

# Interaction Between *Meloidogyne incognita* and *Thielaviopsis basicola* on Cotton (*Gossypium hirsutum*)<sup>1</sup>

N. R. WALKER,<sup>2</sup> T. L. KIRKPATRICK,<sup>3</sup> AND C. S. ROTHROCK<sup>2</sup>

**Abstract:** The effects of *Meloidogyne incognita* and *Thielaviopsis basicola* on the growth of cotton (*Gossypium hirsutum*) and the effects of *T. basicola* on *M. incognita* populations were evaluated in a 2-year study. Microplots were infested with *M. incognita*, *T. basicola*, or a combination of *M. incognita* and *T. basicola*. Uninfested plots served as controls both years. Seedling survival was decreased by the *M. incognita* + *T. basicola* treatment compared to the control. *Meloidogyne incognita* alone and *M. incognita* + *T. basicola* reduced plant height-to-node ratio for seedlings in both years. Seed cotton yield was reduced, and the length of time required for boll maturation was lengthened by *M. incognita* + *T. basicola* in 1994 and *M. incognita* both alone and with *T. basicola* in 1995. Position of the first sympodial node on the main stem was increased by *M. incognita* in both years and was higher for plants treated with *M. incognita* + *T. basicola* in 1995 in comparison to the control. The number of sympodial branches with bolls in the first and second fruiting position and the percentage of bolls retained in the second position were reduced both years by *M. incognita* + *T. basicola* compared to either the control or *T. basicola* alone. Orthogonal contrasts indicated that effects on height-to-node ratio, number of days to first cracked boll, and yield were significantly different for combined pathogen inoculations than with either pathogen alone. *Meloidogyne incognita* eggs at harvest were reduced by *T. basicola* in 1994 and 1995 compared to *M. incognita* alone. The study demonstrated a significant interaction between *M. incognita* and *T. basicola* on cotton that impacted the survival and development of cotton and the reproduction of *M. incognita* on cotton.

**Key words:** black root rot, *Chalara elegans*, cotton, *Gossypium hirsutum*, interaction, *Meloidogyne incognita*, nematode, root-knot, *Thielaviopsis basicola*.

The root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, is a serious pathogen of cotton (*Gossypium hirsutum* L.) (Bridge, 1992) throughout the U.S. cotton belt. *Thielaviopsis basicola* (Berk. and Broome) Ferris (*Chalara elegans* Nag Raj & Kendrick), causal agent of black root rot of cotton, was first described in a field in Arizona in 1942 (King and Barker, 1942) and has been reported from many cotton-growing regions (Farr et al., 1989). Both pathogens have been found widely distributed in cotton fields throughout Arkansas (Kirkpatrick et al., 1992; Robbins et al., 1989; Rothrock and Wells, 1992).

*Thielaviopsis basicola* overwinters as dark, thick-walled chlamydospores that germinate in the presence of the host (Tsao and

Bricker, 1966; Candole and Rothrock, 1997). The fungus colonizes the cortical tissues of cotton seedlings and causes a characteristic dark-brown to black discoloration of the root and hypocotyl resulting in stunted, less vigorous seedlings (Watkins, 1981). Black root rot is most severe early in the growing season when soil temperature is less than 24 °C and soil water content is high (Rothrock, 1992). As soil temperatures increase and the plant develops, the diseased cortical tissue sloughs off and secondary root growth occurs (Mathre et al., 1966; Mauk and Hine, 1988).

Plants affected by *T. basicola* or *M. incognita* are often misdiagnosed as suffering environmental or nutritional problems. However, certain cotton fields in Arkansas have suffered dramatic early-season stand losses and yield reductions when both organisms were present (Kirkpatrick and Rothrock, 1995). The objectives of this study were to elucidate i) the effects of the combination of *T. basicola* and *M. incognita* on cotton development and ii) the effects of *T. basicola* on the population dynamics of *M. incognita*. A preliminary report of results has been presented (Walker et al., 1997).

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<sup>2</sup> Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

<sup>3</sup> University of Arkansas Southwest Research and Extension Center, Hope, AR 71801-9729.

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E-mail: tkirkpat@uaex.edu

## MATERIALS AND METHODS

Concrete microplots (76 cm diam.  $\times$  80 cm), located at the University of Arkansas Southwest Research and Extension Center at Hope, Arkansas, were used in 1994 and 1995 for this study. The microplots, filled with Smithdale fine sandy loam soil (fine loamy siliceous, thermic Typic Paleudult), were fumigated with methyl bromide (100g/m<sup>2</sup>) and covered with plastic film 4 weeks before use each year. One week after fumigation, the plastic film was removed from the plots and the plots were allowed to aerate for 3 weeks prior to infestation and planting. Inoculum of *T. basicola* consisted of chlamydospores harvested from 6-week-old cultures grown on 10% carrot juice agar. Chlamydospore chains were harvested from plates in sterile-deionized water with a rubber policeman and digested with chitinase according to Candole and Rothrock (1997). Inoculum of *M. incognita* race 3 was obtained from stock cultures maintained in a greenhouse on tomato (*Lycopersicon esculentum* Mill. cv. 'Rutgers'). Nematode inoculum was prepared by cutting the infected tomato root systems (60 days old) into segments 1–2 cm long and combining them with the soil in which the plants had been grown. Composite soil samples (250 cm<sup>3</sup>) were collected from the mixture of soil and root segments to quantify the inoculum. The samples were processed by sieving and centrifugal flotation (Ayoub, 1980) to collect second-stage juveniles (J2). Root segments collected during sieving were processed by extraction for 4 minutes in 0.05% NaOCl (Hussey and Barker, 1973) to collect eggs.

Soil was infested with 20 spores of *T. basicola*/g soil in 1994, and 20 or 100 spores/g soil in 1995. The spores were applied to each plot in 250 cm<sup>3</sup> water with a sprinkle can. Soil and tomato root segments containing *M. incognita* were added to microplots to a final density of 10 eggs and J2/cm<sup>3</sup> of soil each year. Control plots received root fragments and soil from uninfected tomato plants. Inoculum of both pathogens was incorporated by mixing thoroughly with a shovel and a rake 10–15 cm deep. Treat-

ments consisted of an uninfested control, *M. incognita* alone, *T. basicola* alone, and *M. incognita* and *T. basicola* in combination in 1994. In 1995, treatments consisted of an uninfested control, *M. incognita* alone, *T. basicola* alone at 20 (Tb20) or 100 (Tb100) spores/g of soil, and *M. incognita* + *T. basicola* at both rates of *T. basicola*. Thirty-two acid-delinted, fungicide-treated [metalaxyl (Apron), carboxin (Vitavax), and PCNB; 0.155, 0.788, and 0.788 g a. i./kg seed, respectively] cotton seeds of the root-knot susceptible cultivar Suregrow 501 were planted in each plot immediately following infestation. Microplot infestation and planting occurred on 2 May 1994 and 14 April 1995, when the average soil temperature at 10 cm was above 16 °C for a 48-hour period. Soil fertility was assayed before planting, and microplots were amended with N, P, and K according to the Arkansas Cooperative Extension Service guidelines for cotton production (Bonner, 1992). Additional NH<sub>3</sub>NO<sub>4</sub> was applied to each plot periodically throughout the growing season to maintain active plant growth. Insect control with esfenvalerate (Asana) and acephate (Orthene) was based on scouting and in accordance with the Arkansas Cooperative Extension Service guidelines for cotton production (Johnson and Jones, 1993).

The number of live plants was determined 28 days after planting (DAP), and plants were thinned to six plants per plot. Root systems from 10 of the plants removed from each plot were rated for disease severity on a 1- to 5-scale, where 1 = 0%, 2 = 1–10%, 3 = 11–25%, 4 = 26–50%, and 5 = 51–100% of root system discolored. The root systems were rinsed in running tap water for 20 minutes, surface-disinfested by immersion for 1.5 minutes in 0.5% NaOCl, blotted dry, and plated on TB-CEN agar (Specht and Griffin, 1985). The percentage of plants from which *T. basicola* grew was recorded after 10 to 14 days. Plant height-to-node ratio (HNR), measured from the cotyledonary node to the tip of the main-stem terminal, was recorded on all plants 21 and 28 DAP. Nematode population densities were evaluated 57 DAP both years and at harvest; harvest was

144 and 133 DAP in 1994 and 1995, respectively. A composite soil sample (250 cm<sup>3</sup>) was removed from each plot and processed by sieving and centrifugal flotation (Ayoub, 1980) to extract J2. Nematode eggs adhering to root fragments collected during soil processing were extracted as described earlier. Entire root systems at harvest were evaluated for nematode galling on a scale of 0 to 5 where 0 = no galls per root system, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = >100 galls/root system.

In both years the number of days to first cracked boll (DTCB) was monitored for each plot. Seed cotton was harvested by hand from each plot 126 and 136 DAP in 1994, and 109, 116, and 133 DAP in 1995. At harvest, plant heights were measured from the cotyledonary node to the tip of the main-stem terminal. Plants were evaluated according to the COTMAP method (Bourland and Watson, 1990) for the position of first sympodial node above the cotyledon, total number of sympodial branches, number of sympodial branches with a boll in the first and second fruiting position, and total number of bolls per plant. Boll retention at first and second fruiting positions on sympodia also was determined for each plant.

Statistical analyses were conducted with SAS (SAS Institute, Cary, NC) to evaluate treatment effects on plant responses and

contrasts between treatments. Orthogonal contrasts consisting of *T. basicola* or *M. incognita* alone vs. the control, and *T. basicola* or *M. incognita* alone vs. *M. incognita* + *T. basicola*, were conducted for all variables for both years. Treatment means were separated with Fisher's protected LSD at  $P \leq 0.05$ . Due to treatment and environmental differences between 1994 and 1995, data were analyzed by individual years.

## RESULTS

*1994 test:* Plots with *M. incognita* + *T. basicola* had the lowest plant stands among the treatments in 1994 (Table 1). Plots with *M. incognita* alone also had reduced stands when compared to the control plots or those infested with *T. basicola* alone. Plant height-to-node ratios at 21 and 28 DAP also were lowest in plots with *M. incognita* + *T. basicola*, with plots infested with *M. incognita* also having reduced ratios compared to the noninfested or *T. basicola*-infested plots (Table 1). When orthogonal contrasts were examined, *M. incognita* + *T. basicola* reduced height-to-node ratio at 21 days compared to the control or either pathogen alone ( $P \leq 0.01$ ). The percentage of roots from which *T. basicola* was isolated was numerically greater and disease severity was greater in plots with *M. incognita* + *T. basicola* than in those with *T.*

TABLE 1. Cotton seedling characteristics and nematode population densities in microplots infested with *Thielaviopsis basicola* (Tb) and *Meloidogyne incognita* (Mi) in 1994.

Treatment	Stand (%) <sup>a</sup>	HNR <sup>b</sup>		Mi/250 cm <sup>3</sup> soil <sup>c</sup>		Tb isolation (%) <sup>d</sup>	Disease severity <sup>d</sup>
		21 days	28 days	J2	Eggs		
Control	75.5	1.47	1.47	—	—	—	—
Tb20	68.9	1.45	1.44	—	—	40.4	1.19
Mi	56.0	1.05	1.25	3,523	8,095	—	—
Mi + Tb20	42.7	0.85	1.07	1,408	4,981	61.6	2.28
LSD	9.5	0.10	0.14	1,297	5,741	26.5	0.48
Contrast							
Tb + Mi vs. Tb or Mi	NS	**	NS	—	—	—	—

Data are means of 10 replications. Means within same column are not significantly different if the magnitude of the difference is not greater than the LSD value according to Fisher's protected LSD at  $P \leq 0.05$ . Orthogonal contrasts between the Tb + Mi treatment and treatments containing either pathogen alone were significant at  $P \leq 0.01$  or nonsignificant at  $P \leq 0.05$  (NS). Dashes indicate no data.

<sup>a</sup> Stand counts were made 28 days after planting.

<sup>b</sup> Height-to-node ratio = plant height per number of nodes.

<sup>c</sup> Counts were made 57 days after planting; J2: second-stage juveniles.

<sup>d</sup> *Thielaviopsis basicola* isolation and disease severity rating were performed 28 days after planting. Disease severity based on a 1-to-5 scale: 1 = 0%, 2 = 1–10%, 3 = 11–25%, 4 = 26–50%, and 5 = 51–100% root discoloration.

TABLE 2. Late-season characteristics of microplot-grown cotton infested with *Thielaviopsis basicola* (Tb) and *Meloidogyne incognita* (Mi) in 1994.

Treatment	Yield <sup>a</sup>	DTCB <sup>b</sup>	Mi		
			J2 <sup>c</sup>	Eggs <sup>c</sup>	Galling <sup>d</sup>
Control	667	112	—	—	—
Tb20	721	112	—	—	—
Mi	649	114	4,819	17,235	4.26
Mi + Tb20	513	118	6,308	8,370	4.83
LSD	107	2	5,741	9,474	1.13
Contrast					
Mi + Tb					
vs. Mi or Tb	*	*	—	—	—

Data are means of 10 replications. Means within same column are not significantly different if the magnitude of the difference is not greater than the LSD value according to Fisher's protected LSD at  $P \leq 0.05$ . Orthogonal contrast between the Tb + Mi treatment and treatments containing either pathogen alone were significant at  $P \leq 0.05$ . Dashes indicate no data.

<sup>a</sup> Seed cotton yield in grams per microplot harvested by hand two times.

<sup>b</sup> Days to first cracked boll.

<sup>c</sup> Second-stage juveniles per 250 cm<sup>3</sup> soil, 144 days after planting.

<sup>d</sup> Galling based on a 0-to-5 scale: 0 = no galls/root system and 5 = >100 galls/root system.

*basicola* alone. Nematode population density (J2 or eggs) was lower in plots receiving *M. incognita* + *T. basicola* than with *M. incognita* alone (Table 1).

Seed cotton yields for the six plants per microplot were similar among the control, *T. basicola*-alone, and *M. incognita*-alone treatments, but the *M. incognita* + *T. basicola* treatment resulted in lower total seed cotton weights than all other treatments in 1994 (Table 2). Plant maturity, as determined by the DTCB, was delayed in the *M. incognita* +

*T. basicola* treatment. When orthogonal contrasts were examined, the combination of the pathogens reduced yield and lengthened DTCB compared to the control or either pathogen alone ( $P \leq 0.05$ ). Nematode J2 population densities were not affected by the presence of *T. basicola* at harvest (Table 2). However, the number of eggs recovered at harvest was lower where *M. incognita* + *T. basicola* was applied than in plots infested with the nematode alone. Root galling in *M. incognita*-infested plots was the same with or without *T. basicola* at the end of the season.

Plant heights at harvest in 1994 were reduced in the *M. incognita* and the *M. incognita* + *T. basicola* treatments compared to the control (Table 3). The position of the first sympodial branch above the cotyledonary node was higher for *M. incognita* plots than the control and *T. basicola* plots but not the *M. incognita* + *T. basicola* plots. Treatments did not affect the total number of sympodial branches per plant at harvest; however, the number of sympodial branches with a boll in both the first and second fruiting positions was reduced by the *M. incognita* + *T. basicola* treatment compared to the uninfested or *T. basicola*-infested plots, but not compared to plots infested with *M. incognita* (Table 3). Total bolls per plant at harvest was reduced by the *M. incognita* and *M. incognita* + *T. basicola* treatments but not by the *T. basicola* treatment compared to the control (Table 3). Bolls retained in the first sympodial fruiting position expressed as the percentage of

TABLE 3. Late-season cotton growth and development characteristics 144 days after planting in microplots infested with *Thielaviopsis basicola* (Tb) and *Meloidogyne incognita* (Mi) in 1994.

Treatment	Plant height (cm)	First sympodial node <sup>a</sup>	Number of sympodial branches	Sympodial branches with 2 bolls	Total bolls	Percent bolls retained at 1st position	Percent bolls retained at 2nd position
Control	96.11	5.43	14.1	6.15	22.8	66.06	48.68
Tb20	99.81	5.32	14.9	5.89	24.1	63.73	46.78
Mi	92.07	7.45	14.0	4.62	18.8	62.13	42.38
Mi + Tb20	90.58	6.74	14.0	4.07	16.1	60.37	34.15
LSD	2.95	1.56	1.21	1.29	3.86	9.99	5.94
Contrast							
Tb + Mi vs. Tb or Mi	NS	NS	NS	NS	NS	NS	NS

Data are means of 10 replications. Means within same column are not significantly different if the magnitude of the difference is not greater than the LSD value according to Fisher's protected LSD at  $P \leq 0.05$ . Orthogonal contrasts between the Tb + Mi treatments and treatments containing either pathogen alone were nonsignificant at  $P \leq 0.05$  (NS).

<sup>a</sup> Nodes to first sympodial branch, excluding cotyledonary node.

TABLE 4. Cotton seedling characteristics and nematode population densities in microplots infested with *Thielaviopsis basicola* (Tb) and *Meloidogyne incognita* (Mi) in 1995.

Treatment	Stand (%) <sup>a</sup>	HNR <sup>b</sup>		Mi/250 cm <sup>3</sup> soil <sup>c</sup>		Tb isolation (%) <sup>d</sup>	Disease severity <sup>d</sup>
		21 days	28 days	J2	Eggs		
Control	75.9	1.62	2.42	—	—	—	—
Tb20	75.0	1.70	2.42	—	—	32.1	2.96
Tb100	66.9	0.64	0.93	—	—	64.2	3.43
Mi	68.6	1.07	1.62	2	4	—	—
Mi + Tb20	66.6	1.02	1.52	14	25	30.4	3.78
Mi + Tb100	57.5	0.43	0.68	26	3	56.0	3.42
LSD	12.4	0.13	0.17	13.7	20.7	22.4	0.76
Contrast							
Tb + Mi vs. Tb or Mi	NS	*	*	—	—	—	—

Data are means of 10 replications. Means within same column are not significantly different if the magnitude of the difference is not greater than the LSD value according to Fisher's protected LSD at  $P \leq 0.05$ . Orthogonal contrasts between the Tb + Mi treatments and treatments containing either pathogen alone were significant at  $P \leq 0.05$  or nonsignificant at  $P \leq 0.05$  (NS). Dashes indicate no data.

<sup>a</sup> Stand counts were made 28 days after planting.

<sup>b</sup> Height-to-node ratio = plant height per number of nodes.

<sup>c</sup> Counts were made 57 days after planting; J2: second-stage juveniles.

<sup>d</sup> *Thielaviopsis basicola* isolation and disease severity rating were performed 28 days after planting. Disease severity based on 1-to-5 scale: 1 = 0%, 2 = 1-10%, 3 = 11-25%, 4 = 26-50%, and 5 = 51-100% root discoloration.

the total number of sympodial branches per plant was not affected by any treatment in 1994. The *M. incognita* + *T. basicola* treatment resulted in the fewest bolls retained in the second fruiting position of all treatments (Table 3).

**1995 test:** In 1995, only *M. incognita* + Tb100 lowered plant stand densities when compared to the control (Table 4). The height-to-node ratios at 21 and 28 DAP were affected similarly, with ratios being significantly less for Tb100 or *M. incognita* than the control and less for the combination of Tb100 + *M. incognita* than for either pathogen alone (Table 4). According to orthogonal contrast analysis, both pathogens reduced height-to-node ratio compared to the control, and *M. incognita* + *T. basicola* was lower than either pathogen alone ( $P \leq 0.05$ ). The percentage of roots from which *T. basicola* was isolated was similar between Tb20 and *M. incognita* + Tb20, and between Tb100 and *M. incognita* + Tb100, but isolation was greater from Tb100 plots than from Tb20 plots (Table 4). In 1995, nematode populations were low in all plots at 57 DAP, but the number of J2 was highest in the *M. incognita* + Tb100 plots compared to other treatments (Table 4). *Meloidogyne incognita* reproduction at 57 DAP was lower in the *M.*

*incognita* + Tb100 or *M. incognita* treatments than the *M. incognita* + Tb20 treatment.

Seed cotton yields for the *T. basicola*-infested plots were not suppressed in 1995 relative to the control, while the treatments

TABLE 5. Late-season characteristics of microplot-grown cotton infested with *Thielaviopsis basicola* (Tb) and *Meloidogyne incognita* (Mi) in 1995.

Treatment	Yield <sup>a</sup>	DTCB <sup>b</sup>	Mi		
			J2 <sup>c</sup>	Eggs <sup>c</sup>	Galling <sup>d</sup>
Control	387	123	—	—	—
Tb20	387	123	—	—	—
Tb100	375	124	—	—	—
Mi	252	130	2,912	27,292	4.97
Mi + Tb20	193	132	3,275	17,017	4.98
Mi + Tb100	238	129	2,209	5,740	4.87
LSD	60	2.4	2,045	23,506	0.13
Contrast					
Tb + Mi vs. Tb or Mi	**	**	—	—	—

Data are means of 10 replications. Means within same column are not significantly different if the magnitude of the difference is not greater than the LSD value according to Fisher's protected LSD at  $P \leq 0.05$ . Orthogonal contrasts between the Tb + Mi treatments and treatments containing either pathogen alone were significant at  $P \leq 0.01$ . Dashes indicate no data.

<sup>a</sup> Seed cotton yield in grams per microplot harvested by hand three times.

<sup>b</sup> Days to first cracked boll.

<sup>c</sup> Second-stage juveniles per 250 cm<sup>3</sup> soil, 133 days after planting.

<sup>d</sup> Galling based on a 0-to-5 scale: 0 = no galls/root system and 5 = >100 galls/root system.

*M. incognita* alone, *M. incognita* + Tb20, and *M. incognita* + Tb100 plots were lower than *T. basicola* or control plots (Table 5). Plant maturity (DTCB) was affected by *M. incognita* + *T. basicola* or *M. incognita* alone, with maturity being delayed by 5 to 9 days (Table 5). According to orthogonal contrasts, *M. incognita* + *T. basicola* reduced yield and delayed maturity compared to the control and reduced yield and delayed maturity to a greater degree than either pathogen alone ( $P \leq 0.01$ ). Nematode populations were much higher at harvest than at 57 DAP, and *T. basicola* did not affect J2 population levels at harvest relative to *M. incognita* alone. The total number of eggs extracted was numerically lowest in the Tb100 treatment. Root galling was not influenced by the presence of *T. basicola* (Table 5).

Plant heights at harvest in 1995 were lowered by the *M. incognita*, *M. incognita* + Tb20, and *M. incognita* + Tb100 treatments compared to *T. basicola* alone at either level and the control (Table 6). Plants in plots treated with *M. incognita* alone or *M. incognita* + *T. basicola* at either level initiated the first sympodial branch higher on the plant stem (Table 6). The number of sympodial branches and the number of sympodial branches with a boll in the first and second position at harvest were not affected by *T. basicola* at either level, but plants in microplots treated with *M. incognita*, *M. incognita* + Tb20, or *M. incognita* + Tb100 had both

fewer branches and fewer branches with two bolls than the control. As in 1994, the treatments with *M. incognita* and *M. incognita* + *T. basicola* at either level lowered the total number of bolls per plant compared to the control or *T. basicola* alone treatments. The percentage of bolls retained in the first fruiting position was not affected by the treatments Tb20, Tb100, *M. incognita*, or *M. incognita* + Tb100 compared to the control, but boll retention in the first fruiting position was reduced by the *M. incognita* + Tb20 and *M. incognita* + Tb100 treatments in comparison to the control or *T. basicola* treatments, but not in comparison to the treatment *M. incognita* (Table 6). Orthogonal comparisons indicated that the combination of *M. incognita* + *T. basicola* reduced plant height, first sympodial node, the number of sympodial branches, and all boll measurements compared to either pathogen alone ( $P \leq 0.05$ ).

## DISCUSSION

Neither *T. basicola* nor *M. incognita* is considered to be an acute pathogen of cotton, and seedling or plant mortality in response to infection by either organism is unusual. The infestation levels of *T. basicola* used in this study are typical of populations that have been found in cotton fields in Arkan-

TABLE 6. Late-season cotton growth and development characteristics 133 days after planting in microplots infested with *Thielaviopsis basicola* (Tb) and *Meloidogyne incognita* (Mi) in 1995.

Treatment	Plant height (cm)	1st sympodial node <sup>a</sup>	Number of sympodial branches	Sympodial branches with 2 bolls	Total bolls	Percent bolls retained at 1st position	Percent bolls retained at 2nd position
Control	86.36 <sup>c</sup>	6.27	15.2	3.43	13.8	52.70	27.90
Tb20	85.09	6.14	15.0	4.02	14.2	56.60	27.35
Tb100	84.07	6.20	15.0	3.10	13.7	56.68	25.34
Mi	78.23	6.92	14.2	2.48	11.6	50.10	22.30
Mi + Tb20	75.95	7.68	13.8	1.62	9.2	41.60	18.10
Mi + Tb100	75.44	7.13	13.5	2.05	10.2	47.58	19.89
LSD	2.08	0.56	0.91	0.74	1.79	7.27	5.98
Contrast							
Tb + Mi vs. Tb or Mi	*	**	*	**	***	**	**

Data are means of 10 replications. Means within same column are not significantly different if the magnitude of the difference is not greater than the LSD value according to Fisher's protected LSD at  $P \leq 0.05$ . Orthogonal contrasts between the Tb + Mi treatments and treatments containing either pathogen alone were significant at  $P \leq 0.05$ , 0.01, or 0.001.

<sup>a</sup> Nodes to first sympodial branch, excluding cotyledonary node.

sas, with numerous fields having populations at levels greater than 100 propagules/g of soil (C. S. Rothrock, unpubl.). Levels of infestation for *M. incognita* vary considerably among fields within the state (Kirkpatrick et al., 1992), with population densities reflecting past cropping history. The primary objective of this study was to determine the nature of the apparent interaction between these pathogens on cotton. Consequently, infestation levels used in the microplots were selected to provide significant disease pressure without being unrealistically high. In addition, because *T. basicola* has been observed to be more severe when soil temperatures are cool (Rothrock, 1992), planting dates for the study were determined based on soil temperature in the microplots rather than calendar dates. In both 1994 and 1995, the experiment was planted when minimum daily soil temperatures at 10 cm had remained above 16 °C for 2 consecutive days. However, conditions for the week following planting varied between the 2 years. In 1994, average soil temperature during the week following planting averaged 16.1 °C, with cumulative rainfall of 7.9 cm, while in 1995 soil in the microplots was both drier and warmer, with an average temperature of 17.8 °C and cumulative rainfall of 5.9 cm. These differences may help explain why effects were seen in 1995 with 100 *T. basicola* propagules/g of soil but not with the lower infestation rate.

The effects seen with the combination of *T. basicola* and *M. incognita* were most severe during the early part of the growing season, and these effects were consistent in both years. Increased seedling mortality and suppression of early seedling growth were more severe where both organisms were present than with either pathogen alone. The primary effect of the pathogen combination appeared to be on early seedling survival and development, although the combination of both pathogens also affected certain season-long plant development characteristics such as the number of sympodial branches with two bolls, percentage of bolls in the second fruiting position, DTCB, and yield. *Thielaviopsis basicola* alone did not significantly affect

cotton seedling mortality, although the higher level of the pathogen suppressed seedling height-to-node ratio in 1995. Plant growth and development throughout the rest of the season generally were not affected by *T. basicola* in the absence of the nematode. Conversely, infection by *M. incognita* alone resulted in suppression of both growth and development of the plants throughout the season, although effects were not as severe as when both pathogens were present. Nematode infestation slowed early seedling growth and development, delayed fruit maturation, reduced number of bolls, and suppressed yield in 1995.

This study showed suppression of *M. incognita* reproduction by *T. basicola*. This effect was seen at 57 DAP in 1994 and at harvest both years. Although there was no difference between the degree of root galling of plants treated with the nematode alone and that of plants with both pathogens, the number of *M. incognita* eggs extracted at harvest was significantly suppressed by *T. basicola*. Although egg counts per unit of root by weight were not determined, no large differences were evident among samples in the volume of roots collected for egg extraction. It is possible that early root system damage due to infection by *T. basicola* may alter the suitability of the root for infection by *M. incognita*, either directly through elimination of infection sites or indirectly through altered host physiology. It is also possible that the fungus may affect the nematode directly through antagonism.

Early-season effects of concomitant populations of *T. basicola* and *M. incognita* can significantly impact development and yield of cotton. Reduced or erratic plant stands and delayed plant growth and development are of particular concern with cotton because cotton is a relatively long-season crop and earliness of maturity allows a timely harvest, particularly in the northern portion of the U.S. cotton production region. Unacceptable levels of seedling mortality may require replanting, which results in delayed crop development. Perhaps of equal concern in most production systems, however, is impeded early seedling growth and develop-

ment, resulting in delays in fruiting and crop maturation. It appears from this study that there is a high risk for adverse economic impact due to a combination of *T. basicola* and *M. incognita* in cotton production systems.

#### LITERATURE CITED

- Ayoub, S. M. 1980. Plant nematology: An agricultural training aid. Sacramento, CA: NemaAid Publications.
- Bonner, C. M. 1992. 1992 cotton production recommendations. Little Rock, AR: University of Arkansas Cooperative Extension Service.
- Bourland, F. M., and C. E. Watson, Jr. 1990. Cotmap, a technique for evaluating structure and yield of cotton plants. *Crop Science* 30:224-226.
- Bridge, J. 1992. Nematodes. Pp. 331-353 in R. J. Hillcocks, ed. Cotton disease. Wallingford, UK: CAB International.
- Candole, B. L., and C. S. Rothrock. 1997. Characterization of the suppressiveness of hairy vetch-amended soils to *Thielaviopsis basicola*. *Phytopathology* 87:197-202.
- Farr, D. F., G. F. Bills, G. P. Chamuris, and A. Y. Rossman. 1989. Fungi on plants and plant products in the United States. St. Paul, MN: APS Press.
- Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter* 57:1025-1028.
- Johnson, D. R., and B. F. Jones. 1993. 1993 insecticide recommendations for Arkansas. Little Rock, AR: University of Arkansas Cooperative Extension Service.
- King, C. J., and H. D. Barker. 1942. An internal collar rot on cotton. *Phytopathology* 29:751.
- Kirkpatrick, T. L., J. D. Barham, and R. J. Bateman. 1992. Incidence of plant-parasitic nematodes in cotton fields in Arkansas. Pp. 57-62 in D. M. Oosterhuis, ed. Proceedings of the 1992 Cotton Research Meeting. Arkansas Agricultural Experiment Station Research Series 158.
- Kirkpatrick, T. L., and C. S. Rothrock. 1995. The interaction between *Thielaviopsis basicola* (black root rot) and the root-knot nematode. Pp. 53-57 in: Proceedings of the 1995 cotton research meeting and 1995 summaries of cotton research in progress. Arkansas Agricultural Experiment Station Special Report 172.
- Mathre, D. E., A. V. Ravenscroft, and R. H. Garber. 1966. The role of *Thielaviopsis basicola* as a primary cause of yield reduction in cotton in California. *Phytopathology* 56:1119-1212.
- Mauk, P. A., and Hine, R. B. 1988. Infection, colonization of *Gossypium hirsutum* and *G. barbadense*, and development of black root rot caused by *Thielaviopsis basicola*. *Phytopathology* 81:946-954.
- Robbins, R. T., R. D. Riggs, and D. Von Steen. 1989. Phytoparasitic nematode surveys of Arkansas cotton fields, 1986-88. Supplement to the *Journal of Nematology* 21:619-623.
- Rothrock, C. S. 1992. Influence of soil temperature, water and texture on *Thielaviopsis basicola* and black root rot of cotton. *Phytopathology* 82:1202-1206.
- Rothrock, C. S., and R. G. Wells. 1992. Prevalence of black root rot, *Thielaviopsis basicola*, on cotton in Arkansas. P. 200 in D. J. Heber and D. A. Richter, eds. 1992 proceedings beltwide cotton conferences. Memphis, TN: National Cotton Council of America.
- Specht, L. P., and G. J. Griffin. 1985. A selective medium for enumerating low populations of *Thielaviopsis basicola* in tobacco field soils. *Canadian Journal of Plant Pathology* 7:438-441.
- Tsao, P. H., and Bricker, J. L. 1966. Chlamydospores of *Thielaviopsis basicola* as surviving propagules in natural soils. *Phytopathology* 56:1012-1014.
- Walker, N. R., T. L. Kirkpatrick, and C. S. Rothrock. 1997. The effects of root-knot (*Meloidogyne incognita*) and black root rot (*Thielaviopsis basicola*) on cotton (*Gossypium hirsutum*). P. 103 in P. Dugger and D. A. Richter, eds. 1997 Proceedings Beltwide Cotton Conferences. Memphis, TN: National Cotton Council of America.
- Watkins, G. M., ed. 1981. Compendium of cotton diseases. St. Paul, MN: APS Press.