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Peach Leaf Senescence Delayed by *Criconemella xenoplax*

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Abstract: Fall annual leaf senescence of peach was delayed in the field and in microplots in the presence of *Criconemella xenoplax*. Soil from the rhizosphere of orchard trees with greener leaves had ca. 2.5× more nematodes than soil around trees in a more advanced state of fall senescence. In microplots, monoclonal antibody enzyme immunoassay (EIA) of leaf cytokinins indicated that concentration of zeatin riboside-like substances and chlorophyll content were greater in leaves of trees growing in nematode-infested soil than in trees in uninfested soil. EIA also indicated the presence of substances resembling trans-zeatin, zeatin riboside, dihydrozeatin, and dihydrozeatin riboside-like substances in whole body homogenates of *C. xenoplax*. Levels of zeatin-like substances were present in the nematode in greater levels than the other related substances.

Key words: chlorophyll, *Criconemella xenoplax*, cytokinin, peach, *Prunus persica*, ring nematode, senescence.

Senescence is "the deterioration that ends the functional life of an organism or organ" (16). In deciduous trees, fall leaf senescence is an annual event that is partially regulated by plant hormones such as cytokinins (26). Even though there is still some controversy regarding the location of all sites of cytokinin synthesis in plants, the current thinking is that roots are a major source of the cytokinin arriving in leaves (10,18).

Nematode-induced pathogenesis has been observed to involve plant hormones in several instances (2,19–21). Root gall formation caused by root-knot nematodes (*Meloidogyne* Goeldi) is associated with growth regulator disorders (9,17,25). The effect of nonsedentary nematodes on plant hormone levels has received little attention (19,22,23); such compounds may be involved in the host response to nematode infection. For example, indoleacetic acid-inactivating agents have been detected in *Criconemella xenoplax* (Raski) Luc & Raski, leading to postulation (21) that this was the reason parasitized roots were coarse and devoid of lateral roots. Such root symptomatology does not appear unless high populations of *C. xenoplax* are present (11,12).

In October 1979, eight of ten 3-year-old Nemaguard peach trees growing in microplots containing soil infested with *C. xenoplax* retained their leaves longer and were greener than five of nine trees in control plots (13). Within the next 3 years, nine of the ten inoculated trees died after exhibiting cold injury symptoms typically associated with the peach tree short life (PTSL) syndrome (14).

The objectives of this study were to determine the influence of *C. xenoplax* on levels of cytokinin-like substances and chlorophyll content in leaves and the presence of cytokinin-like substances in the nematode homogenate.

Materials and Methods

Field: In 1984 peach trees near Byron, Georgia, retained their foliage until early December. Two 5-year-old peach orchards with a history of PTSL had trees with greener foliage and less senescence than others in the same orchard. Five trees were randomly selected from the two senescence groups in each orchard. Soil samples (eight cores, each 2.5 cm d × 30 cm deep) were collected beneath the canopy of each tree. Nematodes were extracted from a 100-cm^3 subsample by elutriation (4) and centrifugation (8) and counted. The same trees were resampled on 16 January 1985. Data were analyzed using SAS procedures for analysis of variance.

Microplot: "Closed-end" lysimeter-type

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TABLE 1. Population density of *Criconemella xenoplax* and *Xiphinema* sp. and degree of leaf senescence of peach on two sampling dates.

Sampling date	Orchard†	Tree age (yr)	No. nematodes/100 cm³ soil			
			Total *C. xenoplax*		*Xiphinema* sp.	
			Green	Yellow	Green	Yellow
14 Dec. 1984	1	5	102**	28	13*	50
	2	5	15	11	126	100
16 Jan. 1985	1	5	697+	227	55	143
	2	5	559	289	382	368

** $P = 0.01$; * $P = 0.05$; + $P = 0.10$ according to analysis of variance procedure using transformed data $\log_{10}$ (no. + 1); actual data in table.

† Five trees with green leaves and five with yellow were sampled per orchard.

microplots (1.2 m d × 1.2 m deep) were established in June 1983 on a site where peaches had not been planted previously. The loamy sand soil (82% sand, 13% silt, 5% clay) was fumigated with 454 g methyl bromide (98% methyl bromide, 2% chloropicrin) per plot under a 0.10-mm polyethylene tarp in September 1983.

Rooted Nemaguard peach cuttings were planted (one seedling per pot) in steam-pasteurized loamy sand in 15-cm-d plastic pots and placed in a greenhouse in August 1983. In April 1984, 12 pots were infested with 5,000 *C. xenoplax* originally from a PTSL orchard in Byron. The nematodes had been cultured on Nemaguard seedlings and extracted as described for field samples. Twelve control pots were treated with a nematode-free soil extract suspension from the same culture. Cuttings and associated soil were transplanted (one cutting per microplot) to the microplots in May 1984. The experimental design was completely randomized with 12 single tree replicates per treatment. All trees received annual applications of fertilizer as recommended by the Georgia Cooperative Extension Service and were watered by trickle irrigation as needed. Nematode populations were monitored annually by sampling (six soil cores per microplot), extracting, and counting as described for field plots.

Microplot leaf assays: Foliage of each tree was subjectively rated as "green" or "yellow" on 3 November 1986. Twelve leaves (four per quadrant) located five nodes below the terminal leaf were collected from each tree on 7 November 1986. Leaves were immediately placed in plastic bags and stored in an ice-chest containing dry ice for transport to a long-term storage freezer maintained at −18 C. A 1.7-cm-d leaf disk was removed 1 cm from the base of each of 10 leaves. Leaf disks from an individual tree were composited, weighed, and placed in a 50-ml Pyrex centrifuge tube. Dihydrozeatin riboside-like and zeatin riboside-like substances were assayed according to methods previously developed for chestnut tissue (24).

Leaf chlorophyll content from the same leaves was determined according to Arnon (1). Remaining leaf tissue was weighed, and the chlorophyll was extracted using 80% acetone. The optical density of the chlorophyll extract was measured immediately at 645 and 663 nm. Arnon's equations were transformed to give molar concentrations ([22.22] O.D. 645 + 9.057 O.D. 663 = μmol Chl^{-1}) as described by Evans (6). Data were analyzed using SAS procedures for analysis of variance.

Nematode enzyme immunoassay: Criconemella xenoplax were extracted by centrifugal-flotation (8) and further separated from roots and silt by sucrose-gradient centrifugation. The gradient consisted of 1.0 M, 0.5 M, and 0.25 M sucrose, and *C. xenoplax* in water was layered on top. *Criconemella xenoplax* was collected from the 0.25-M and 0.5-M layers following centrifugation at 920 *g* for 1 minute. Nematodes were rinsed free of sugar by back washing specimens

TABLE 2. Population density of *Criconemella xenoplax*, leaf tissue content of zeatin riboside-like (ZR) and dihydrozeatin riboside-like (DZR) substances, and leaf chlorophyll of 2-year-old Nemaguard peach trees grown in microplots.

	C. xenoplax (no./100 cm³ soil)		Concentration of cytokinin-like compounds in leaf tissue (pmole/mg)		Leaf chlorophyll†
	12 Nov. 1985	4 Dec. 1986	ZR	DZR	
C. xenoplax	2,408	4,385	0.68 a	0.13 a	127.4 a
Control	0	0	0.35 b	0.09 a	100.0 b

Column numbers followed by different letters are significantly different ($P = 0.01$) according to analysis of variance procedure.

† Leaf chlorophyll content expressed as percentage of chlorophyll detected in control leaves. Mean chlorophyll content in control leaves was 171 μmol/m².

collected on a 25-μm-pore (500-mesh) sieve. Approximately 90,000 nematodes were macerated in a ground-glass homogenizer in 1 ml cold 80% methanol. The homogenate was purified by Sep-Pak C-18 column and thin-layer chromatography and assayed for dihydrozeatin, dihydrozeatin riboside, and trans-zeatin and zeatin riboside as described for leaf disks (24).

RESULTS

Field: Criconemella xenoplax and *Xiphinema* sp. were the two most abundant plant-parasitic nematodes detected in both orchards and under trees with both types of leaf senescence (Table 1). Greater numbers of *C. xenoplax* were recovered under green leaf than under yellow leaf trees. There were 3.6 and 1.4 times more *C. xenoplax* under green leaf trees than under yellow leaf trees in orchards 1 and 2, respectively, on 14 December 1984 (Table 1). The number of *C. xenoplax* in both orchards increased by the second sampling, but the population density ratio associated with leaf color remained relatively stable for both orchards (3.1 and 1.9). Significant differences between nematode number and the degree of leaf senescence classes were evident in orchard 1 on both sampling dates, with greater significance ($P = 0.01$) occurring on the first date and marginal ($P = 0.10$) on the second. None of the trees sampled in the two orchards developed symptoms of PTSL in the spring of 1985, although some surrounding trees did.

Microplot leaf assays: There were no striking differences in leaf color between the two treatments on 24 October 1986. Relative differences in leaf color were first apparent on 3 November. Based on leaf samples obtained on 7 November, 8 of 12 (67%) nematode-inoculated trees were visibly greener than 5 of 12 (42%) control trees. *Criconemella xenoplax* was well established in infested plots by the autumn of 1986 but was not detectable in control plots (Table 2). The level of zeatin riboside-like substances was almost two times greater ($P = 0.01$) in leaves of trees growing in nematode-infested soil than in leaves of control trees. No significant differences in dihydrozeatin riboside-like substances were detected between treatments. Chlorophyll content was greater ($P = 0.01$) in leaves of trees growing in nematode-infested soil than in leaves of control trees (Table 2), thus, substantiating our subjective tree rating on 3 November. Two trees growing in nematode-infested soil had succumbed to PTSL by the spring of 1987; all control trees remained alive.

Nematode enzyme immunoassay: All four cytokinin-like compounds were detected in *C. xenoplax,* but a zeatin-like substance was present in highest levels (Table 3). The riboside forms appeared to be less prevalent than the riboside-free molecule.

DISCUSSION

Parasitism of peach roots by *C. xenoplax* delayed leaf senescence. Three criteria were used to measure senescence: leaf color, chlorophyll content, and content of

TABLE 3. Cytokinin-like substances extracted from the bodies of *Criconemella xenoplax* that had fed on Nemaguard peach roots.†

Cytokinin	Amount per nematode ($\times 10^{-17}$ moles)
Trans-zeatin	13.9
Zeatin riboside	7.0
Dihydrozeatin	6.8
Dihydrozeatin riboside	3.3

† Quantitative analysis was based on enzyme immunoassays utilizing monoclonal antibodies to the four cytokinins.

zeatin riboside-like substances in leaves. Since leaf color is related to senescence, more chlorotic-appearing leaves indicated an advanced stage of senescence and conversely, greener leaves (more chlorophyll) indicated less senescence. Leaf cytokinin content should reflect the progression of senescence, since leaf cytokinin levels are closely related to the level of leaf senescence (26). The association between leaf senescence and *C. xenoplax* population levels in the orchard suggests that nematodes affect tree physiology. This effect also was observed in the microplot experiment where nematode levels were the only variable and foliage zeatin riboside-like substance levels were higher in nematode-infested than in the control plots. Additional studies would be necessary to prove that *C. xenoplax* increases leaf cytokinin levels, but presence of the nematode does appear at least to retard the senescence-related loss of cytokinins from peach leaves. The mechanism by which the nematode alters cytokinin content is unclear. It is possible that the nematode increases synthesis or decreases catabolism of cytokinin in the tree. It is also possible that *C. xenoplax* may synthesize and inject cytokinin into the plant (3,5).

Peach tree short life (PTSL) involves changes in tree physiology in such a way that affected trees are more susceptible to cold injury in late winter and early spring (15). In the present study, results provide direct evidence that cytokinin levels in the host plant are related to the feeding by *C. xenoplax* on the root system and to leaf senescence. This suggests that peach dormancy physiology is altered (7,10,16) so that trees would be more susceptible to PTSL. The effect of *C. xenoplax* on cytokinin levels over time and the significance of cytokinin in PTSL pathogenesis warrant additional research.

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