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A Survey of Plant-Parasitic Nematodes Associated with Cotton in Alabama

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ABSTRACT

A survey was undertaken to determine the frequency and abundance of plant-parasitic nematodes associated with cotton (*Gossypium hirsutum* L.) in Alabama. In the fall seasons of 1998-2000, 969 soil samples were collected from cotton fields representing 8613 ha, or 4.3% of the cotton hectares in the state. Composite soil samples were collected from 8 ha in each field. Nematodes were extracted by gravity screening and sucrose centrifugation, identified to genus, and quantified. Eleven genera of plant-parasitic nematodes were identified. *Rotylenchulus reniformis* was found in 46% of the fields sampled. Percentages of fields with low, moderate, high, or very high populations of juveniles and vermiform adult stages per 100 cm³ were 44%, 9%, 14%, and 33%, respectively. *Meloidogyne incognita* was identified in 7% of the fields sampled. Percentages of fields with low, moderate, and high populations of juveniles of *M. incognita* per 100 cm³ were 57%, 16%, and 27%, respectively. *Hoplolaimus columbus* was identified in 0.3% of the fields surveyed. *Rotylenchulus reniformis* occurred at population levels above the economic threshold in 47% of the infested fields. *Meloidogyne incognita*, although found less frequently, was found above the economic threshold in 70% of the fields infested. This survey indicates that *Rotylenchulus reniformis* and *Meloidogyne incognita* are widespread in the cotton production regions of Alabama and nematode management strategies must become a part of production practices.

Cotton (*Gossypium hirsutum* L.) is the most important row crop in Alabama. In 1998, 1999, and 2000, an average of 206,000 ha of cotton was

harvested in 25 of the 67 counties of Alabama. The Tennessee Valley (north Alabama) and the prairie and coastal plain regions of central and south Alabama are the largest production areas in this state (Anonymous, 2001).

More than 90% of cotton in Alabama is monocultured, which creates a favorable environment for the buildup of nematodes and other pests. Although nine species of plant-parasitic nematodes have been reported to be associated with cotton (Lawrence and McLean, 1995), only three damage cotton in Alabama (Blasingame and Patel, 2002), *Meloidogyne incognita* (Kofoid & White) Chitwood, *Rotylenchulus reniformis*, Linford and Oliveira, and *Hoplolaimus columbus* Sher.

Hoplolaimus columbus is a serious nematode pest in North Carolina, South Carolina, and parts of Georgia (Mueller, 1993). It has been identified only in isolated cotton fields in central Alabama and thus has little impact on overall cotton production in Alabama (Gazaway and Armstrong, 1994). *Hoplolaimus magnistylus* and *H. galeatus* have been found on cotton in Alabama but have not been shown to be economically damaging.

Meloidogyne incognita causes serious yield losses on cotton and occurs in all 16 cotton producing states (Starr et al., 1993). It is considered a major economic nematode pest in most states and is estimated to reduce annual cotton production in Alabama by 1%. This nematode is found primarily along river bottoms and in fields with sandy soils in central and south Alabama, but is seldom a problem in the Tennessee Valley (Gazaway, unpublished).

Rotylenchulus reniformis occurs in 11 cotton-producing states, and is the most dominant and most damaging nematode species on cotton in Alabama (Gazaway et al., 2001), Louisiana (Overstreet, 1999), and Mississippi (Lawrence and McLean 1999). In Alabama *R. reniformis* is estimated to suppress yields by 9% statewide (Gazaway et al., 2001). It was first discovered in Alabama in 1958 in a few cotton fields in eastern central Alabama (Minton and Hopper, 1959), but was not recognized as a serious

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nematode pest on cotton until 1986 when it caused substantial yield losses in a few fields in south Alabama. By 1990, *R. reniformis* had become widespread and established in south and central Alabama and began appearing in some northern Alabama fields (Gazaway, unpublished). Analyses of soil samples for nematodes collected in "problem" fields during the 1990's revealed that *R. reniformis* continued to spread in cotton production areas throughout the state. A survey was conducted in 1989 and 1990 that found 4 and 9% of the fields in north and central Alabama were infested with *R. reniformis* at populations above the economic threshold. Consequently, this survey was undertaken to determine the current distribution and population levels of *R. reniformis* and other plant-parasitic nematode species in the central and northern cotton production areas of Alabama.

MATERIALS AND METHODS

Cotton fields in the northern valley and the prairie and coastal plain regions of Alabama were surveyed for plant-parasitic nematodes in September, October, and November 1998, 1999, and 2000. More than 900 soil samples were collected from cotton fields representing 8,613 ha in the 12 counties (Fig. 1). Each composite sample consisted of 20 soil cores (2.5-cm-diam. x 20-cm deep) taken in a systematic, zigzag-sampling pattern from an 8-ha section of each field. Composite soil samples were sealed in plastic bags and stored in a cooled ice chest (less than four hours) as they were collected from the field until being transferred to a 5° C refrigerator prior to extraction. Each sample was thoroughly mixed and a 100 cm³ sub-sample was collected for nematode extraction. All samples were processed within 14 days of collection. Nematodes were separated by gravity screening and sucrose centrifugation (Jenkins, 1964). Plant-parasitic nematodes were identified and enumerated using a stereomicroscope. The three major plant-parasitic nematode genera that limit cotton production were identified to species. These were *Rotylenchulus reniformis* (Linford and Oliveira, 1940), *Meloidogyne incognita* (Chitwood, 1940), and *Hoplolaimus* spp. (Robbins, 1982; Sher, 1963).

Since *M. incognita* is the only *Meloidogyne* species known to parasitize cotton in the United States, the presence of galls or swellings on cotton roots could be used to identify *M. incognita* (southern root-

knot nematode). In this survey, *Meloidogyne incognita* was confirmed by examining cotton roots for the presence of galls in fields where the soil samples contained *Meloidogyne* juveniles. Fields were revisited and cotton roots were removed to confirm galling in those fields where *M. incognita* juveniles were identified in the soil sample. *Rotylenchulus reniformis* was confirmed by taxonomic characteristics including the presence of males and stylet length (Linford and Oliveira, 1940; Robinson et al., 1997). *Hoplolaimus columbus* was confirmed by Dr. S. A. Lewis of Clemson University.

RESULTS AND DISCUSSION

The 1998-2000 survey encompassed 4.3% of the cotton hectares in northern and central Alabama. Eleven genera of plant-parasitic nematodes were identified: *Criconemella* spp., *Helicotylenchus* spp., *Hoplolaimus* spp., *Meloidogyne incognita*, *Pratylenchus* spp., *Paratrichodorus* spp., *Rotylenchulus reniformis*, *Scutellonema* spp., *Tylenchorhynchus* spp., *Tylenchus* spp., and *Xiphinema* spp.

Rotylenchulus reniformis and *M. incognita* are the major nematode pathogens of cotton and occurred in 46% and 7% of the fields sampled, respectively. Two other pathogens of cotton, *Hoplolaimus* spp. and *Pratylenchus* spp., were recovered from 9 and 11% of all fields sampled, respectively. *Helicotylenchus* was the most common nonpathogenic or weakly pathogenic genera occurring in 81% of all fields sampled. *Tylenchus*, *Xiphinema*, *Scutellonema*, *Criconemella*, and *Tylenchorhynchus* were other weak or nonpathogenic genera that occurred in 21, 23, 11, 7, and 6% of all fields sampled. *Heterodera* spp., which are pathogenic on other hosts (especially legumes), but not cotton, were recovered from 11% of all fields sampled.

Thirty-eight percent of the soil samples contained only a single genus of plant-parasitic nematodes, while 61% of the soil samples contained multiple populations of plant-parasitic nematodes. Where more than one genus was found, 34% of the samples contained two genera, 15% contained three genera, and 12% contained more than four genera. Similar ratios of single and multiple species of plant-parasitic nematodes were found in surveys of cotton fields conducted in Louisiana (McLean and Lawrence, 2000) and Missouri (Wrather et al., 1992).

Lance nematodes, *Hoplolaimus* spp., were recovered in 8.6% of the fields sampled or 740 ha (Table 1). The three *Hoplolaimus* spp. observed were *H. magnistylus* (Robbins), *H. galeatus* (Cobb) Thorne, and *H. columbus* (Sher). *Hoplolaimus columbus* was found only in central Alabama in Autauga County. *Hoplolaimus magnistylus* and *H. galeatus* were detected with the highest frequency in Limestone County in northern Alabama with 7.8% of the samples infested. Population densities of *H. magnistylus* and *H. galeatus* ranged from 0 to 80 per 100 cm³ of soil, with a mean density of 10 juve-

niles and adults in the fields where they were identified. Lance nematodes were detected less frequently than *R. reniformis* and *M. incognita*.

Meloidogyne incognita was identified in 7% of fields sampled or 603 ha. Population densities of *M. incognita* in the infested fields varied, with 57% of fields having light infestations, 16% of fields being moderately infested, and 26% showing high infestation levels (Table 2). In two previous studies in Louisiana, 27% and 23% of the samples were infested with *M. incognita* (McLean and Lawrence 2000; Overstreet and McGawley, 1997). In Florida,

Table 1. Distribution of *Hoplolaimus* spp. and population levels per 100 cc of soil in Alabama in 1998 - 2000.

County	Total Fields Sampled	Number of fields with infestation levels ²		
		Light	Moderate	High
Autauga	94	8	6	0
Chilton	19	2	0	0
Colbert	46	2	0	0
Dallas	18	3	1	0
Elmore	36	1	0	0
Lauderdale	6	0	0	0
Lawrence	174	3	4	0
Limestone	386	24	6	0
Macon	5	2	0	0
Madison	181	18	4	0
Montgomery	3	0	0	0
Morgan	1	0	0	0
Total	969	63	21	0

² Infestation level, where light = 1 to 40 nematodes per 100 cm³ soil; moderate = 41 to 80 nematodes per 100 cm³ soil; and high = more than 81 nematodes per 100 cm³ soil.

Table 2. Distribution and infestation levels of *Meloidogyne incognita* in 12 Alabama counties in 1998 - 2000.

County	Total Fields Sampled	Number of fields with infestation levels ²		
		Light	Moderate	High
Autauga	94	12	5	8
Chilton	19	7	3	2
Colbert	46	1	0	0
Dallas	18	1	0	8
Elmore	36	2	0	0
Lauderdale	6	0	0	0
Lawrence	174	1	0	0
Limestone	386	10	3	0
Macon	5	0	0	0
Madison	181	5	0	0
Montgomery	3	0	0	0
Morgan	1	0	0	0
Total	969	39	11	18

² Infestation level where light = 1 to 40 nematodes per 100 cm³ soil; moderate = 41 to 80 nematodes per 100 cm³ soil; high = more than 81 nematodes per 100 cm³ soil.

M. incognita was found in 61% of the fields infested (Kinloch and Sprenkel, 1994), while in Georgia, it was detected in 38% of the fields (Baird et al., 1996). Typically, cotton production areas in Florida and Georgia are in soils that contain higher percentages of sand than soils in most cotton production regions. *Meloidogyne incognita* has also been recovered from 30% of cotton fields in Missouri (Wrather et al., 1992), 14% in Arkansas (Robbins et al., 1989), and 10% in Mississippi (Lawrence et al., 1997).

Rotylenchulus reniformis was found to be the most widespread of the three major nematode pathogens of cotton in Alabama. According to our surveys, it has continued to spread and increase in population densities across the cotton-producing areas of northern and central Alabama during the past 10 years. *Rotylenchulus reniformis* populations were found in 46.5% of the fields sampled, or 4005 ha. These populations were all confirmed to be *R. reniformis* based on the taxonomic criteria previously stated. Population densities of *R. reniformis* varied, with 43% of infested fields having light infestations; 9% being moderately infested; 14% being highly infested; and 33% showing very high infestations (Table 3.) Twenty-two percent of fields infested with *R. reniformis* contained population levels above the economic threshold of 1,000 juveniles and vermiform adults per 100 cm³ of soil established by the Alabama Cooperative Extension

Service (Gazaway, 1996). This is an increase from the 1989/1990 survey that indicated only 9% of cotton fields had damaging levels of *R. reniformis* (Table 4). The populations of *R. reniformis* appear to have increased in infested counties in this survey compared to the original 1989/1990 survey (Fig. 1). Although yield losses of up to 75% have been documented under extremely adverse growing conditions, it is estimated that a 20% reduction in yield is more common most years in fields where *R. reniformis* population densities are above the economic threshold in Alabama (Gazaway et al., 2001). Previous loss studies and the results from this survey indicate that *R. reniformis* is likely to be of economic concern wherever cotton is grown in Alabama.

An incidence of 46% of cotton fields infested with *R. reniformis* in this survey is much higher than a previous survey ten years ago, confirming that this nematode is spreading at an alarming rate in this state. The increasing infestation levels and spread of *R. reniformis* in Alabama appear to be following trends observed in two other southeastern states, Louisiana and Mississippi. In Louisiana, 57% of the fields sampled were infested with *R. reniformis* (Overstreet and McGawley, 1997). In a more recent survey, 68% of the fields surveyed in that state were found to be infested (McLean and Lawrence, 2000). In Mississippi, Lawrence reported that 15% of cotton fields were infested with *R. reniformis* in 1996 (Lawrence et al., 1997). A 2001 survey of three major cotton

Table 3. Distribution and infestation levels of *Rotylenchulus reniformis* in 12 Alabama counties in 1998 - 2000.

County	Total Fields Sampled	Number of fields with infestation levels ²			
		Light	Moderate	High	Very High
Autauga	94	15	1	5	11
Chilton	19	6	0	0	0
Colbert	46	24	5	8	6
Dallas	18	1	0	0	1
Elmore	36	1	6	7	20
Lauderdale	6	2	0	0	0
Lawrence	174	54	7	22	50
Limestone	386	70	14	17	45
Macon	5	0	0	0	1
Madison	181	23	6	4	13
Montgomery	3	1	1	0	0
Morgan	1	0	0	1	0
Total	969	197	40	64	147

² Infestation level where light = 1 to 249 nematodes per 100 cm³ soil; moderate = 250 to 499 nematodes per 100 cm³ soil; high = 500- 999 nematodes per 100 cm³ soil; very high = more than 1000 nematodes per 100 cm³ soil.

Table 4. Fields in north and central Alabama with levels of *Rotylenchulus reniformis* above the damage threshold of (>1000/100 cm³ of soil) in two surveys conducted in 1989/1990 and 1998/2000.

Region	County ^z	1989-1990 Survey		1998-2000 Survey	
		Fields sampled	% fields > threshold	Fields sampled	% fields > threshold
Tennessee Valley or North Alabama	Colbert	20	20	46	13
	Lawrence	25	4	174	29
	Limestone	23	0	386	12
	Madison	30	0	181	9
Central Alabama	Autauga	14	0	94	12
	Elmore	22	23	36	56
	Dallas	20	10	-	-
	Lee	20	20	-	-
	Montgomery	1	0	3	0
Total		175	9	920	22

^z Although damaging population levels of reniform were not found in Limestone County in the 1989/1990 survey, a few fields had been identified with low levels of reniform from routine samples received in the plant diagnostic laboratory; Dallas and Lee Counties were not included in the 1999/2000 survey.



Figure 1. Counties in Alabama (highlighted in green) surveyed for plant-parasitic nematodes. Top number indicates bales produced and the lower number indicates hectares planted in 2000.

Click the map to see a larger version.

production counties in the Mississippi Delta found that an average of 45.8% of cotton fields sampled in the three county area were infested with reniform nematodes (Sciombato et al., 2002). *Rotylenchulus reniformis* has also been found in 15% of the fields in Florida (Kinloch and Sprenkel, 1994), 5% in Georgia (Baird et al., 1996), 3% in Missouri (Wrather et al., 1992), and 1% in Arkansas (Robbins et al., 1989). In Louisiana and Texas, *R. reniformis* has been associated with fine textured soils (Starr et al., 1993), but it has also been found in soils having a higher percentage of sand than in other regions of the southern United States (Heald and Robinson, 1990).

Nematode infestations are usually poly-specific; however, *R. reniformis* is characteristically mono-specific (Starr et al., 1993; Lawrence et al., 1997; McLean and Lawrence, 2000). Concomitant infestations of *R. reniformis*, *M. incognita*, and *H. columbus* were found in only one field sampled in this survey. In that field, populations of all three species were below economic thresholds. *Rotylenchulus reniformis* and *M. incognita* were found concomitantly in only 1.6% of the fields. Population levels of *M. incognita* were above the established economic thresholds in all of the fields that were concomitantly infested with *R. reniformis* and *M. incognita*. However, population levels of *R. reniformis* were below economic thresholds in all but two of the fields. In three counties in central and northern Alabama (Autauga, Elmore, and Limestone counties), only 4.2, 6.2, and 1.2% of cotton fields were infested with both *M. incognita* and *R.*

reniformis, respectively. This infrequent, natural, concomitant occurrence of the two species has been observed in Texas (Starr et al., 1993), Mississippi (Hankins et al., 1997), and Louisiana (McLean and Lawrence, 2000). *Meloidogyne incognita* and *H. columbus* were found in concomitant association in 1% of fields, while *R. reniformis* and *H. columbus* were found in concomitant association in 0.5% of the fields sampled in this survey. Population levels of *M. incognita* were above the established economic thresholds in half of the concomitantly infested fields. Population levels of *R. reniformis* were below economic thresholds in all but one of the concomitantly infested fields, while *H. columbus* was above economic thresholds in all of the concomitantly infested fields.

CONCLUSIONS

In the 12 county survey, 53% of the samples contained either *R. reniformis*, *M. incognita*, *H. columbus*, or combinations of the three species. *Meloidogyne incognita* population numbers were above established economic threshold levels in 48 of the 68 samples in which it was found in the 12 county survey. *Rotylenchulus reniformis* numbers were above the economic threshold in 147 of the 448 samples containing *R. reniformis*. Thus, it is estimated to be at economically damaging levels in 30,533 ha in the twelve counties. In Alabama, the economic threshold population levels of *R. reniformis* have been estimated to reduce cotton yields by 20%, causing losses greater than 271 kg of lint per ha. (Gazaway et al., 2001). Cotton has remained the dominant agricultural crop in the counties in northern Alabama (Anonymous, 2001). Initially limited to a few scattered cotton fields in north Alabama, *Rotylenchulus reniformis* has spread rapidly and become established in the state's most productive cotton regions of north Alabama since the 1989-1990 survey. This nematode pest is now a major, yield-limiting factor, and threatens cotton production throughout Limestone, Colbert, and Madison counties, three of the top five cotton producing-counties in the state. Moreover, the reniform nematode is beginning to move into Lauderdale, another major cotton-producing county.

The reniform nematode, which can survive over two years in stored soil (Carter et al., 2002), is thought to spread from farm to farm in soil particles or clods clinging to farm equipment, tools, and farm

vehicles. Once introduced and established in a field, the reniform nematode cannot be eradicated and must be managed through the use of nematicides or rotation with nonhost crops. Both measures are expensive and often ineffective. Excluding the nematode from "reniform free" fields is the most effective way to manage the reniform nematode. Unfortunately, a strong statewide educational program stressing voluntary quarantines and sanitation programs to restrict the reniform nematode's movement between farms and other regions of the state has not been successful. This survey points out the need for more stringent quarantines, possibly state or federally mandated, to prevent further contamination of fields that remain free of reniform nematodes.

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