

## Survey of *Meloidogyne incognita* and *Thielaviopsis basicola*: Their Impact on Cotton Fruiting and Producers' Management Choices in Infested Fields

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**Abstract:** A survey of 100 cotton fields selected randomly in 1995 and 1996 was conducted in the High Plains of Texas to determine the incidence and potential severity of *Meloidogyne incognita* and *Thielaviopsis basicola*. Information was obtained from producers for each field on their nematicide application rates and fungicide seed treatments. The percent of squares and bolls set was evaluated for 20 plants in each field during late July 1995 and early August 1996. *Thielaviopsis basicola* was identified in 55% of the fields in 1995 and 73% of the irrigated fields in 1996. *Meloidogyne incognita* was found in 39% and 43% of the fields in 1995 and 1996, respectively. Both *M. incognita* and *T. basicola* were found together in approximately 30% of the fields. The average rate of aldicarb used in 1995 and 1996 was higher when fields were infested with both *T. basicola* and *M. incognita* than for fields infested with none or only one of the pathogens. However, there was no relationship between the use of fungicide treatments active against *T. basicola* and the potential for root necrosis, or presence of either or both pathogens (*T. basicola* and *M. incognita*). Aldicarb rates and usage of fungicide seed treatments were chosen by producers before fields were surveyed (i.e., the survey did not influence grower practices). In 1995, but not in 1996, the association of *M. incognita* and potential root necrosis (based on a bioassay from soil samples) was negatively correlated with the number of squares, percentage of squares set, and percentage of bolls set. The association between *M. incognita* and *T. basicola*, or potential severity of root necrosis, could not be correlated with fruit attributes in 1996 under warm spring conditions but was negatively correlated with fruit attributes in 1995 under cool spring conditions.

**Key words:** cotton, disease survey, management practices, *Meloidogyne incognita*, *Thielaviopsis basicola*.

Cotton is grown on 1.2 to 1.4 million ha in the High Plains of Texas each year, accounting for about 12% of the total agricultural cash receipts for the state in 1996 (Anonymous, 1996). Soilborne diseases, such as black root rot, caused by *Thielaviopsis basicola* (Berk. & Broome) Ferraris and the root-knot nematode (*Meloidogyne incognita* (Kofoid & White) Chitwood) limit yield in this region (Minton and Garber, 1983; Orr and Robinson, 1984). While no surveys have been published on the incidence of fields with *T. basicola* in this region, *M. incognita* has been found in 40% of the hectareage on the High Plains (Robinson et al., 1987).

Damage caused by *M. incognita* on cotton can result in a reduction of plant height and

suppress fruit set (Smith et al., 1991; Thomas and Smith, 1993; Veech and Starr, 1986). Preplant damage thresholds in cotton as low as 50 to 100 *M. incognita* eggs/500 cm<sup>3</sup> soil have been reported (Starr et al., 1989). *Thielaviopsis basicola* colonizes the cortical tissue of cotton seedlings, resulting in a taproot that is reduced in diameter and shriveled, a delay in plant growth because the necrotic epidermal and cortical tissues must first be sloughed off before lateral roots can be produced, and reduced yield (Minton and Garber, 1983; Kaufman et al., 1998). Black root rot is more severe when soil temperatures are <24 °C (Blank et al., 1953; Rothrock, 1992).

Management of *M. incognita* can involve host resistance, crop rotation, or use of nematicides. There are no root-knot nematode-resistant cultivars adapted for the short growing season that exists in the High Plains of Texas. There is, however, some crop rotation practiced with peanut, which is a non-host for *M. incognita* (Kirkpatrick and Sasser, 1984). Management of root-knot nematodes with nematicides is the primary method of control in cotton in this region. Nonfumi-

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gant nematicides that are currently labeled for use as in-furrow applications at planting on cotton include aldicarb and fenamiphos.

There are no management strategies that are highly effective for control of black root rot of cotton. Crop rotation with corn or grain sorghum may be somewhat effective by not allowing the fungus to reproduce (Johnson, 1916). There are no cultivars available with resistance to the pathogen. Fungicide seed treatment using products shown to have activity against *T. basicola*, (myclobutanil (Butler et al., 1996) and triadimenol (Arthur, 1996)) may suppress black root rot. Most of the seed planted in the High Plains of Texas before 1998 was saved by producers from the previous crop. The producers selected the fungicides, which were then applied at an acid-delinting facility. This was in contrast to most areas of the United States where producers purchased all their seed already commercially treated. The practice among Texas growers of saving seed is now on the decline since the introduction of transgenic cultivars adapted to the High Plains region in 1997–1998. The objectives of this study were to determine the: (i) incidence and potential severity of *T. basicola* and *M. incognita* in the High Plains of Texas, (ii) frequency and type of chemical control routinely used by producers for disease control, and (iii) impact of these pathogens both alone and in combination on cotton fruit development.

#### MATERIALS AND METHODS

*Survey:* One hundred cotton fields in the High Plains of Texas were selected at random in both 1995 and 1996, using line intercept sampling (Gates, 1979) from county maps. In 1995 fields were selected regardless of irrigation practices, and five were chosen in each of 20 counties. In 1996, only irrigated fields were randomly selected unless the county was almost entirely dryland production (95% of the surveyed fields were irrigated). The number of fields per county in 1996 were not equal. Counties with a higher incidence of *M. incognita* based on the 1995 results had more fields selected in 1996

(maximum of eight fields and minimum of three fields per county). A questionnaire was sent to the producers of the surveyed fields to determine nematicide usage and rates, seed treatments, and irrigation practices. Fields sampled in 1995 were excluded from the 1996 survey.

*Fruit development:* Twenty plants were examined along a transect across each field, and the following attributes were measured: number of squares, number of missing squares, number of bolls, and number of missing bolls. The percentage of squares and bolls set was then calculated. Fruiting information was collected during the last week in July 1995 and during the first week of August 1996.

*Soil samples and assays:* Soil samples for nematode and *T. basicola* assays were taken in the first 2 weeks of August both years. Each field was divided into thirds, and a composite soil sample was taken from each third. Composite soil samples consisted of 20 soil cores, with 10 paces (6 to 9 m) between each soil core. A core of soil consisted of 50 cm<sup>3</sup> taken at a 10 to 20-cm depth near the taproot of a plant. Soil was assayed for plant-parasitic nematodes using two methods—one to extract second-stage juveniles (J2) (pie-pan method, Thistlethwayte, 1970) and one to extract eggs of root-knot nematode (Hussey and Barker, 1973). Eggs were extracted by adding 2 liters of water to 500 cm<sup>3</sup> of soil and root fragments, stirring for 15 seconds, and allowing 15 seconds for settling before pouring the water and organic matter over a 0.23-mm-pore sieve. The eggs were extracted with sodium hypochlorite from the residue collected on the sieve.

Detection of *T. basicola* was based on a bioassay where the seedling disease pathogens in the soil were baited by planting susceptible cotton seeds. In 1995, soil from each sample was placed into a pot (Stuewe and Sons, Corvallis, OR) holding 110 cm<sup>3</sup> of soil and planted with two seeds of cotton cv. Paymaster HS-26. Plants that emerged were rated on a scale of 0 = healthy, 1 = 1 to 20% root necrosis, 2 = 21 to 50% root necrosis, and 3 = >50% root necrosis. Necrotic root tissue was microscopically examined at ×400

for the presence of chlamydospores of *T. basicola*. In addition, in 1995 soil was assayed with a dilution series on a medium that is semi-selective for *T. basicola* (Specht and Griffin, 1985). Soil (10 cm<sup>3</sup>) was placed in 90 ml of water and stirred. Ten ml was removed and stirred in 90 ml of water, and 1-ml aliquots were pipeted into petri plates that contained the selective medium that was warm and still molten, forcing the *T. basicola* colonies to grow through the medium. Plates were stored under cool conditions (<22 °C) for 21 days, and then colonies of *T. basicola* were identified and counted.

The bioassay was modified in 1996. Each soil sample was divided among four pots, which were each planted with two seeds with one of the following treatments: no fungicide, metalaxyl (0.14 ml a.i./kg seed), carboxin-PCNB (0.33 ml a.i./chemical/kg seed), or myclobutanil (0.2 ml a.i./kg seed). Metalaxyl is selectively active against *Pythium* spp. (Nelson, 1988), carboxin-PCNB is active against *R. solani* (Borum and Sinclair, 1968), and myclobutanil is active against both *R. solani* and *T. basicola* (Butler et al., 1996). Plants in each pot were rated for percent emergence and root necrosis as in 1995, and the presence of *T. basicola* was confirmed by microscopic examination and identification of chlamydospores on roots. Pots were maintained under cool (17–20 °C), wet conditions (watered to saturation every other day) for three weeks during both 1995 and 1996 before evaluating seedlings for root necrosis.

*Analyses:* The entire data set was used to calculate incidence of *T. basicola* and *M. incognita* and determine usage rates or frequencies of nematicide and fungicide seed treatments. Correlation analysis was conducted using only those fields that had either *T. basicola* or *M. incognita*. Fields with neither pathogen were eliminated from the analyses. Correlation analysis was used to relate fruit attributes to *M. incognita* log<sub>10</sub> transformed density + 1 (LMi); presence or absence of *T. basicola* (Tb); colony-forming units of *T. basicola*/cm<sup>3</sup> soil (Tbsoil) which was done only in 1995; root necrosis rating (NEC) from the bioassay; the association be-

tween root-knot nematode density and presence or absence of *T. basicola* (LMi × Tb); association between LMi and Tbsoil (LMi × Tbsoil); and the association between LMi and severity of black root rot based on the bioassay (LMi × NEC). Correlations were considered significant at  $P \leq 0.05$ .

*Weather data:* Climatic information was obtained from U.S. Department of Agriculture records at six locations across the High Plains (Big Spring, Seminole, Brownfield, Crosbyton, Levelland, and Tulia).

## RESULTS

*Growing season:* In 1995, planting conditions were cool and average minimum air temperature collected at the six weather locations during May ranged from 9 to 13 °C. The cool temperatures continued into June, with average minimum air temperature of 14 to 17 °C. In July and August, temperatures were high, and water was limited, with most regions receiving <2.5 cm of precipitation in July and <5 cm in August. Yields in 1995 ranged between 204 and 611 kg of lint/ha across the surveyed region and averaged 397 kg/ha (Anonymous, 1996).

Temperatures during May 1996 were warmer, with average minimum air temperature ranging from 14 to 19 °C, and rainfall was variable, ranging from 0.25 to 4.57 cm. During June, average minimum air temperature ranged from 17 to 21 °C with variable rainfall (0 to 9.9 cm). Yields from the 20 counties were much higher in 1996 than in 1995 and ranged from 236 to 1,037 kg of lint/ha, with an average yield of 666 kg/ha (Anonymous, 1996).

*Pathogen assays:* *Thielaviopsis basicola* was identified in 55% of the fields in 1995 and 73% of the fields in 1996 based on microscopic identification of *T. basicola* from bioassays. In 18% of the soil samples collected in 1995, no plants emerged from the bioassay, so the presence of *T. basicola* could not be determined and is probably underestimated in 1995. Soil assays were conducted on all samples in 1995 for *T. basicola*, but the assays were less sensitive to the presence of *T. basicola* than the bioassays. Only 34% of

the fields were identified with *T. basicola* based on soil assays, with an average density of 8.6 propagules/cm<sup>3</sup> soil and a range of 0 to 120 propagules/cm<sup>3</sup> soil. Root necrosis ratings averaged 1.3 in both 1995 and 1996, and only 21 to 26% of the fields were completely free of the potential for root necrosis in both years (Fig. 1A). *Meloidogyne incognita* was found in 39% of the fields in 1995 and 43% of the irrigated cotton fields in 1996. *Meloidogyne incognita* density ranged from 0 to 177,700 eggs + J2/500 cm<sup>3</sup> soil (averaged across three soil samples per field). Of the root-knot nematode infested fields, approximately one half of the fields had moderate to high potential for causing yield losses (>500 eggs + J2/500 cm<sup>3</sup> soil) (Fig. 1B). Approximately 25% of the fields had neither *T. basicola* nor *M. incognita*, 40 to 45% of the fields had either *T. basicola* or *M. incognita* (but not both), and approximately 30% of the fields had both pathogens present (Fig. 1C).

In the bioassays during 1996, plants from seed not protected against *T. basicola* had an average root rating of 1.06, and ratings for plants from seed treated with myclobutanil averaged 0.74. Plant emergence from untreated seed averaged 30% (standard deviation [SD] = 28), while seed protected against *Pythium* with metalaxyl averaged 67% emergence (SD = 20) and seed protected against *R. solani* with carboxin-PCNB averaged 50% emergence (SD = 30). Seed protected against both *R. solani* and *T. basicola* averaged 56% emergence (SD = 25).

*Management practices:* Aldicarb was the only nematicide used by producers in the survey, and half of the producers did not use a nematicide (Fig. 1D). Approximately 17% of the producers that used aldicarb indicated that they used rates of <0.57 kg a.i./ha. In 1995 and 1996, 30 and 50% of the producers, respectively, did not apply aldicarb in fields with *M. incognita* densities that were <500 eggs + J2/500 cm<sup>3</sup> soil. In fields where the density of *M. incognita* was >500 eggs + J2/500 cm<sup>3</sup> soil, 27 to 38% of the producers did not apply aldicarb.

Seed treated with fungicides that were active against *T. basicola* was planted in 26% of the fields in 1995 and 23% of the fields in

1996 (Fig. 1D). In 43 to 54% of the fields with *T. basicola*, no fungicide seed treatment with activity against this pathogen was applied (Fig. 1E). Where fields had a combination of *T. basicola* and *M. incognita*, a higher rate of aldicarb was generally applied than for fields with *M. incognita* alone (Fig. 1F). Use patterns of aldicarb appeared to be related to the presence of both *M. incognita* and *T. basicola*, but use patterns of seed treatment fungicides that are active against *T. basicola* were not related to either the presence or density of this pathogen or to the presence of both *T. basicola* and *M. incognita*.

*Fruit development as affected by pathogens:* *Meloidogyne incognita* population density was negatively correlated with the number of squares per plant and positively correlated with the number of bolls per plant (Table 1). There was no correlation between *M. incognita* population density and fruit production in 1996.

The presence or absence of *T. basicola* in fields was not correlated with fruit production in either year (Table 1). However, the density of *T. basicola* propagules/cm<sup>3</sup> soil was negatively correlated with squares/plant in 1995 (Table 1). Root necrosis ratings from the bioassays were not correlated with any fruit measurements in 1995 or 1996 (Table 1).

The association of the two pathogens, which was represented in several different ways (LMi × Tb, LMi × Tbsoil, and LMi × NEC), was negatively correlated with either squares/plant, percent squares set, or percent bolls set in 1995 (Table 1). The combination of root-knot nematode density and root necrosis rating (LMi × NEC) was also correlated positively with bolls/plant in both years (Table 1).

*Management practices:* Aldicarb rate was negatively correlated with the percent of squares set and positively correlated with the number of bolls/plant in 1995 (Table 1), but the rate of aldicarb used was not correlated with fruit attributes in 1996. Aldicarb rate was positively correlated with root-knot nematode density in 1995 (Table 2) but not in 1996. However, the aldicarb rate used by

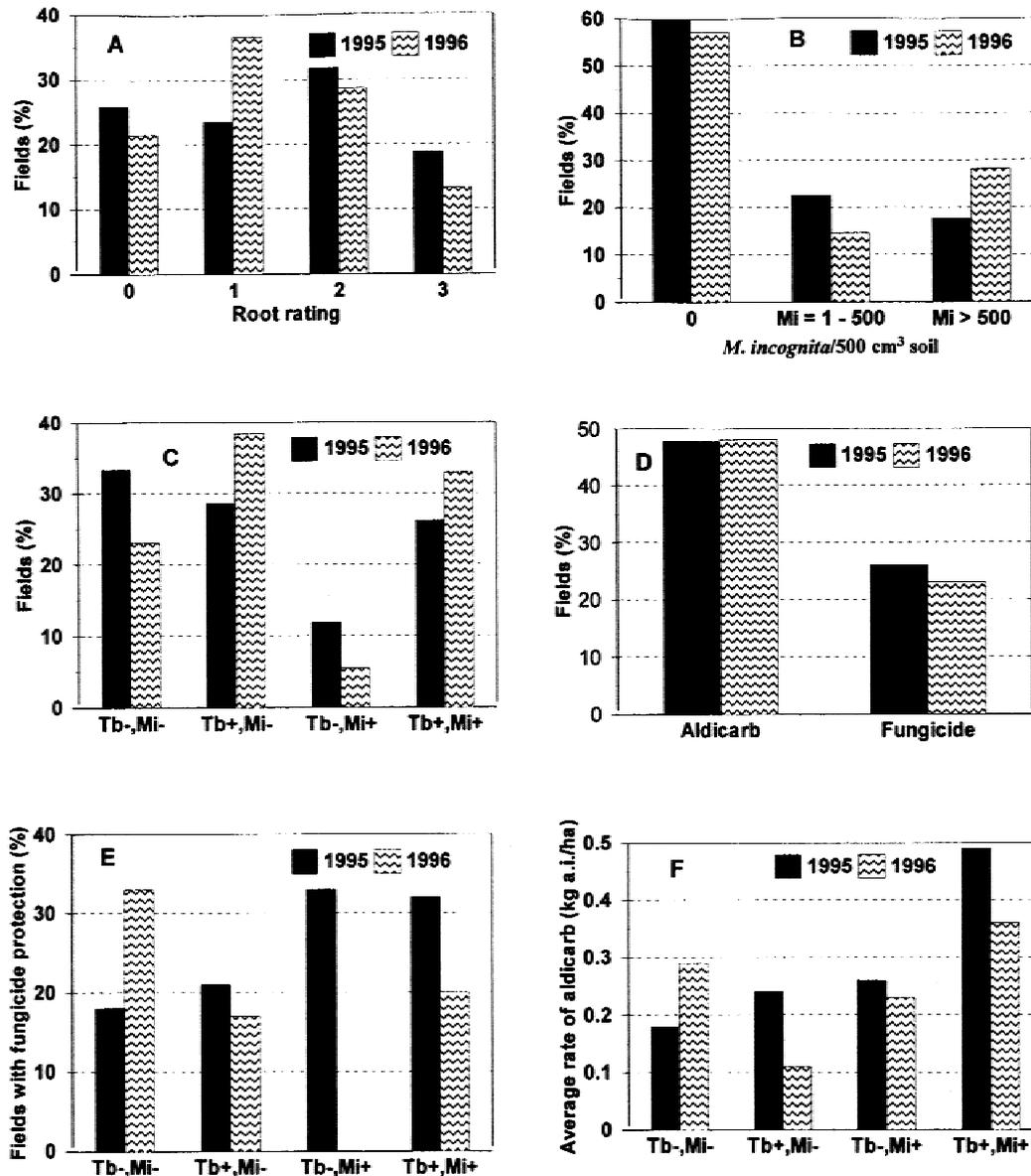


FIG. 1. Summary of fields surveyed during 1995 and 1996 (100 fields/year) for *Meloidogyne incognita* (Mi), *Thielaviopsis basicola* (Tb), and chemical controls practiced by producers in the High Plains of Texas: A) The incidence of fields with black root rot potential, based on a root necrosis index obtained from cotton seed not protected against *T. basicola*, where 0 is no root necrosis, 1 = 1–20% root necrosis, 2 = 21–50% root necrosis, and 3 = >50% root necrosis. B) The incidence of fields with *M. incognita* divided into categories of none, low density (1–500 eggs + second-stage juveniles [J2]/500 cm<sup>3</sup> soil), and moderate to high density (>500 eggs + J2/500 cm<sup>3</sup> soil). C) The incidence of fields with different combinations of *T. basicola* and *M. incognita*. D) The incidence of fields where producers chose aldicarb and a fungicide seed treatment with activity against *T. basicola*. E) The relationships between *M. incognita* and *T. basicola* presence or absence and fungicide seed treatment usage by producers. Fungicide seed treatments were rated as 0 or 1 depending on activity against *T. basicola*. F) The relationships between *M. incognita* and *T. basicola* presence or absence and average aldicarb rate.

producers was correlated in both years to the association between *M. incognita* and *T. basicola* (Table 2). Fungicide seed treat-

ments were not correlated to fruit attributes pathogen severity, or the association between pathogens in either year.

TABLE 1. Correlation coefficients for cotton fruiting parameters, pathogens, and management attributes from a survey of 100 cotton fields in 1995 and 1996.

Pathogen and management attributes <sup>a</sup>	Squares/plant		% Squares set		Bolls/plant		% Bolls set	
	1995	1996	1995	1996	1995	1996	1995	1996
LMi	-0.32** <sup>b</sup>				0.50***			
Tb								
Tbsoil	-0.31*							
Necrosis								
LMi × Tb			-0.46**					
LMi × necrosis	-0.39**		-0.41**		0.33*	0.22*	-0.29*	
LMi × Tbsoil	-0.33**							
aldicarb rate			-0.26*		0.38**			
Tb seed treatment								

<sup>a</sup> LMi is  $\log_{10}(Mi + 1)$ , where Mi is *Meloidogyne incognita* eggs + second-stage juveniles/500 cm<sup>3</sup> soil; Tb is *Thielaviopsis basicola*, represented as present or absent from a field (1 or 0); Tbsoil are the colony-forming units of *T. basicola*/cm<sup>3</sup> soil, assayed only in 1995; Necrosis is root necrosis rating of bioassay cotton seedlings on a scale of 0 (no disease) to 3 (>50% root necrosis); Tb seed treatment is application of a fungicide seed treatment active against *T. basicola* (1) or no treatment applied with activity against *T. basicola* (0).

<sup>b</sup> Significant correlation coefficients are listed. Blanks indicate coefficients that were not significant at  $P = 0.05$ . Asterisks indicate significance level: \* $P = 0.05$ , \*\* $P = 0.01$ , and \*\*\* $P = 0.001$ .

DISCUSSION

The cool spring temperatures in 1995 favored black root rot development. Limited rainfall, particularly in July and August, may have contributed to severe damage by root-knot nematode. Water stress can impact the ability of plants to tolerate root-knot nematode (Kirkpatrick et al., 1995). Temperatures in 1996 were less conducive to black root rot. The greater impact of the presence of both *M. incognita* and *T. basicola* on plant fruiting characteristics in 1995 was likely due to an interaction between these pathogens. Walker et al. (1999) found that fresh weight of 42-day-old cotton seedlings was significantly lower when both pathogens were combined than when either pathogen occurred alone. The effects were more pronounced at 20 and 24 °C than at 28 °C. The cooler spring temperatures in 1995 may have been more conducive for this interaction than the warmer spring in 1996, where the effects of both pathogens on plant fruiting were much less obvious. Cotton yield was significantly lower, and number of days to the first cracked boll were significantly higher, for the combination of *M. incognita* and *T. basicola* than for the pathogens alone in a microplot study (Walker et al., 1998). Any situation that results in a delay in maturity (i.e., delay in the first cracked boll)

would be especially serious in a region with a short growing season, such as the High Plains of Texas.

In our survey, square and boll shedding may have been, in part, a response to the interaction of *M. incognita* and *T. basicola*. When water stress reaches -190 MPa, small boll retention declines, and at -250 MPa squares can be shed, resulting in a reduction of yield potential (Hake and Kerby, 1996).

TABLE 2. Correlation coefficients between *Thielaviopsis basicola* (Tb), *Meloidogyne incognita* (Mi), and the rate of aldicarb applied by producers.

Pathogens <sup>a</sup>	Rate of aldicarb	
	1995	1996
LMi	0.37** <sup>b</sup>	
Tb		
Tbsoil	0.38**	
Necrosis		
LMi × Tb	0.48***	0.30*
LMi × Necrosis	0.49***	0.31*
LMi × Tbsoil	0.42***	

<sup>a</sup> LMi is  $\log_{10}(Mi + 1)$ , where Mi is *Meloidogyne incognita* eggs + second-stage juveniles/500 cm<sup>3</sup> soil; Tb is *Thielaviopsis basicola*, represented as present or absent from a field (1 or 0); Tbsoil is the colony-forming units of *T. basicola*/cm<sup>3</sup> soil, assayed only in 1995; Necrosis is root necrosis rating of bioassay cotton seedlings on a scale of 0 (no disease) to 3 (>50% root necrosis).

<sup>b</sup> Significant correlation coefficients are listed. Blanks indicate coefficients that were not significant at  $P = 0.05$ . Asterisks indicate significance level: \* $P = 0.05$ , \*\* $P = 0.01$ , and \*\*\* $P = 0.001$ .

The interaction between *M. incognita* and *T. basicola* could increase water stress to a greater degree than for each pathogen alone due to severe root damage. Cotton plants infected by *M. incognita* alone often show symptoms of water-deficit stress (O'Bannon & Reynolds, 1965). When soil moisture was allowed to fall to -30 kPa before application of irrigation, then *M. incognita*-infected plants had more pronounced symptoms of drought stress than uninfected plants (Kirkpatrick et al., 1995). Irrigated cotton in the High Plains of Texas is deficit-irrigated due to limited water and high evapotranspiration rates.

Cotton producers in the High Plains of Texas generally applied higher rates of aldicarb in fields where both *M. incognita* and *T. basicola* were present than in fields where either of the pathogens were found alone. It is likely that interaction between these pathogens, frequently resulting in severe disease, increased their awareness of problems in these fields. There was no indication from our survey, however, that growers were more apt to use a fungicide seed treatment with activity against *T. basicola*—even in fields where both pathogens were found together. The reasons that growers were more inclined to use nematicides rather than fungicide seed treatment to minimize disease effects in their problem fields are unclear. One possibility may be that detection of nematodes is relatively straightforward and may be more familiar to producers, or that root-knot problems are more easily diagnosed by producers in the field. It is also possible that nematode suppression using aldicarb or other nematicides may also suppress disease severity due to the interaction between the pathogens. Conversely, detection of *T. basicola* and diagnosis of black root rot is more difficult and may be less familiar to growers. It is also possible that relatively minimal visible plant response to seed treatment with fungicides may mask the effect of the treatment, although recent studies indicate that seed treatment with materials active against *T. basicola* resulted in a 12% improvement in yield in the High Plains (Kaufman et al., 1998). More research is needed

to fully define the impact of the interaction between the root-knot nematode and *T. basicola* in cotton and to evaluate the efficacy of both nematicide and fungicide use for control of both pathogens.

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