of *C. xenoplax.* This reduction in reducing sugars may help explain how *C. xenoplax* predisposes peach to cold injury (20). Additional experiments are required to determine if similar changes occur under controlled field conditions (e.g., field microplots). However, such greenhouse data on *C. xenoplax* population dynamics and plant growth reduction as related to time and pruning may prove useful in establishing a rootstock screening program of *Prunus* spp. for tolerance or resistance to *C. xenoplax.*

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