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PLANT PATHOLOGY & NEMATOLOGY

Extracting *Hoplolaimus columbus* from Soil and Roots: Implications for Treatment Comparisons

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INTERPRETIVE SUMMARY

The Columbia lance nematode, *Hoplolaimus columbus,* is a microscopic, nonsegmented parasitic worm that causes significant cotton yield losses in the southeastern U.S.. This nematode damages root tissue as it moves in and out of the root while feeding and laying eggs. If only the soil fraction of a sample is assayed, nematodes inside the root will not be observed and researchers fear the total population of *H. columbus* may be underestimated. For this reason, many researchers use one extraction to remove *H. columbus* from the soil and another to remove it from plant roots. Each extraction method requires time, labor, and equipment. The two extraction procedures become inconvenient or even impossible when a large number of samples must be processed.

The objective of this study was to determine whether extracting nematodes only from the soil could provide accurate comparisons among treatments in field tests of cotton. A secondary objective was to determine whether the relative proportion of nematode populations in the soil vs. root components varied among treatments at a single sample date and whether this proportion varied among sampling dates within a growing season.

In this study, identical mean separations ($LSD_{0.05}$) among treatments were obtained regardless of whether soil counts alone or total counts (soil + root components) were used. This result suggests that there is limited advantage to using total counts instead of using soil counts alone when all plots in an experiment are planted with cotton.

The proportion of *H. columbus* in the soil component of assay samples may change significantly from one sampling date to another within a growing season. Changes in proportions could affect conclusions relative to *H. columbus* population dynamics if population levels are high and the proportions of *H. columbus* in the soil are different among sampling times. Such differences in proportions were not observed in this study.

The proportion of *H. columbus* in the soil component of assay samples increased between the midseason sample and the late-season sample. These changes were independent of nematicide treatment. The movement of *H. columbus* out of the roots late in the cotton growing season has been observed previously and likely occurs every year in response to the changing physiology of the plants. This study also indicated that most of the *H. columbus* present were in the soil even at midseason. When a single soil sample was divided into root and soil fractions, a majority of the *H. columbus* in the sample were consistently found to be in the soil fraction.

Samples collected from cotton fields in mid or late season provided useful information about *H. columbus* population levels even where nematodes were extracted only from the soil and not the roots. The probability of failing to identify fields with high levels of *H. columbus* based on soil samples alone is low.

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ABSTRACT

The Columbia lance nematode (*Hoplolaimus columbus* Sher) can cause significant yield suppression in cotton (*Gossypium hirsutum* L.) when it is present in the soil and inside roots. This study was undertaken to determine whether extracting nematodes from only the soil was sufficient to make accurate comparisons among treatments in cotton. We also studied the proportion of *H. columbus* present in the soil to determine whether that proportion varied among treatments at a single

sample date and from one sample date to another during a growing season. This study utilized data sets from three field tests in three years in which nematodes were extracted from both soil and root fractions. Statistical comparison ($LSD_{0.05}$) of the mean number of *H. columbus* extracted per treatment resulted in identical separations, regardless of whether soil counts alone or total counts were used. The mean proportion of *H. columbus* present in the soil across all treatments in the midseason samples was 0.74 in 1988, 0.80 in 1989, and 0.67 in 1998; the mean proportion at harvest was 0.93 in 1988 and 0.98 in 1989. Although the proportion of *H. columbus* in the soil increased between midseason and late-season samples in 1988 and 1989, these changes were not affected by nematicide treatment. A majority of the *H. columbus* population was consistently found to be in the soil fraction. Extraction of *H. columbus* from soil alone appears to be sufficient for comparing treatment effects on nematode populations in cotton field plots.

The Columbia lance nematode is a serious pathogen of cotton and soybean (*Glycine max* L. Merr.) in parts of Georgia, North Carolina, and South Carolina (Davis et al., 1996; Ferris and Ferris, 1998; Kraus-Schmidt and Lewis, 1979; Noe et al., 1991; Starr, 1998). It can cause significant yield loss and economic damage in infested fields.

The nematode extraction procedure used can influence the number of nematodes recovered at different sampling times (Powell and Nusbaum, 1963). The numbers of nematodes found in soil-assay samples may be affected greatly by extraction method (Barker et al., 1969). This is especially true for nematodes that move inside the roots (endoparasites) because there are times when the majority of the population may be primarily inside the roots, thereby making soil-flotation extraction methods unsuitable (Barker et al., 1969).

Because *H. columbus* is a migratory ecto/endoparasite (Kinloch, 1998; Lewis and Fassuliotis, 1982; Starr, 1998), some studies report the extraction of nematodes from both root and soil fractions of a single sample to determine nematode population densities (Appel and Lewis, 1984; Hussey, 1977; Noe, 1990, 1993; Schmitt and Bailey, 1990; Schmitt and Imbriani, 1987). In some studies, the roots used were collected separately from the soil (Kraus-Schmidt and Lewis, 1981; Mueller and Sanders, 1987; Mueller and Sullivan, 1988; Nyczepir and Lewis, 1979). Other studies, including most surveys, rely on extraction from soil alone (Baird et al., 1996; Bird et al., 1974; Martin et al., 1994; Minton et al., 1979; Motsinger et al., 1974, 1976).

The total population of *H. columbus* present in a field is comprised of nematodes in both the soil and roots. Nematode extraction from roots is more time consuming than extraction from soil, and requires equipment that may not be available in all nematology laboratories. The conflicting goals of minimizing labor and ensuring accurate nematode counts has caused debate about the utility of soil extraction alone and the necessity of extraction from soil and roots to accurately measure *H. columbus* population densities.

Using data that previously had been used to evaluate nematicide efficacy (Noe, 1990) and host plant tolerance in cotton cultivars (Davis, unpublished), this study was undertaken to determine whether assaying for *H. columbus* from the soil fraction alone was sufficient to make accurate comparisons among treatments. A secondary objective of this research was to determine whether the proportion of the *H. columbus* population present in the soil changed significantly in field plots during the growing season.

MATERIALS AND METHODS

The data used in this study were derived from previously reported experiments for the evaluation of fumigant nematicides (Noe, 1990) and a study of tolerance to *H. columbus* in cotton (Davis, unpublished). Soil samples were collected from cotton research plots on the Southeast Georgia Branch Experiment Station in Midville, naturally infested with *H. columbus*.

The soil was characterized as a Dothan sandy loam (fine-loamy, kaolinitic, thermic Plinthic Kandiudults; 69% sand, 13% silt, 18% clay; pH 5.8). Tests in 1988 and 1989 were done in the same field on adjacent sites.

To sample each plot, 12 individual soil cores (2.5 cm diam., 20 cm deep) were collected in a systematic pattern from the center two rows of each plot and combined for analysis. Plant-parasitic nematodes were extracted from 500 cm^3 soil by semi-automatic elutriation and sucrose centrifugation

(Barker, 1985). Root fragments were collected on 500 μm upper sieves and nematodes were collected on 38 μm lower sieves during the elutriation process. Nematodes were extracted by centrifugal flotation (Jenkins, 1964) from 400 cm^3 of soil in 1998. All root material collected on sieves during nematode extraction from soil then was placed into a mist chamber (Barker, 1978) for 48 h at an ambient average temperature of 26 °C (Barker, 1985) to collect nematodes from the root fraction.

Samples were collected at midseason and at harvest in 1988 and 1989 from field tests, with five treatments designed to evaluate nematicide efficacy on the cotton cultivar Deltapine Acala 90. Cotton was planted on 18 May 1988 and sampled on 10 August (midseason) and 6 December (harvest). Cotton was planted on 26 May 1989 and sampled on 10 August (midseason) and 16 November (harvest).

Nematicide treatments both years included a nontreated control, 1,3–dichloropropene applied preplant at 31.9 kg a.i. ha^{-1}, and an experimental formulation of methyl bromide (bromomethane) at 33.6, 67.2, and 134.4 kg a.i. ha^{-1}. Each test had three replications. Eight subsamples were collected from each plot on each sampling date, so each sampling date had a total of 120 data points. Subsamples were included in analyses of variance when comparing *H. columbus* population densities from soil and root fractions or proportion data at a single sample time, but subsample means (cell means) were used when comparing the proportion of *H. columbus* in the soil fraction at different sample times within a year.

Samples were collected on 22 July (midseason) in 1998 from four treatments in a field test planted 14 May designed to evaluate *H. columbus* tolerance in cotton varieties. The four treatments sampled were the maximum nematicide treatment (1,3–dichloropropene applied at 31.9 kg a.i. ha^{-1} + aldicarb (2–methyl–2–(methylthio)propionaldehyde O– (methylcarbamoyl)oxime) applied in furrow at 0.84 kg a.i. ha^{-1} + oxamyl (methyl *N',N'*–dimethyl–*N*–[(methylcarbamoyl)-oxy]–1–thiooxamimidate) applied post emergence 27 d after planting at 0.56 kg a.i. ha^{-1} + aldicarb applied side dress 40 d after planting at 0.84 kg a.i. ha^{-1}) and the minimum nematicide treatment (aldicarb applied in furrow at 0.59 kg a.i. ha^{-1}) plots for two of the genotypes in the test, NuCotn 35B and Deltapine 5690. Each treatment was replicated six times.

The number of *H. columbus* extracted from the soil was added to the number collected from the roots to calculate the total number present in a sample. The number of *H. columbus* extracted from the soil was divided by the sample total to determine the proportion of the nematode in the soil fraction for each sample.

Treatments were evaluated by analysis of variance on each sample date by comparing both the number of *H. columbus* in the soil fraction and the total number per sample. Analysis of variance also was used to determine whether the treatments in a test affected the proportion of *H. columbus* extracted from the soil. For the 2 yr in which multiple sample dates were available for a single test, a split-plot in time-of-sampling analysis of variance was used to determine whether the proportion of *H. columbus* extracted from the soil remained constant during the growing season within a single test. Only observations with 15 or more total *H. columbus* (soil fraction plus root fraction) were included in analyses involving proportions to minimize large changes in proportion resulting from relatively small changes in nematode counts. All data were used in analyses not involving proportions.

RESULTS AND DISCUSSION

Differences ($\alpha = 0.05$) were detected among treatments in the number of *H. columbus* extracted from soil in the 1988 and 1989 midseason and the 1988 harvest samples (Tables 1, 2). The total number of *H. columbus* (the sum of the number extracted from both soil and roots) also differed significantly among treatments on those dates. Comparisons of treatment means ($LSD_{0.05}$) resulted in identical separations regardless of whether soil counts alone or total counts were used. The 1989 harvest samples had statistically similar ($\alpha = 0.05$) levels of *H. columbus* in all treatments for both soil counts and total counts (Table 2), as did the 1998 midseason samples from the tolerance study (Table 3). Conclusions about treatment differences were the same regardless of whether soil counts alone or total counts were used.

Similarities in the statistical analysis of soil counts and total counts for all five sampling dates

Table 1. Comparison of numbers and proportions of *Hoplolaimus columbus* in soil and root fractions from 1988 midseason and harvest samples in a fumigant nematicide evaluation experiment.

Treatment and rate	Total *H. columbus*		Proportion *H. columbus* in soil fraction ‡ {no. subsamples used to calculate mean}
	In soil fraction	In soil + root fraction	
	100 cm^3 soil †		
Midseason			
1,3-dichloropropene			
28.1 L a.i. ha^{-1}	25 b	32 b	0.79 b {14}
Methyl bromide			
33.6 kg a.i. ha^{-1}	9 b	13 b	0.76 b {7}
67.2 kg a.i. ha^{-1}	4 b	6 b	0.56 c {3}
134.4 kg a.i. ha^{-1}	4 b	4 b	1.00 a {2}
Control	98 a	133 a	0.70 bc {21}
Harvest			
1,3-dichloropropene			
28.1 L a.i. ha^{-1}	23 b	27 b	.86 b {11}
Methyl bromide			
33.6 kg a.i. ha^{-1}	18 bc	19 bc	.94 a {11}
67.2 kg a.i. ha^{-1}	12 bc	12 bc	.95 a {6}
134.4 kg a.i. ha^{-1}	2 c	3 c	.96 a {1}
Control	106 a	111 a	.96 a {23}

† Means within a sample group followed by the same letter are not statistically different according to Fisher's protected $LSD_{0.05}$.

‡ Only subsamples with 15 or more total *H. columbus* were used to calculate mean proportions.

Table 2. Comparison of numbers and proportions of *Hoplolaimus columbus* in soil and root fractions from 1989 midseason and harvest samples in a fumigant nematicide evaluation experiment.

Treatment rate	*H. columbus*		
	In soil fraction	In soil + root fraction	Proportion in soil fraction ‡ {no. subsamples used to calculate mean}
	100 cm^3 soil†		
Midseason			
1,3-dichloropropene			
28.1 L a.i. ha^{-1}	51 b	66 b	0.80 a {11}
Methyl bromide			
33.6 kg a.i. ha^{-1}	60 b	70 b	0.79 a {11}
67.2 kg a.i. ha^{-1}	64 b	79 b	0.82 a {16}
134.4 kg a.i. ha^{-1}	34 b	42 b	0.77 a {11}
Control	117 a	141 a	0.79 a {17}
Harvest			
1,3-dichloropropene			
28.1 L a.i. ha^{-1}	83 a	84 a	0.98 b {14}
Methyl bromide			
33.6 kg a.i. ha^{-1}	72 a	72a	0.97 a {18}
67.2 kg a.i. ha^{-1}	111 a	112 a	0.98 a {19}
134.4 kg a.i. ha^{-1}	69 a	70 a	0.99 a {17}
Control	113 a	113 a	1.00 a {20}

† Means within a sample group followed by the same letter are not statistically different according to Fisher's protected $LSD_{0.05}$.

‡ Only subsamples with 15 or more total *H. columbus* were used to calculate mean proportions.

confirm that soil counts are sufficient for making comparisons among treatments if all the plots are planted in cotton. These results do not address whether soil samples alone are sufficient for comparing treatments in crop rotation studies that utilize other host or nonhost plants. It is possible that other hosts for *H. columbus*, such as soybean or corn (*Zea mays*, L.), would have a different proportion of the total in the soil (Fassuliotis, 1974; Lewis and Smith, 1976).

Differences among host plant species may account for previous reports that most of the *H. columbus* present in soybean and cotton fields would be in the roots until plants near harvest (Lewis and Fassuliotis, 1982). *Hoplolaimus columbus* could feed preferentially as an ectoparasite or as a migratory endoparasite on different plant species. With poor-host or nonhost plants, such as rye (*Secale cereale* L.) or peanut (*Arachis hypogaea* L.) (Powell, 1990), it seems likely that nearly all *H. columbus* would be in the soil rather than in plant roots at any sampling date.

The proportion of *H. columbus* in the soil differed ($\alpha = 0.05$) among treatments in the 1988 midseason and the 1988 and 1989 harvest samples (Tables 1, 2). The proportion of *H. columbus* in the soil did not differ among treatments in the 1989 or 1998 midseason samples (Tables 2, 3).

The proportion of *H. columbus* extracted from the soil fraction varied during the five sampling dates in this study, from a low treatment mean of 0.56 (Table 1) to a high of 1.00 (Tables 1, 2). In the raw data, which included 504 observations, the lowest proportion of *H. columbus* in the soil fraction was 0.11, though the mean proportion across all treatments was 0.74 for the 1988 midseason sample, 0.93 for the 1988 harvest sample, 0.80 for the 1989 midseason sample, 0.98 for the 1989 harvest sample, and 0.67 for the 1998 midseason sample.

The proportion of *H. columbus* in the soil can change significantly from midseason to harvest within a year in one set of cotton plots. This shift in proportions could affect conclusions in studies of *H. columbus* population dynamics if the proportions in the soil differ greatly among sampling times. The

Table 3. Comparison of numbers and proportions of *Hoplolaimus columbus* in soil and root fractions from 1998 midseason sample in an *H. columbus* tolerance experiment.

Cultivar and treatment †	*H. columbus*		
	In soil fraction	Total soil + root fraction ‡	Mean proportion in soil fraction § {no. subsamples used to calculate mean}
	100 cm^3 soil ‡		
NuCotn 35B Min	25 a	52 a	0.63 a {4}
NuCotn 35B Max	7 a	24 a	0.67 a {4}
Deltapine 5690 Min	35 a	88 a	0.67 a {5}
Deltapine 5690 Max	23 a	79 a	0.73 a {3}

† Min = minimal nematicide = aldicarb applied in furrow at 0.59 kg a.i. ha^{-1}. Max = maximum nematicide = (1,3-dichloropropene applied at 31.9 kg a.i. ha^{-1} + aldicarb applied in furrow at 0.84 kg a.i. ha^{-1} + aldicarb applied side dress at 0.84 kg a.i. ha^{-1} + oxamyl applied post emergence at 0.56 kg a.i. ha^{-1}).

‡ Means within a sample group followed by the same letter are not statistically different according to Fisher's protected $LSD_{0.05}$.

§ Only subsamples with 15 or more total *H. columbus* were used to calculate mean proportions.

effect of a change in proportion would become more significant as the total population level increased. The proportion of *H. columbus* in the soil increased ($\alpha = 0.05$) between midseason and harvest in 1988 and 1989, but the magnitude of the changes in proportion was relatively small.

Changes in *H. columbus* population levels were similar whether measured by soil counts or total counts. In contrast, fluctuations in the total population of a related species, *H. galeatus*, was reported to be less than the fluctuations of the population in the soil (Chapman, 1976). The data presented in this study include mid- and late-season samples but do not include early season samples, so no conclusions can be drawn about the magnitude of differences in proportion of *H. columbus* in the soil between early season and late season.

A split-plot-in-time analysis of variance verified that the proportion of *H. columbus* in the soil increased ($\alpha = 0.05$) between the midseason sample and the late-season sample. There was no statistical interaction between treatment and sample time in the split-plot-in-time analysis of variance in the 1988 or 1989 data sets, which indicates that changes in the proportion of *H. columbus* in the soil between midseason and harvest were independent of nematicide treatment. The movement of *H. columbus* out of the roots late in the cotton growing season is likely a response to the cotton plant's changing physiology as it nears harvest, which should make it a predictable and consistent phenomenon.

Hoplolaimus columbus is reported to migrate out of cotton roots in the fall (Kraus-Schmidt and Lewis, 1979). Lewis and Fassuliotis (1982) found that levelsof *H. columbus* inside soybean and cotton roots are higher than the levels in the soil until harvest, but this is contradicted by a study that found 50% or more of the *H. columbus* in a sample to be in the soil fraction (Perez et al., 1994). The study reported here supports the conclusion that *H. columbus* is more prevalent in the soil as cotton plants approach the end of the season, but our midseason observations indicated that most of the *H. columbus* present were in the soil even in midseason. When a single soil sample was divided into root and soil fractions, a majority of the *H. columbus* in the sample was consistently found in the soil fraction.

Late-season sampling of cotton fields provides useful information about *H. columbus* population levels even if nematodes are extracted only from the soil and not the roots. In this study, the proportion of the *H. columbus* population in the soil at harvest ranged from 0.86 to 1.00 in 1988 and 1989 (Tables 1, 2). Because assaying cotton fields for plant-parasitic nematodes to help determine management actions is recommended to be done near harvest (Davis et al., 1996; Kraus-Schmidt and Lewis, 1979), there is little chance of failing to identify fields with high levels of *H. columbus* because a large proportion of *H. columbus* is in the roots.

CONCLUSIONS

The number of *H. columbus* extracted from soil alone or from both soil and roots differed among treatments in the 1988 and 1989 midseason and the 1988 harvest samples. Identical mean separations were obtained ($LSD_{0.05}$) regardless of whether soil counts alone or total counts were used. The 1989 harvest samples and the 1998 midseason samples were concluded to have similar ($\alpha = 0.05$) levels of *H. columbus* in all treatments regardless of whether soil counts alone or total counts were used to make that determination. The coefficients of variation in the analyses of variance were reduced for four of the five sampling times when total counts were used

instead of soil counts, but the difference was less than 5 percent for three of the five sampling times.

All the above suggests that there is little or no advantage to using total counts instead of soil counts alone when all sampled plots are planted with cotton. These results do not address whether soil samples alone are sufficient for comparing treatments in studies that utilize other host or nonhost plants.

The proportion of *H. columbus* in the soil increased between the midseason sample and the late-season sample. These changes were independent of nematicide treatment. The movement of *H. columbus* out of the roots late in the cotton growing season is probably a response to the changing physiology of plants nearing harvest and should occur every year. This study also indicated that most of the *H. columbus* present will be in the soil even in midseason. When a single soil sample was divided into root and soil fractions, a majority of the *H. columbus* in the sample were consistently found in the soil fraction.

Samples collected from cotton fields in midseason or late season provide useful information about *H. columbus* population levels even if nematodes are extracted only from the soil and not the roots. The chance of failing to identify fields with high levels of *H. columbus* because a large proportion of *H. columbus* were in the roots is low.

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