Soil Texture Influence on *Meloidogyne incognita* and *Thielaviopsis basicola* and Their Interaction on Cotton

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Abstract

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Microplots were used to evaluate the impact of soil texture on *Meloidogyne incognita*, *Thielaviopsis basicola*, and their interaction on cotton. A native silt loam soil (48% sand) and four different artificial soil textures produced by mixing native soil with sand (53, 70, 74, and 87% sand) were studied. Each soil texture was infested with 0, 4, or 8 *M. incognita* eggs and 0 or 20 *T. basicola* chlamydospore chains per gram of soil in a factorial treatment arrangement. Plots were watered when soil moisture fell below –10 joules/kg for the first 21 days and –30 joules/kg from 22 days to harvest. Plant growth was suppressed early in the season and midseason by *T. basicola*. *M. incognita* suppressed plant growth and delayed plant development late in the season

across all soil textures. Cotton yield was lower in the presence of either *T. basicola* or *M. incognita*. An interaction between *M. incognita* and *T. basicola*, which decreased plant growth and yield, occurred in 2006 when neither pathogen caused substantial plant damage. Plant growth, development, and yield were lowest in soils with >74% sand. Root colonization by *T. basicola* and fungal reproduction and survival decreased in soil having 87% sand. *M. incognita* generally caused more galling and reproduction in soils as sand content increased. Root galling severity and *M. incognita* reproduction were suppressed by the presence of *T. basicola* in soil at sand contents lower than 87%. Soil texture had a greater impact on *T. basicola* than on *M. incognita* in this study.

The root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood and the soilborne fungus *Thielaviopsis basicola* (Berk. & Broome) Ferraris (syn. *Chalara elegans* Nag Raj & W.B. Kendr.), the causal agent of black root rot, are important plant pathogens of upland cotton (*Gossypium hirsutum* L.) (16). *M. incognita* is the most widely-distributed nematode pest of cotton worldwide (18). Black root rot has become an important seedling disease on cotton throughout the world (1,20).

Root-knot nematodes emerge from eggs as second-stage juveniles (J2s) and penetrate cortical root tissue immediately behind the root cap. After migrating to the vascular tissue, they establish a permanent feeding site, eliciting morphological and physiological changes that include the formation of root galls (21). Both soil temperature and texture influence root-knot nematode damage and reproduction. Soil temperatures of 25 to 30°C favor nematode embryogenesis and egg hatch (36), while temperatures below 10°C are inhibitory (8). Population densities of the nematode are inversely related to silt and clay contents (10,17,28). Reproduction of *M. incognita* was greater in soils with sand contents of 58 or 91% than soils with lower sand contents (17), and damage to cotton increased at greater sand content (24,27). Soil moisture may also affect survival of the nematode, with survival being greater in soils at water pressures of –0.11 MPa than in soil at –0.016, –0.03, –1.5, or –9.2 MPa (35).

T. basicola overwinters as thick-walled chlamydospores that germinate in the presence of the host and adequate soil water (11,25). *T. basicola* usually colonizes the root cortical tissue, causing dark-brown to black discoloration of the roots and hypocotyl (1,19,20).

Under high disease pressure, hyphae and chlamydospores of the fungus have been observed within the vascular system of upland cotton roots (15,20). Black root rot is most severe when soils are cool (below 24°C) and wet (above –20 joules/kg) (14,30,38). As soil temperatures increase and roots mature, affected cortical tissues are sloughed off and secondary root growth occurs (19,20). Within a field, lower soil populations of *T. basicola* were associated with soil textures having 65% sand or greater (22). In controlled studies using soils infested with a benomyl-resistant strain of *T. basicola* at 100 chlamydospores/g of soil, soils with sand contents of 56% or greater suppressed black root rot compared with soils with lower sand content (4).

A synergistic interaction between *M. incognita* and *T. basicola* has been described in cotton (23,37–39). Concrete microplots infested with both *T. basicola* and *M. incognita* showed an increase in seedling death and a decrease in plant growth compared with microplots infested with either pathogen alone. Plants affected by both the root-knot nematode and *T. basicola* had fewer bolls per plant, a reduction in boll retention at the second fruiting position, and reduced seed-cotton yields compared with plants grown in soils infested with either pathogen alone or in noninfested plots (37). Plant damage associated with this interaction increased as population densities of the individual pathogens increased (39). Infection of cotton by both *T. basicola* and *M. incognita* appeared to increase the effective range of temperatures at which both pathogens caused damage (23,38). Histological studies detected the presence of *T. basicola* frequently in the vascular root tissue in plants grown in soils infested with *M. incognita*, suggesting that cotton roots affected by the nematode allowed *T. basicola* to colonize vascular tissues that are not normally colonized in the absence of the nematode (38). The presence of *T. basicola* reduced nematode reproduction (23,37–39). The objective of this study was to examine the influence of soil texture on the reproduction and damage potential of *M. incognita* and *T. basicola* and their interaction on cotton.

Materials and Methods

A native silt-loam soil (48% sand, 50% silt, and 2% clay) from a field at the Delta Branch Station, Clarkedale, AR (Dubbs-Dundee

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*The e-Xtra logo stands for “electronic extra” and indicates that four supplementary tables are available in the online edition.

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complex mixed, thermic Typic Hapludalfs), with a long history of cotton monoculture was used to make four artificial soil textures (53, 70, 74, or 87% sand) by mixing the native soil with sand (>2 mm = 6.3%, 1 to 2 mm = 11.0%, 0.5 to 1 mm = 21.6%, 0.25 to 0.5 mm = 41.5%, 0.1 to 0.25 mm = 16.9%, and 0.05 to 0.1 mm = 2.75%) using an electric cement-mixer (model CM350A; Northern). Texture for each soil was determined using the hydrometer particle-size method (2). Microplots consisting of clay flue tiles (45 by 30 cm wide and 75 cm deep) that had previously been established at the Arkansas Agricultural Research and Extension Center, Fayetteville, were used. The empty tiles were disinfested by spraying the walls with 0.5% NaOCl until run-off. The soil mixtures were steam pasteurized for 30 min at 70°C prior to use. Prior to placing the soil into the microplots, samples were taken from each soil texture and oven dried to calculate soil water content. Soil for each microplot was divided into five portions by weight, added in 15-cm increments, and packed to a bulk density of 1.1 g/cm³. After a layer was compacted, the surface was disturbed with a rake before the addition of soil for the next layer.

Inoculum. *T. basicola* isolate 3N-25B, recovered from cotton seedlings at the Delta Branch Station near Clarkedale, AR, was used to prepare the fungal inoculum. Chlamydospore chains were obtained by the screening procedure described by Candole and Rothrock (6). Briefly, 6-week-old cultures of *T. basicola* grown on 10% carrot juice agar were flooded with sterile distilled water and decanted repeatedly to dislodge endoconidia. Chlamydospore chains and other fungal structures were removed from plates with a rubber policeman, suspended in sterile distilled water, and passed through a monofilament nylon fabric with 53-µm openings (Tetko, Inc.). The suspension was then filtered through a monofilament nylon fabric with 20-µm openings. Chlamydospores chains retained on the nylon fabric were transferred into a sterile 50-ml centrifuge tube containing about 20 ml of sterile distilled water and stored in a refrigerator at 2 to 5°C. The suspension containing chlamydospore chains were plated on carrot juice agar, incubated for 24 h, and examined to determine percent chlamydospore germination.

M. incognita, host race 3, was provided by the Arkansas Nematode Diagnostic Clinic, University of Arkansas Southwest Research and Extension Center at Hope. The nematode population was originally collected from cotton in a producer's field in Arkansas. For microplot inoculum, the isolate was increased on tomato (*Lycopersicon esculentum* L. 'Rutgers') in a greenhouse. Eggs were collected from infected roots after 75 d using NaOCl as described by Hussey and Barker (12).

Microplot establishment. Microplots were infested with *T. basicola* to obtain 20 chlamydospore chains (approximately 100 individual chlamydospores) per gram of oven dry weight (ODW) equivalent of soil in the top 15-cm layer of soil. Chlamydospores were suspended in 50 ml of sterile distilled water and applied with a hand trigger sprayer to each soil texture, and the soil was mixed in a cement mixer. Infested soils were placed in microplots above the four layers of noninfested soil and packed to a depth of 15 cm. *M. incognita* eggs and J2s were suspended in sterile distilled water, and applied into two holes (1 cm in diameter by 5 cm deep) for each microplot at planting to obtain a final rate of 4 or 8 eggs and J2s/g of soil (ODW) in the top 15 cm of soil.

Six pathogen treatments were included for each soil texture: a noninfested control, *T. basicola* alone, the two rates of *M. incognita* alone, and all combinations of *M. incognita* and *T. basicola* in 2006 and 2008. The microplots were planted in 2007 without reinfesting plots, using the surviving populations from the 2006 season as inoculum. Prior to planting in 2008, soil from each microplot was removed to a depth of 75 cm and steam pasteurized. The pasteurized soil for each texture was used to refill the microplots as described for 2006 and the top 15 cm was infested at appropriate pathogen levels, as described.

Fourteen acid-delinted seeds of 'DP 444 BG/RR' cotton (Delta and Pine Land) were planted in each plot immediately following infestation. Planting dates were 16 May in both 2006 and 2007. In 2008, 'DP 555 BG/RR' was used and the planting date was 17

May. Seed were treated with the fungicides triadimenol, thiram, and metalaxyl (0.107, 0.326, and 0.152 g a.i./kg of seed, respectively; RTU Baytan-Thiram and Allegiance, Bayer CropScience). Soil fertility analysis was performed on a composite soil sample taken from each soil texture prior to planting, and each microplot was fertilized with 0.7, 0.6, and 0.15 g of N, K, and S, respectively, 15 and 50 days after planting (DAP) based on recommendations of the University of Arkansas Division of Agriculture Soil Testing and Research Laboratory, Marianna. Plants were sprayed with MiracleGro water-soluble fertilizer (24-8-16) at 4 g/liter if nitrogen deficiency symptoms were observed. Cotton plants were sprayed with the insecticide dicofol at 277.5 g a.i./ha (Kelthane 18.5 EC; Rohm and Haas Company) to control mites in 2006 and 2007.

Soil temperature and moisture data were collected using soil temperature and watermark soil moisture sensors connected to a data logger (WatchDog, model 450) in one microplot for each soil texture. Soil moisture also was measured in each texture with a ThetaProbe ML2 (Delta-T Devices). Air temperature and rainfall data were obtained from a weather station at the Arkansas Agricultural Research and Extension Center, Fayetteville (26). All microplots for each soil texture were watered when they reached a matric potential of approximately -10 joules/kg for the first 21 days and -30 joules/kg from 22 days to harvest by watering with the equivalent of 1.5 cm of rainfall. Percent water for each matric potential was determined for each soil texture using a Haines' apparatus, a Buchner funnel with a fine sintered-glass base plate, prior to initiating the study (9).

Plant and disease assessment. At 12 DAP, seeding emergence was assessed, and the number of plants was thinned to six plants per plot. Two plants were arbitrarily sampled from each microplot at 22 to 28 DAP (early-season) and at 45 to 50 DAP (midseason), and 2 plants were left until harvest. Plants were measured for height from the cotyledonary node to the tip of the main stem terminal and the number of main stem leaf nodes was counted for each plant. The portion of plant above the cotyledonary node was removed, dried at 60°C for 48 h, and weighed. Root systems from each plant were rinsed with tap water for 20 min, immersed for 2 min in 0.5% NaOCl, blotted dry in a paper towel, and weighed. Roots were rated for root discoloration using a scale of 0 to 10, where 0 = 0%, 1 = 1 to 10, 2 = 11 to 20, 3 = 21 to 30, 4 = 31 to 40, 5 = 41 to 50, 6 = 51 to 60, 7 = 61 to 70, 8 = 71 to 80, 9 = 81 to 90, and 10 = 91 to 100% of the root system discolored. Root systems then were evaluated for nematode galling on a scale of 0 to 5, where 0 = 0, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 50, and 5 = 51 to 100 galls/root system. Roots were plated on the medium TB-CEN (32), amended with Penicillin G (ICN Biomedicals Inc.) at 60 mg/liter. Plates were kept in the dark at room temperature (20 to 23°C) and, after 12 to 14 days, the percentage of root systems with growth of *T. basicola* on the medium was rated using the 0-to-10 scale described previously for root discoloration. At harvest, cotton plants were cut below the cotyledonary node and mapped for growth and development using COTMAP (3). Lint and seed were hand harvested from each microplot to assess seed cotton production per plant.

Pathogen populations. *M. incognita* and *T. basicola* populations were assessed from soils at 22 to 28, 45 to 50, and 120 to 130 DAP (late-season) and in spring (2 April 2007 and 2008 and 5 April 2009). A composite soil sample taken with a 2.5-cm-diameter sampling tube to a depth of 15 cm was collected from each plot. Population density of *T. basicola* was determined by the pour-plate technique using amended TB-CEN medium. Briefly, 27 g of soil were suspended in 237 ml of sterile water agar (0.15%) for a total of 250 ml. Soil suspensions were shaken on a wrist action shaker for 20 min, and 1 ml of the sample was pipetted into each of the six petri plates (100 by 15 mm) for a sample. Molten TB-CEN medium (approximately 45°C) was poured into each plate and plates were swirled to uniformly distribute the soil suspension. Plates were kept at room temperature (20 to 23°C) in the dark and the number of colonies of *T. basicola* counted 12 to 14 days after plating. Counts were adjusted to CFU per gram of soil (ODW).

Soil nematode populations were extracted by the Arkansas Nematode Diagnostic Clinic Laboratory, University of Arkansas Southwest Research and Extension Center. A 100-cm³ subsample of soil from each plot was processed using a semiautomatic elutriator (5) followed by centrifugal flotation (13). *M. incognita* root populations were estimated by agitating the roots collected from the 100-cm³ sample in a 0.5% NaOCl solution (12) and collecting the eggs on a 500-mesh (26-μm) sieve. Nematodes and eggs were counted with a stereoscope at a ×40 to ×60 magnification.

Statistics. The experiment was arranged in a randomized complete block design using a factorial arrangement of texture and pathogen treatments and four replications. Statistical analyses were conducted using the GLM procedure in SAS 9.2 (SAS Institute Inc.). Data were analyzed by year. Root discoloration, colonization, and galling were analyzed as midvalues. Pathogen populations were transformed using log₁₀ (x + 1) for statistical analyses. Treatment means were separated with Fisher's protected least significant difference (LSD) at *P* = 0.05. When interactions were significant (*P* ≤ 0.05), appropriate interaction means were examined and LSDs were calculated. Treatments not receiving *M. incognita* were omitted from analyses for nematode populations and root galling, and treatments not receiving *T. basicola* were omitted from analyses for root discoloration and colonization.

Results

In 2006, daily soil temperatures at 10 cm averaged 22.1, 24.6, and 28.6°C and rainfall totals were 22.9, 7.7, and 10.9 mm from the time of planting in May, June, and July, respectively. In 2007, daily soil temperatures averaged 20.3, 23.5, and 26.3°C and rainfall was 5.2, 14.1, and 12.6 mm for the same periods of time, respectively. In 2008, soil temperatures averaged 23.5, 25.3, and 27.8°C and rainfall 13.7, 14.9, and 19.4 mm from the time of planting in May, June, and July, respectively. Soil temperatures did not differ consistently among soil textures. Soil bulk density in the top 15 cm averaged 1.12, 1.10, 1.10, 1.17, and 1.10 g/cm³ for the soils with 48, 53, 70, 74, and 87% sand content, respectively, when assessed after harvest in 2008.

Plant growth. Fewer differences were seen among treatments for early-season plant growth than at midseason or at harvest. *T. basicola* reduced plant height compared with plots that were not infested with *T. basicola* over all soil textures at 22 to 28 DAP in

both 2007 and 2008: Infested height was 0.4 cm and noninfested height was 0.8 cm in 2007 (*P* = 0.0002), and 3.4 and 4.4 cm, respectively, in 2008 (*P* = 0.0056). Similarly, numbers of nodes were 0.7 in infested and 1.3 in noninfested microplots in 2007 (*P* = 0.0001) and 2.1 and 2.4 in 2008 (*P* = 0.0195). *M. incognita* reduced plant height and the number of nodes compared with plots that were not infested with the nematode over all soil textures in 2008 (3.7 or 3.0 to 5.1 cm [*P* < 0.0001], and 2.1 or 2.1 to 2.6 [*P* < 0.0001], respectively). *T. basicola* in 2007 reduced root weight compared with plots not infested with *T. basicola* over all soil textures at 0.175 to 0.301 g (*P* < 0.0001), respectively. In 2008, a soil texture–*T. basicola* interaction occurred (*P* = 0.0187), with *T. basicola* reducing root weight compared with plots not infested with *T. basicola* at sand contents of 48, 53, or 74% (data not presented). No consistent effects were seen on early plant growth for soil texture (data not presented).

At midseason (45 to 50 DAP), soil texture, *M. incognita*, and *T. basicola* all affected plant height in all 3 years (Supplementary Table S1). Effects were generally independent, although a soil–*T. basicola* interaction and an *M. incognita*–*T. basicola* interaction were observed in 2006 for plant height. Both *M. incognita* and *T. basicola* reduced midseason plant height in 2007 and 2008 compared with the noninfested treatment (Table 1). *T. basicola* reduced plant heights 40% in 2007 and 9% in 2008, while plant height reductions with *M. incognita* averaged 40% in 2007 and 31% in 2008. Sand contents of 53 and 70% supported greater plant heights than 87% sand in both 2007 and 2008 (Table 1). *T. basicola* reduced plant height in soil with the lowest sand content (48%) compared with plots not infested with *T. basicola* in 2006. Co-infection by *T. basicola* and *M. incognita* caused a greater suppression of plant height than either *T. basicola* or *M. incognita* alone in 2006 (Table 1). *M. incognita* also reduced plant height in the absence of *T. basicola* compared with the noninfested treatment.

For main stem leaf nodes, soil texture significantly affected growth in all 3 years and *M. incognita* and *T. basicola* significantly affected growth in 2007 and 2008. Interactions between soil texture and *M. incognita* and between *M. incognita* and *T. basicola* were present in 2007. No consistent effects of soil texture were observed for nodes (Table 1). Both *M. incognita* and *T. basicola* reduced the number of main stem nodes in 2008 compared with the noninfested

Table 1. Main and interaction effects of soil texture (sand), *Meloidogyne incognita*^a (Mi), and *Thielaviopsis basicola*^u (Tb) at midseason season on plant height and number of main stem nodes^v

Main effect	Plant height (cm)			Main stem nodes		
	2006	2007	2008	2006	2007	2008
Sand (%)	0 ----- Tb ----- 20			Mi		
48	36.8 a ^w	26.0 cd	23.6 a ^x	7.6 a ^x	7.7 a ^y	6.9 abcd
53	32.5 ab	28.5 bc	23.3 a	6.7 b	7.6 ab	6.8 abcd
70	26.3 cd	28.5 bc	22.7 ab	7.0 ab	7.4 ab	5.4 de
74	28.2 bc	24.1 cde	18.9 bc	7.1 ab	7.4 ab	5.7 cde
87	22.1 de	19.5 e	17.1 c	6.6 b	7.2 abc	5.1 e
Mi	0 ----- Tb ----- 20			0 ----- Tb ----- 20		
0	33.8 a ^z	33.2 a	29.1 a	7.3 a	7.7 a ^z	7.2 a
4	26.3 b	21.9 c	17.2 b	7.0 a	6.9 a	5.1 b
8	27.4 b	20.7 c	18.0 b	6.7 a	7.2 a	4.9 b
Tb						
0		26.2 a	22.5 a	7.2 a		7.4 a
20		15.7 b	20.4 b	6.8 a		7.0 b

^t Soils were infested at planting with 4 or 8 eggs and second stage juveniles (J2) of *M. incognita*/g of soil.

^u Soils were infested at planting with 20 chlamydospore chains of *T. basicola*/g of soil.

^v Plant growth variables were measured 45 to 50 days after planting.

^w Interaction means for any sand and Tb combination followed by the same letter are not significantly different, Fisher's protected least significant difference (*P* ≤ 0.05).

^x Means for a year and main effect followed by a common letter are not significantly different, Fisher's protected least significant difference (*P* ≤ 0.05).

^y Interaction means for any sand and Mi combination followed by the same letter are not significantly different, Fisher's protected least significant difference (*P* ≤ 0.05).

^z Interaction means for any Mi and Tb combination followed by the same letter are not significantly different, Fisher's protected least significant difference (*P* ≤ 0.05).

treatment midseason regardless of differences in soil texture (Table 1). *M. incognita* at either or both infestation rates reduced the number of plant nodes in soils with a sand content >48% compared with plots not infested with *M. incognita* in 2007 (Table 1). The number of nodes was lower in soil infested with *M. incognita* and *T. basicola* than in plots infested with either the nematode or the fungus, regardless of differences in soil texture in 2007 (Table 1).

At midseason, soil texture, *M. incognita*, and *T. basicola* affected root weight in all 3 years. A significant interaction between *M. incognita* and *T. basicola* or *T. basicola* and soil texture occurred for root weight in 2006 and 2007, respectively. *M. incognita* in 2007 and 2008 and *T. basicola* in 2008 reduced root weight compared with the noninfested treatment over all soil textures (Table 2). Root weights were lower for plants grown in soils infested with both pathogens than for plants grown in noninfested soil or soils with *M. incognita* or *T. basicola* alone in 2006. *T. basicola* reduced root weight in soil with sand contents of 74% or lower but not at 87% sand content in 2007 compared with treatments not infested with *T. basicola*. Soil textures of 74

or 87% sand had lower root weights compared with 53% sand in 2006 and 2008, and in 2007 for the noninfested soils only (Table 2).

Effects of *T. basicola*, *M. incognita*, and soil texture were independent for crop growth and development at the end of the season, except for a soil-*T. basicola* interaction in 2008 for plant height (Supplementary Table S2). Plants were shorter in 87% sand than other soil textures in both 2006 and 2007 and except for 74% sand in the presence of *T. basicola* in 2008 (Table 3). *M. incognita* reduced plant height in both 2007 and 2008. The nematode also influenced initiation of fruiting all 3 years, delaying the formation of the first sympodial branch on the main stem in plants grown in soil treated with one or both rates of *M. incognita* compared with plots not infested with *M. incognita* (Table 3). The position of the first sympodial branch was also higher in plants grown in soils infested with *T. basicola* in 2008.

Seed-cotton yield was affected by *T. basicola*, *M. incognita*, and soil texture in each year, with a significant *M. incognita*-*T. basicola* interaction in 2006. Yield was lower at sand contents of 74 or 87% than in 53 or 70% sand all 3 years (Table 3). Yield was

Table 2. Main and interaction effects of soil texture (sand), *Meloidogyne incognita*^u (Mi), and *Thielaviopsis basicola*^v (Tb) at midseason season on fresh root weight^w

	Fresh root weight (g)				
Main effect	2006		2007		2008
Sand (%)	0 ----- Tb ----- 20		0 ----- Tb ----- 20		
48	2.362	ab ^x	2.408	ab ^y	1.596 b ^x
53	2.496	a	3.125	a	2.737 a
70	2.290	ab	2.186	bc	2.787 a
74	2.075	b	2.097	bc	0.968 c
87	1.586	c	1.463	cd	1.412 bc
Mi	0 ----- Tb ----- 20		0 ----- Tb ----- 20		
0	2.322	a ^z	2.767	a	2.758 a
4	2.448	a	1.159	b	1.641 b
8	2.367	a	1.345	b	1.432 b
Tb	0 ----- Tb ----- 20		0 ----- Tb ----- 20		
0					2.134 a
20					1.737 b

^u Soils were infested at planting with 4 or 8 eggs and second stage juveniles (J2) of *M. incognita*/g of soil.

^v Soils were infested at planting with 20 chlamydo spores chains of *T. basicola*/g of soil.

^w Root weight was measured 45 to 50 days after planting.

^x Means for a year and main effect followed by a common letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

^y Interaction means for any sand and Tb combination followed by the same letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

^z Interaction means for any Mi and Tb combination followed by the same letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

Table 3. Main and interaction effects of soil texture (sand), *Meloidogyne incognita*^v (Mi), and *Thielaviopsis basicola*^w (Tb) on cotton development and yield

Main effect	Plant height (cm)				First fruiting node			Seed cotton (g)		
	2006	2007	2008	2008	2006	2007	2008	2006	2007	2008
Sand (%)	0 ----- Tb ----- 20				0 ----- Tb ----- 20			0 ----- Tb ----- 20		
48	77.9 b ^x	84.6 a ^x	77.3 bcd ^y	79.2 bc	4.5 a ^x	6.0 a ^x	7.4 a ^x	37.6 a ^x	39.7 a ^x	27.6 ab ^x
53	84.5 a	81.0 ab	77.0 bcd	85.7 ab	4.4 a	6.1 a	6.9 b	40.4 a	46.2 a	32.6 a
70	74.8 b	77.1 bc	79.5 bc	91.2 a	4.3 a	6.0 a	7.3 ab	38.3 a	40.8 a	33.7 a
74	72.2 b	71.1 c	72.7 cd	73.31 cd	4.7 a	6.3 a	7.2 ab	29.8 b	29.1 b	19.6 b
87	59.7 c	55.7 d	80.8 bc	70.0 d	4.8 a	5.9 a	7.3 ab	24.9 b	22.4 b	23.9 b
Mi	0 ----- Tb ----- 20				0 ----- Tb ----- 20			0 ----- Tb ----- 20		
0	76.8 a	85.1 a	88.4 a ^x		4.2 b	5.5 b	7.0 b	40.6 a ^z	34.2 ab	48.4 a
4	71.3 a	69.3 b	74.0 b		4.8 a	6.6 a	7.1 b	32.6 ab	33.4 ab	30.1 b
8	73.4 a	67.7 b	73.5 b		4.8 a	6.5 a	8.0 a	36.4 ab	28.6 b	21.8 b
Tb	0 ----- Tb ----- 20				0 ----- Tb ----- 20			0 ----- Tb ----- 20		
0	74.5 a	74.0 a			4.4 a	5.9 a	7.0 b		39.1 a	30.9 a
20	73.2 a	74.3 a			4.7 a	6.2 a	7.4 a		33.3 b	24.5 b

^v Soils were infested at planting with 4 or 8 eggs and second stage juveniles (J2) of *M. incognita*/g of soil.

^w Soils were infested at planting with 20 chlamydo spore chains of *T. basicola*/g of soil.

^x Means for a year and main effect followed by a common letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

^y Interaction means for any sand and Tb combination followed by the same letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

^z Interaction means for any Mi and Tb combination followed by the same letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

also lower in soils infested with either *M. incognita* or *T. basicola* in 2 of the 3 years of this study (Table 3). In 2006, the greater inoculum rate of the nematode with *T. basicola* reduced yields compared with the noninfested treatment.

Pathogen responses. Root galling was affected by *T. basicola* early in the season in both 2007 and 2008 and midseason in 2007 (Supplementary Table S3). Soil texture influenced galling at mid-season in 2008 and *M. incognita* infestation level influenced mid-season galling in 2006. Significant interactions between soil texture and *M. incognita* were observed midseason in 2008 and a soil texture–*T. basicola* interaction occurred midseason in 2007. In 2008, galling tended to be numerically greater at higher sand contents (Table 4). Where *T. basicola* was present, root galling by *M. incognita* was suppressed both early in the season in 2007 and 2008 and at midseason in 2007 (Table 4).

M. incognita population density at harvest was affected by soil texture in both 2007 and 2008, while *T. basicola* had an effect on nematode reproduction in 2006 and 2007. In both 2006 and 2008, a soil–*T. basicola* interaction was present for nematode population density at harvest. *T. basicola* reduced *M. incognita* populations over all soil textures in 2007 compared with plots that were not infested with *T. basicola* (Table 5). In 2006, where *T. basicola* and

M. incognita were both present, nematode reproduction was suppressed in plots with sand content lower than 87%, with significant nematode population reductions in soils with sand contents of 48, 53, and 74% compared with soils that were not infested with *T. basicola*. In 2008, significant population density reductions were observed for *M. incognita* in the presence of *T. basicola* for 48% sand compared with other soil textures with or without *T. basicola*, except for 74% sand (Table 5). In 2007, nematode reproduction was greater in the soils with 53, 74, and 87% sand than with 48 or 70% sand and the response was similar in 2008 in the presence of *T. basicola*.

Soil texture influenced root colonization by *T. basicola* in both 2006 and 2008 (Supplementary Table S4). *M. incognita* did not affect root colonization, and there was no interaction between soil texture and the nematode. Root colonization by *T. basicola* was very high in 2007 and was not influenced by soil texture (Table 6). In 2006, root colonization was lower in the 87% sand compared with the other textures. In 2008, root colonization by *T. basicola* varied greatly but was lower in soils with 53, 70 and 87% sand than in 48 and 74% sand. Soil populations of *T. basicola* were affected by soil texture at midseason in all 3 years and at harvest in 2007 and 2008. Soil populations of *T. basicola* at midseason and at

Table 4. Main and interaction effects of soil texture (sand), *Meloidogyne incognita* populations^u (Mi), and *Thielaviopsis basicola*^v (Tb) on cotton root gall ratings^w

Main effect	Galling, early season			Galling, midseason		
	2006	2007	2008	2006	2007	2008
Sand (%)					0-----Tb-----20	4-----Mi-----8
48	18.3 a ^x	15.3 a ^x	30.8 a ^x	10.6 a ^x	48.4 bc ^y	12.6 bcd ^z
53	9.9 a	12.6 a	40.9 a	2.9 a	70.7 ab	43.7 ab
70	10.6 a	12.4 a	35.7 a	2.3 a	57.9 abc	56.9 a
74	11.0 a	26.5 a	51.3 a	4.0 a	75.0 a	36.1 abc
87	10.6 a	19.6 a	64.0 a	5.8 a	39.9 c	65.6 a
Mi						
4	12.3 a	11.9 a	49.8 a	23.0 a	28.0 a ^x	
8	14.5 a	25.0 a	39.3 a	7.3 b	33.4 a	
Tb						
0	15.0 a	24.4 a	50.8 a	4.2 a		37.7 a ^x
20	11.8 a	2.7 b	38.3 b	6.1 a		41.5 a

^u Soils were infested at planting with 4 or 8 eggs and second stage juveniles (J2) of *M. incognita*/g of soil.

^v Soils were infested at planting with 20 chlamydospore chains of *T. basicola*/g of soil.

^w Root galling was rated: 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-50, 5 = 50-100 galls/root. Analyses were conducted using midpoint values. Treatments without *M. incognita* were dropped from the analysis.

^x Means for a year and main effect followed by a common letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

^y Interaction means for any sand and Tb combination followed by the same letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

^z Interaction means for any sand and Mi combination followed by the same letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

Table 5. Main and interaction effects of soil texture (sand), *Meloidogyne incognita*^v (Mi), and *Thielaviopsis basicola*^w on *M. incognita* soil populations^x

Main effect	Harvest <i>M. incognita</i> population			Preplant <i>M. incognita</i> population		
	2006	2007	2008	2007	2008	2009
Sand (%)	0-----Tb-----20		0-----Tb-----20			
48	2.9 ab ^y	1.7 cd	1.3 b ^z	2.5 a ^z	2.2 a ^z	1.8 c ^z
53	2.8 abc	1.6 d	2.6 a	2.7 b	1.9 a	2.4 ab
70	2.8 abc	1.9 bcd	1.3 b	2.7 b	1.7 a	2.3 bc
74	3.1 a	1.4 d	2.4 a	2.9 ab	1.9 a	2.1 bc
87	2.4 abc	2.8 ab	2.6 a	3.0 ab	1.9 a	2.9 a
Mi						
4	2.4 a ^z	2.1 a	2.8 a ^z	2.2 a	2.1 a	2.3 a
8	2.3 a	1.9 a	2.7 a	2.2 a	1.9 a	2.3 a
Tb						
0		3.2 a		2.2 a	2.1 a	2.3 a
20		0.8 b		2.1 a	1.8 a	2.3 a

^v Soils were infested at planting with 4 or 8 eggs and second stage juveniles (J2) of *M. incognita*/g of soil.

^w Soils were infested at planting with 20 chlamydospore chains of *T. basicola*/g of soil.

^x Treatments without *M. incognita* were dropped for this analysis. Data transformed, $\log_{10}(x + 1)$.

^y Interaction means for any sand and Tb combination followed by the same letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

^z Means for a year and main effect followed by a common letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

harvest were lower in 87% sand than the other soil textures, except at harvest in 2006 (Table 7).

The preplant populations of *M. incognita* were affected by soil texture in 2009. Population densities were greater for 87% sand than all but the 53% sand treatment (Table 5). The populations of *T. basicola* the following spring were affected by soil texture in 2007 and 2009. In 2007, *M. incognita* also affected preplant populations and there was a texture–nematode interaction. In 2007, *T. basicola* survival was greater when the nematode was present in most soil textures, except in soil with 70 and 74% sand content. Survival of *T. basicola* was greater in soil with sand contents below 87% in 2009 (Table 7).

Discussion

T. basicola reduced plant height, the number of main stem nodes, and root weight in 2 of the 3 years of the study (2007 and 2008) both early in the season and at midseason. In earlier studies in microplots, Walker et al. (37,39) documented few effects of *T. basicola* on plant growth in the absence of the nematode, with the height-to-node ratio being less than that in the noninfested control in 1 year at an infestation rate of 100 chlamydospores/g of soil at 21 or 28 DAP (39). In a later study, Walker et al. (37) again observed that *T. basicola* had little impact on plant growth in the absence of the nematode except to reduce plant weight in one sea-

son at 56 DAP (39). The reduction in plant growth early in the season in soils infested with *T. basicola* in the current study corresponded to lower seed-cotton yield in both 2007 and 2008. Yield reductions from *T. basicola* have not been observed in the absence of the nematode in previous microplot studies (37,39). Severity of black root rot on cotton increases at soil temperatures below 24°C and in poorly drained soils (20,23,30,38). The variable impact of *T. basicola* on disease development and severity and cotton growth and yield in the current study may be related to differences in soil environment at or shortly after planting among the 3 years of this study. *T. basicola* had the greatest root colonization and the greatest impact on cotton growth and yield in 2007. The 2007 season was characterized by lower soil temperatures in May and June than in the other years of the study. Soil temperatures early in the season were similar to or lower than the 2007 temperatures in the previous microplot studies; however, root discoloration and colonization or incidence was much lower in the former studies (37,39). These results suggest that the greater level of early-season colonization accounted for the yield losses observed with *T. basicola* in this study. The year 2006 had the lowest levels of root colonization (52% colonization) and also was the year with a *T. basicola*–*M. incognita* interaction that resulted in greater losses than *T. basicola* alone. This is similar to previous work. The other soil factor that may have impacted the level of black root rot observed in this study was the soil texture used in these studies. The base soil for this study was a silt-loam soil compared with a fine-sandy-loam soil that was used in the previous microplot studies (71% sand, 17% silt, and 12% clay) (37,39); a soil that would favor *T. basicola*.

The effect of soil texture on *T. basicola* was evident, with soils having 87% sand content exhibiting lower root colonization and reproduction of the *T. basicola* in both 2006 and 2008 compared with other soil textures. The effects of soil texture on *T. basicola* were also evident for root weight. The interaction between soil texture and *T. basicola* for root weight early in the 2008 season or midseason in 2007 showed that the fungus had no effect on root weight for soil textures with a high sand content. Buchanan (4), using a benomyl-resistant isolate of *T. basicola*, reported that the isolation frequency of *T. basicola* from cotton decreased in field soil using the same pathogen population as sand content increased to 76% compared with soil textures with soils with lower sand contents from the same field. Monfort (22) observed that populations of *T. basicola* were lower in areas of a field with the greatest sand content (50.1 to 81.7% sand) compared with other classifications of 22 to 50% sand.

M. incognita also affected plant growth early in the season but the nematode effects were more consistent on plant growth later in the season, when soil temperatures were warmer. *M. incognita* reduced early-season plant height and number of nodes only in

Table 6. Main effects of soil texture (sand) and *Meloidogyne incognita*^x (Mi) on percent root colonization by *Thielaviopsis basicola*^y

Main effect	2006	2007	2008
Sand (%)			
48	57.0 a ^z	95.0 a	86.2 a
53	61.2 a	80.0 a	19.2 b
70	57.5 a	92.5 a	8.4 b
74	60.8 a	86.1 a	88.3 a
87	23.6 b	80.0 a	6.2 b
Mi			
0	63.5 a	88.9 a	45.5 a
4	45.0 a	83.3 a	43.6 a
8	48.2 a	76.8 a	37.7 a

^x Soils were infested at planting with 4 or 8 eggs and second stage juveniles (J2) of *M. incognita*/g of soil.

^y Soils were infested at planting with 20 chlamydospore chains of *T. basicola*/g of soil. Root discoloration and colonization: 0 = none, 1 = 1–10%, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, 9 = 81–90%, and 10 = 91–100%. Analyses were conducted using midpoint values. Treatments without *T. basicola* were dropped from the analyses.

^z Means in a column for a year and main effect followed by a common letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

Table 7. Main effects and interaction of soil texture (soil) and *Meloidogyne incognita*^w (Mi) on *Thielaviopsis basicola* populations^x

Main effect	Midseason			Harvest			Preplant				
	2006	2007	2008	2006	2007	2008	2007	2008	2009		
							Mi				
							0-----4-----8				
Sand (%)											
48	2.8 a ^y	2.5 bc ^y	2.6 a ^y	4.0 a ^y	2.8 ab ^y	2.6 a ^y	1.6 bc ^z	3.2 a	3.2 a	2.7 a ^y	5.3 a ^y
53	2.5 a	3.0 a	1.6 b	5.1 a	2.5 bc	2.5 a	0.0 d	2.4 a	3.2 a	2.6 a	5.2 a
70	2.5 a	2.9 ab	1.7 bc	4.1 a	2.8 a	2.2 b	3.2 a	3.1 a	3.05 a	2.7 a	5.0 a
74	2.2 a	2.9 ab	2.7 a	4.8 a	2.7 ab	2.6 a	3.1 a	3.1 a	3.1 a	2.8 a	4.8 a
87	1.4 b	2.3 c	1.1 c	4.1 a	2.4 c	1.6 c	1.1 c	2.3 ab	2.3 a	2.5 a	3.6 b
Mi											
0	2.4 a	2.7 a	1.7 a	4.3 a	2.7 a	2.1 a				2.7 a	4.6 a
4	2.3 a	2.7 a	2.1 a	4.7 a	2.5 a	2.3 a				2.5 a	5.0 a
8	2.1 a	2.7 a	2.1 a	4.3 a	2.7 a	2.4 a				2.7 a	4.8 a

^w Soils were infested at planting with 4 or 8 eggs and second stage juveniles (J2) of *M. incognita*/g of soil.

^x Soils were infested at planting with 20 chlamydospore chains of *T. basicola*/g of soil. Data transformed, $\log_{10}(x + 1)$. Treatments without *T. basicola* were dropped from the analyses.

^y Means for a year and main effect followed by a common letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

^z Interaction means for any sand and Mi combination followed by the same letter are not significantly different, Fisher's protected least significant difference ($P \leq 0.05$).

2008, which was the warmest year early in the season. By midseason, when the soils were warmer, the nematode lowered all growth measurements in both 2007 and 2008. At harvest, the presence of *M. incognita* delayed initiation of the first sympodial fruiting branch in all 3 years and suppressed plant height, boll number, and seed cotton in 2007 and 2008. *M. incognita* is a warm-season, chronic pathogen that would likely become increasingly severe later in the growing season, when soils temperatures are warmer (38).

Soil texture had little effect on severity of the *M. incognita* over the range of sand content in this study. Soil textures with greater sand contents enhanced root galling in 2008. Koenning et al. (17) observed that the reproduction of *M. incognita* was greater in soils with 91% sand than in soils with sand contents of 48 or 53%, and that the population density of the nematode was negatively related to the percent of clay and silt. The clay content in soils used by Koenning et al. (17) was greater (29 to 30%) than the clay content in the soil used in this study (2%) and may explain why few differences in populations of the nematode were found in this study. In surveys in the lower Rio Grande Valley in Texas, Robinson et al. (29) reported that *M. incognita* was associated with sandy-loam and silt-loam soils and, in a similar survey from across Texas, *M. incognita* rarely occurred in soil with greater than 40% clay (33). Monfort et al. (24) documented that *M. incognita* population densities at planting and the soil sand content explained from 65 to 86% of the variation seen in cotton yield, depending on the year. O'Bannon and Reynolds (27) also reported greater losses from *M. incognita* in cotton in loamy-sand and sandy-loam soils, with no losses in a silt loam or loam when comparing fumigated and non-fumigated soils.

Soil texture had some impact on plant growth in this study, with root weight being reduced at soil textures of 74 and 87% sand and height being less at 87% sand. Seed-cotton yields also were often the lowest for these soil textures. Water was managed for each soil texture in this study. As plant development progressed through the season, the amount of water added if rainfall did not occur may not have been sufficient to move into the lower soil profile, resulting in plant water stress and accounting for these soil texture effects. In the field, Monfort et al. (24) suggested that differences in plant growth and yield may have reflected changes in soil factors such as water and nutrient availability for growth, where these inputs are applied uniformly across the field. Water management by soil texture may partially explain why damage was less across the soil textures in this microplot study.

Interactions between *T. basicola* and *M. incognita* were not consistently detected in the 3 years of this study but the effects were consistent whenever they did occur. An interaction between the fungus and the nematode for seedling height and root weight and for yield occurred in 2006, the year in which severity of nematode galling was the lowest and *T. basicola* caused little damage in the absence of the nematode. Disease severity was greater when the interaction occurred than where either pathogen was present alone, as has been shown in previous studies (37–39). In the previous microplot studies, *T. basicola* and *M. incognita* did not cause appreciable damage by themselves and the interaction between *M. incognita* and *T. basicola* was observed (37,39). Nematode infection did not increase root discoloration or colonization of *T. basicola* in these studies. Root galling was, however, reduced by the presence of *T. basicola* in 2007 at both early and midseason. The 2007 season was also the year with the greatest colonization of roots by the fungus. Soil conditions after planting that were favorable for *T. basicola* may have allowed the fungus to colonize and damage the root tissue sufficiently to lower the suitability of the host for the nematode. *T. basicola* affected harvest populations of *M. incognita* all 3 years, with populations being influenced by a significant soil texture–*T. basicola* interaction in 2 of the 3 years. Nematode populations were reduced by *T. basicola* in 2006 for all textures below 87% sand and the 48% sand soil in 2008. However, this did not affect spring populations of the nematode.

No consistent soil texture–*T. basicola*–*M. incognita* interaction was observed for plant development or yield. The most important

texture effect appears to be limiting the activity of *T. basicola* at increased sand contents. Previous work examining the role of soil texture on potato early dying caused by *Pratylenchus penetrans* and *Verticillium dahliae* did not find that the disease complex was affected in Wooster silt loam, Spinks fine sand, and Rifle peat in microplots, even though *P. penetrans* caused more yield loss in the sandy soil (7). *M. incognita* and *T. basicola* are widely distributed in Arkansas cotton fields at levels that are capable of affecting plant growth and yield (24,31). However, the presence of these pathogens in the soil may not be the limiting factor for yield due to environment. These results support previous studies showing that *T. basicola* and *M. incognita* distribution in cotton fields is influenced by sand content (22,24). Population densities of *T. basicola* are more likely to be present in areas with lower sand contents, while soils with greater sand contents would be more conducive to larger populations of *M. incognita*. Areas with concomitant infestation by both pathogens may increase disease severity and damage to cotton, because these pathogens are known to interact in a synergistic manner (37–39). Management of *M. incognita* using chemical nematicides may be an important strategy to reduce the damage caused whenever these two pathogens are present in the same soil (24). The use of triazole fungicides to suppress *T. basicola* may also minimize losses (34). Because soil texture is a stable soil property, and the association of the nematode population densities to sand particle distribution in soils remains similar over the years, as demonstrated by Monfort (24), soil textural maps may be useful for making management decisions. The use of field textural maps in the management of *M. incognita* also may allow growers to include site-specific nematicide application for anticipating areas where losses from this nematode–fungus interaction would increase losses under a favorable environment.

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