Nematodes Attacking Cultivars of Peach in North Carolina[1]

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Abstract: Criconemoides xenoplax and *Meloidogyne incognita* were the nematode species most frequently associated with peach in North Carolina. Other nematodes often found in high numbers on that crop were *Pratylenchus vulnus, Helicotylenchus* spp., *Trichodorus christiei, Xiphinema americanum* and *Tylenchorhynchus claytoni. P. vulnus* and *P. penetrans* reproduced well on rootstocks of 21 peach cultivars tested in the greenhouse. *P. zeae, P. brachyurus, P. coffeae* and *P. scribneri* decreased or increased only slightly in most instances. *C. xenoplax* increased as much as 330-fold and reproduced on all cultivars tested. In a field experiment with six peach cultivars and moderate numbers of *P. brachyurus, P. vulnus, C. xenoplax,* and *M. incognita*, only *M. incognita* caused significant stunting in 30 months. This nematode increased only on root-knot susceptible cultivars, whereas the other nematodes followed the same patterns observed in the greenhouse. In a second field experiment, seedlings were stunted significantly by high numbers of *C. xenoplax* during an 18-month period. *Key Words: Criconemoides xenoplax, Meloidogyne incognita, Pratylenchus vulnus, P. scribneri, P. coffeae, P. zeae, P. brachyurus.*

Most of the nematological research on peach, *Prunus persica* (L.) Batsch, in the Southeastern United States has concerned the effects of *Meloidogyne* spp. and the development of rootstocks resistant to this pest. Although rootstocks such as 'Okinawa' and 'Nemaguard' are resistant to most populations of *M. incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood, slight galling has been observed, primarily under greenhouse conditions (20, 22). Other nematodes reported from peach are *Criconemoides xenoplax* Raski, *Pratylenchus vulnus* Allen & Jensen, *P. penetrans* (Cobb) Chitwood & Oteifa, *P. scribneri* Steiner, *P. brachyurus* (Godfrey) T. Goodey, *P. coffeae* (Zimmerman) T. Goodey, *P. crenatus* Loof, *P. pratensis* (deMan) Filipjev and *P. zeae* Graham (2, 4, 5, 6, 7, 14, 15, 16, 18, 21).

A very serious problem encountered in peach production is "short life" (1) of the trees. Factors implicated in the etiology of this disease are nutritional deficiencies and excesses, cold injury, toxins, fungi such as *Pythium* spp., bacterial canker, damage to roots caused by disking and attack by nematodes (10, 17). By 1965, in Georgia, an estimated 33% of all peach trees were dead, and an additional 17% diseased (10). Losses in North Carolina range from 10-15% per year. Although the etiology of this disease has not been resolved fully, certain cultural practices including pruning in the Spring, use of 'Lovell' seedlings for rootstocks, and pre- and post-plant fumigation with dibromochloropropane and other materials in N.C. and other areas (1, 2, 8, 10, 14), prolong tree life from a few years up to 20 years. Chemical soil treatments greatly increase root density of peach and reduce the invasion of roots by *Pythium* spp. (1). In addition to fungi and nematodes, fumigation may also control *Pseudomonas syringae* Van Hall (6). High numbers of *C. xenoplax* resulted in poor growth of peach and predisposed plants to attack by *P. syringae* as did *Pythium* spp. (12).

Because the effects of *Pratylenchus* and *Criconemoides* species on peach have not been clearly elucidated, and because no rootstocks are known to be resistant to these nematodes, this investigation was initiated: (i) to determine the relative frequency of occurrence and densities of these and other plant parasitic nematodes on peach in North Carolina; (ii) to determine the host suitability of available rootstocks to six species of *Pratylenchus* and to *C. xenoplax*; and (iii) to measure responses of selected cultivars to these nematodes and to *M. incognita* in field plots.

MATERIALS AND METHODS

Experiments conducted: Twenty orchards

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were surveyed to determine the frequency of occurrence of various nematodes on peach in North Carolina (N. C.). Three greenhouse experiments involved six species of *Pratylenchus* on 21 cultivars and *C. xenoplax* on five cultivars. In two field experiments, selected cultivars were inoculated with *M. incognita, P. vulnus, P. brachyurus* and *C. xenoplax*. Nematode species used, source, and host from which it was isolated, if known, were: *P. scribneri,* California; *P. penetrans,* Canada; *P. coffeae* Florida; *P. brachyurus,* N. C. (tobacco); *P. zeae,* N. C. (corn); *M. incognita,* N. C. (tobacco); *P. vulnus,* N. C. (boxwood); and *C. xenoplax,* N. C. (peach).

Culture of nematodes and plants: The first three species of *Pratylenchus* were increased monoxenically on tissues of alfalfa, *Medicago sativa* L. 'DuPuits', grown on medium described by Lownsbery et al. (13). Alfalfa seeds were surface-sterilized with a 1:1 mixture of 95% ethanol and commercial 5.25% sodium hypochlorite solution. Nematodes for these cultures were initially surface-sterilized with Hibitane®. Subsequent transfers were made by introducing small sections of infected tissues to new "callus" cultures of alfalfa. The remaining species were increased in the greenhouse on the following plants: *P. brachyurus* - cowpea, *Vigna sinensis* (Torner) Savi 'Early Ramshorn'; *P. zeae* - corn, *Zea mays* L. 'N. C. 270'; *M. incognita* - *Lycopersicon esculentum* Mill. 'Floradel'; and P. vulnus and *C. xenoplax* on Lovell peach. All plants were grown in 15- or 20-cm pots filled with a 1:1 sandy loam soil and sand previously fumigated with methyl bromide.

Peach seedlings were grown initially in nematode-free soil or sand until they were 12-16 cm high. Open pollinated root-knot resistant cultivars were: Nemaguard, 'S37-1' and North Carolina selections numbered 73, 73-1, 74, 79, 83, 83I-1, 88, 88I-2, 92, 95, 228, 232, 240, 295I-5, 319, 325, 332I-1 and 333RL. Root-knot susceptible seedlings were: 'Lovell RK', Lovell, 'Lovell-y', 'Elberta', 'Carolina Natural' (Na-2), 'NL3', 'NRL-3', and 'NRL-4'.

Unless indicated otherwise, plants were inoculated by adding appropriate numbers of nematodes in aqueous suspension to 8-10 holes about 5-cm deep within 5-8 cm of a given seedling. Then the soil surface was covered with a 2-cm layer of 35-mesh or 65-mesh silica sand. Hoagland's nutrient solution was added as needed in the greenhouse experiments. Normal fertilization practices were followed in the field plots.

Sampling and extraction of nematodes: In routine sampling, twenty 2.5-cm borings about 15- to 20-cm deep were collected randomly from each of 20 orchards. In addition to these soil samples, roots were collected from 10 trees/orchard for additional nematode assays. Five borings were collected within 20 cm of the trunk of each tree from experimental plots. Soil samples were mixed by passing them through a sample divider three to four times or by sieving through a screen with 6-mm openings.

A centrifugal flotation extraction method (11) was used to recover nematodes from soil, unless indicated otherwise. A subsample of 50 cc was used for this method and for sugar-flotation-sieving (3) which was also utilized as indicated. Nematode numbers are expressed as the number/pot or number/500 cc of soil. Nematodes were extracted from roots in a Seinhorst mist chamber for 2 weeks, with nematodes collected every 4-5 days. Attempts to recover *Pratylenchus* species from roots by combining blender and Baermann funnel failed. The Baermann funnel was used for extracting nematodes from the monoxenic cultures.

Experimental designs and statistical analyses: All experiments were factorials, involving the suitability of various rootstocks as hosts for given numbers of one or more species of nematodes. Number of replicates ranged from 3-10/treatment. Plant growth in the greenhouse was measured by recording fresh root and top weights as well as plant height. In the field tests, basal cross-sectional trunk area was determined at the end of each growing season. Only the final trunk areas and the top weights obtained upon termination of the experiments are included. Standard analyses of variance were done on data from all experiments, and least significant difference (LSD) values computed where warranted.

RESULTS

Survey of orchards: Criconemoides xenoplax and *M. incognita* were the nematode species which occurred most frequently on peach in N. C.; *C. xenoplax* occurred in greatest numbers (Table 1). Of the four *Pratylenchus* species found, only *P. vulnus* occurred in high numbers, and it was limited in distribution. The type of assay used to

TABLE 1. Frequency of occurrence and relative densities of nematodes in 20 peach orchards in North Carolina.

Nematode	Orchards infested (%)	Mean nematode density in infested orchards[a] (500 cc soil)
Criconemoides spp.[b]	90	1997
Meloidogyne spp.[b]	80[c]	260
Pratylenchus spp.	45[d]	155
P. vulnus	20	488
P. brachyurus	20	0[a]
P. zeae	30	25
P. neglectus	10	0[a]
Helicotylenchus spp.[b]	45	250
Xiphinema americanum	20	125
Tylenchorhynchus claytoni	20	406
Trichodorus christiei	55	50
Hemicycliophora spp.	10	125

[a]*P. brachyurus* and *P. neglectus* were only recovered (in low numbers) by incubating roots in a mist chamber.
[b]Most frequently encountered species of respective genera were: *C. xenoplax, M. incognita* and *H. dihystera.*
[c]Comparisons of assay methods for *Meloidogyne* spp. gave the following results: examination of roots of 10 orchards, 10/10; bioassay (tomato) of soil from remaining 10 orchards, 8/10; root samples incubated in a mist chamber, 18/20.
[d]Frequency of occurrence was 14/20 for *Pratylenchus* spp. when root samples were incubated in mist chamber (species identified from 10 fields).

detect *Pratylenchus* determined the results obtained with incubation of root samples giving the greatest detection rates. Additional ectoparasitic nematodes associated with peach were: *Helicotylenchus* spp. [primarily *H. dihystera* (Cobb) Sher.]; *Trichodorus christiei* Allen; *Tylenchorhynchus claytoni* Steiner; *Xiphinema americanum* Cobb and *Hemicycliophora* spp.

Greenhouse experiments: The first of these tests involved nine replicates of each of six *Pratylenchus* species (*P. scribneri, P. penetrans, P. coffeae, P. brachyurus, P. zeae* and *P. vulnus*) on each of six cultivars of peach (Nemaguard, Elberta, Lovell, and N. C. numbered selections 88, 240 and 73). Three replicates of each treatment were harvested at 2, 4 and 8 months after inoculation with 500 nematodes/pot. Nematodes were extracted from soil by sugar-flotation-sieving and from roots in a mist chamber. Additional cultivars tested with two replicates/nematode and harvested after 8 months were: North Carolina rootstock selections 332I-1, 228, 79, 88I-2, 142, 83, 83I-1, 232, 325, 74, 95, 92, 319, 73-1, Lovell-y and 2951-5.

Because all cultivars responded very similarly to these nematodes, only the nematode population means for all cultivars are given (Fig. 1-A, B). When the data were analyzed on the basis of numbers of nematodes/plant, there were significant differences among cultivars, but not when nematodes were expressed as numbers/g of root. Only *P. vulnus* and *P. penetrans* increased significantly on peach. Of the remaining species, only *P. scribneri* increased more than 2-fold on any cultivar. Five cultivars had 2000-7000 nematodes/plant, but all others supported little reproduction. Populations of *P. zeae, P. coffeae* and *P. brachyurus* decreased in most instances.

Plant growth in this experiment of limited duration, and because initial inocula were low, it was affected very little. Plants inoculated with *P. penetrans* had slightly lower root and top weights than plants in the other treatments. Both *P. penetrans* and *P. vulnus* caused only slight discoloration of the root systems.

In the first greenhouse experiment with *C. xenoplax*, seedlings of cultivars 88 and 96 were inoculated with 0, 500, 5000 and 10,000 nematodes per plant in 20-cm pots. The rate of increase in this experiment ranged from 26- to 330-fold during a 7-month period. Root and top weights of plants inoculated with 10,000 nematodes were about one-half

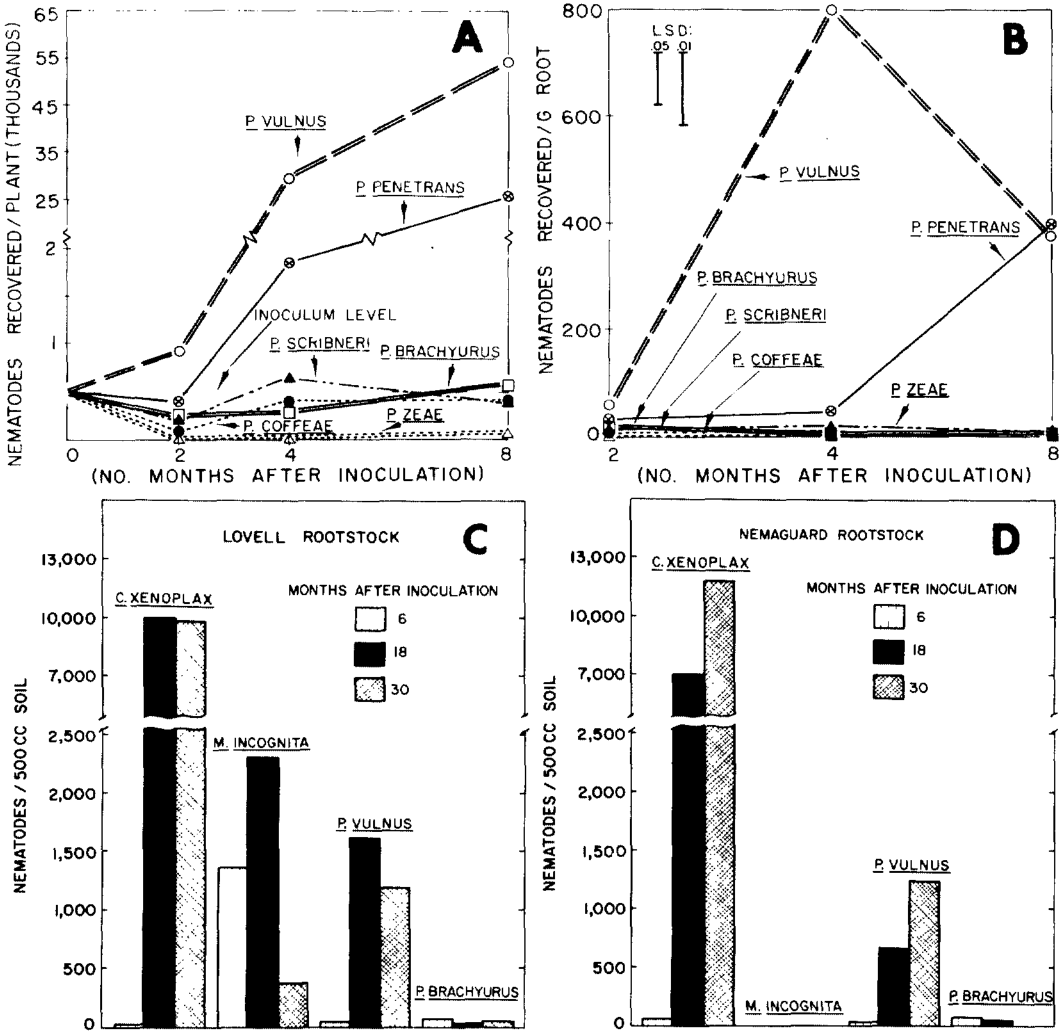

FIG. 1. Reproduction of certain nematodes on peach. **A.** Mean numbers of six *Pratylenchus* spp. recovered per plant on six cultivars in the greenhouse [LSD (at P=0.05) = 4,560, and (at P=0.01) = 6,000] **B.** Numbers of these six *Pratylenchus* species recovered per gram of root. **C.** Numbers of *Pratylenchus brachyurus, P. vulnus, Meloidogyne incognita,* and *Criconemoides xenoplax* on root-knot susceptible 'Lovell' in field plots at three sampling times; and **D.** Numbers of these four nematodes on root-knot resistant cultivar 'Nemaguard' at the three sampling times.

that of the controls (Table 2).

The second greenhouse experiment with *C. xenoplax* involved five cultivars (Lovell RK, 333RL, Lovell, S37-1 and Na-2) at three inoculum levels (0, 5000 and 50,000 nematodes/20-cm pot). As with the *Pratylenchus* species, there were significant differences among cultivars in final numbers of nematodes/plant after 4 months, but not in numbers of nematodes/g of root. Only the high inoculum level resulted in a significantly smaller root system for all cultivars as compared to the controls (Table 2). Top weights and heights were very similar at all inoculum levels.

Field experiments: In a 30-month field test, seedlings (50-cm height) of six cultivars (Nemaguard, 88, 88I-8, Na-2, Lovell and Lovell - y) were inoculated with four nematode species in methyl bromide-treated clay loam soil. Four two-tree plots were inoculated with a given nematode species by adding infested soil to a 12-cm depression (40-cm diam) around a given tree as follows: *C. xenoplax* 10,000; *M. incognita* - 160,000 eggs; *P. vulnus* - 11,000 or *P. brachyurus* -

TABLE 2. Increase of *Criconemoides xenoplax* on peach and resulting host growth responses to various inoculum levels under greenhouse conditions at different harvest times.

Inoculum level (nematodes/pot)	Nematode reproduction: No. recovered in thousands			Plant response		
	No./pot	No./gram root	R value[a]	Root wt (g)	Top wt (g)	Top height (cm)
Experiment 1 (Harvested 7 months after inoculation)[b]:						
Control	0	0	0	92	59	89
500	164.9	2.1	330	109	62	72
5000	232.2	2.1	46	121	75	79
10,000	259.4	6.5	26	39[c]	26	40
LSD P=0.05	74.2	2.5	128	N.S.[c]	30	13
P=0.01	N.S.	3.8	194		N.S.	19
Experiment 2 (Harvested 4 months after inoculation)[d]:						
Control	0	0	0	30	27	83
5000	50.8	1.9	10.1	28	27	88
50,000	221.5	10.1	4.4	23	25	84
LSD P=0.05	20.4	1.3	1.0	2	N.S.	4
P=0.01	27.3	1.7	1.3	3	N.S.	N.S.

[a]R value (reproduction factor) = final population/initial population.
[b]Data for Experiment 1 are means of four replicates of cultivar '88', except that cultivar '96' was used in two replicates of the 500 and 5000 inoculum levels.
[c]When compared with control only, high inoculum level resulted in significantly (P=0.05) lower root weight.
[d]Data for Experiment 2 are means of 10 replicates for each of five rootstocks, giving a total of 50 replicates.

1500. Soil with chopped healthy peach roots was added to control plots. Trees were transplanted 150 cm apart in 150-cm rows, with 4-mm plastic shields used as barriers between plots.

There were striking differences in numbers of *M. incognita* on the susceptible cultivars (Na-2, Lovell, and Lovell-y), as compared to the resistant cultivars (Nemaguard, 88, and 88I-8). *M. incognita* failed to reproduce on the resistant rootstocks and declined after 18 months on the susceptible plants. Otherwise, rootstock cultivar had little effect on numbers of nematodes recovered at 6, 18 and 30 months after inoculation. Figure 1-C, D show nematode populations associated with Lovell and Nemaguard rootstocks. *P. brachyurus* failed to increase on any cultivar, but *P. vulnus* reproduced on all plants tested, with the greatest numbers occurring after 30 months in most cases (Fig. 1-C, D). *C. xenoplax* had the highest rates of reproduction, increasing up to 10-16 thousand/500 cc of soil after 18 to 30 months (data for only two cultivars given in Fig. 1-C, D).

The only nematode to affect tree growth significantly was *M. incognita*, and this was limited to the susceptible cultivars (Table 3).

In a second field experiment 40- to 60-cm tall seedlings of five cultivars (N13, Lovell RK, NRL-3, NRL-4 and Na-2) were inoculated with 0, 500, 5000, 50,000 or 500,000 *C. xenoplax*/single tree plot. Infested soil was added to a 15-cm depression (40-cm diam) around each tree. Rows were 150 cm apart with 130 cm between trees. Plots had been fumigated 1 month earlier with methyl bromide at 673 kg/hectare (600 lb/acre). All plants supported high numbers of *C. xenoplax* with no significant differences among cultivars. After 5 months the greatest numbers of nematodes were recovered from trees inoculated with 500,000. However, after 18 months, the highest populations were from trees initially receiving 5000 nematodes, followed by those inoculated with 500/tree (Table 4).

The highest inoculum level had a highly significant detrimental effect on trunk diameter (Table 4). The moderate inoculum levels also caused some stunting as compared to the controls.

DISCUSSION

Our survey and many previous field experiments indicate that *Meloidogyne incognita* is the most destructive nematode on

TABLE 3. Growth of peach cultivar seedling in field plots infested with various nematodes[a].

	Cultivar top weights (Kg)						Basal cross-sectional tree trunk area (cm^2)					
Nematode[b]	Nema-guard	88	881-8	Na-2	Lovell	Lovell-y	Nema-guard	88	881-8	Na-2	Lovell	Lovell-y
None (Control)	3.8	1.6	1.4	1.3	2.1	2.1	16.6	6.0	7.2	7.4	10.9	10.8
Criconemoides xenoplax	3.1	1.5	1.0	1.4	1.7	1.4	15.0	7.2	6.3	7.5	8.8	8.9
Pratylenchus brachyurus	2.5	1.7	1.5	2.0	1.8	1.6	12.2	7.7	7.2	9.5	9.4	8.9
Pratylenchus vulnus	3.6	1.9	1.2	1.5	1.5	1.6	14.6	8.8	6.5	9.0	8.0	8.9
Meloidogyne incognita	2.9	1.1	0.9	0.3	0.7	0.7	13.1	6.2	5.3	2.5	5.1	5.7
LSD P=0.05	N.S.	N.S.	N.S.	1.0	0.8	N.S.	N.S.	N.S.	N.S.	4.5	2.9	N.S.

[a]Data were taken 30 months after inoculation.
[b]Initial inoculum levels/tree/nematode were: *C. xenoplax* – 10,000; *P. brachyurus* – 1500; *P. vulnus* – 11,000; and *M. incognita* – 160,000 eggs.

peach in North Carolina. The decrease in numbers of this nematode between 18 and 30 months in the field tests, compared to the constant or increasing numbers of *C. xenoplax* and *P. vulnus*, reflects the striking damage to root systems caused by *M. incognita*. However, the high numbers of *C. xenoplax* found in most orchards may be causing damage directly and possibly predisposing peach to attack by other organisms such as *P. syringae* as Lownsbery et al. (12) have observed. The striking responses of peach to preplant treatments with dibromochloropropane obtained in North Carolina and other states (1, 2, 8, 10, 14) may involve a reduction of such interactions as suggested by DeVay et al. (6) and Lownsbery et al. (12) as well as providing nematode control.

Although excellent sources of resistance to *M. incognita* and *M. javanica* are available, our tests indicate that developing cultivars with high resistance to *P. vulnus*, *P. penetrans* and *C. xenoplax* will be difficult, since all rootstocks evaluated were good hosts for these nematodes. Minor differences in reproduction of these pests on a tree basis can be attributed primarily to different growth rates among cultivars. Fortunately, the *Pratylenchus* species are limited in their distribution in North Carolina. Some of the previous research on *C. xenoplax* and *C. ornatus* indicated that peach is a poor host for these nematodes (19). Host differences for populations of *C. xenoplax*, inoculum density and duration of experiments may be responsible for these discrepancies.

Although *P. vulnus* caused little damage in our tests, it has been associated with decline in Georgia (7) and California (14). Apparently, high numbers of this species, as well as *P. penetrans*, are required to cause direct damage to peach. However, they may be causing indirect damage by interacting with various organisms or by predisposing peach to cold or drought injury. Griffin and Epstein (9) found *Xiphinema americanum* to be associated with winter-kill of ornamental spruce. Several ectoparasitic as well as endoparasitic nematodes might be involved in similar interactions on peach.

TABLE 4. Increase of *Criconemoides xenoplax* on peach and resulting host response in field plots.

Initial nematodes per tree	Mean nematodes per 500 cc soil (× 10^3)		Mean plant size after 18 months	
	After 5 mos.	After 18 mos.	Top wt. (Kg)	Trunk area (cm^2)
Control	0	0.04	3.1	13.3
500	0.2	13.4	2.8	12.4
5000	0.6	19.2	2.6	11.7
50,000	2.1	9.8	2.5	10.7
500,000	8.3	8.0	1.9	8.8
LSD P=0.05	4.2[a]		0.8	2.3
P=0.01	5.6[a]		N.S.	3.2

[a]These LSD values may be used for making comparisons in either direction.

Our results with *P. brachyurus* support the conclusions drawn by Stokes (21) that this species apparently has little effect on peach. Malo (16), on the other hand, may have been working with a different race of this nematode when he found it to be associated with severe damage of peach roots. Our survey and greenhouse tests indicate that *P. zeae*, sometimes found in the rhizosphere of peach roots (7), may be feeding on associated grasses rather than on peach. Separation of grass and peach roots from the same sites showed that this nematode was in the grass roots. *P. coffeae* and *P. scribneri* also appear to be of limited importance on peach.

Thus, our results indicate that *C. xenoplax* as well as *Meloidogyne* spp., primarily *M. incognita*, may be causing damage on peach in North Carolina. We have not observed *M. incognita* to break the resistance of Nemaguard as reported by Wehunt (22). The primary species of *Pratylenchus* on peach in North Carolina is *P. vulnus* which, however, is of minor importance. Further work is needed to determine the significance of the other ectoparasitic nematodes associated with peach and to determine the possible interactions of these with other pathogens as well as the environment in the "short life" of peach trees.

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