Evaluation of Several Approaches to Manage *Meloidogyne incognita* and Cotton Seedling Disease Complexes in the High Plains of Texas

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Abstract: Field experiments were conducted for control of the southern root-knot nematode (*Meloidogyne incognita*) and cotton seedling disease fungi (primarily *Thielaviopsis basicola*) in one naturally infested field during 1999 and 2000 and in three additional fields in 2000. Treatments included: seed-applied fungicides (triadimenol + mefenoxam + thiram and carboxin + PCNB + mefenoxam), cultivars (Paymaster [PM] 2326 RR and PM 2200 RR), and a nematicide (aldicarb at 0.83 kg a.i/ha). Plant stands were higher (P = 0.02) in the presence of aldicarb (77% emergence) than in its absence (74% emergence). Hypocotyl disease symptom ratings were lower (P = 0.0001) following triadimenol + mefenoxam + thiram seed treatment (0.53) as compared with carboxin + PCNB + mefenoxam (0.93). Root necrosis was lower (P = 0.002) following triadimenol + mefenoxam + thiram seed treatment (27%) as compared with carboxin + PCNB + mefenoxam (34%). In one field, in both years, aldicarb was associated with more root necrosis (58%) than in its absence (46%) (P = 0.004). At three other sites aldicarb did not affect root necrosis. Population densities of *Meloidogyne incognita* eggs and juveniles at midseason were greater (P = 0.005, P = 0.003, respectively) on PM 2200 RR (less resistant) than on PM 2326 RR (more resistant). Yield was affected by the plant genotype by aldicarb interaction (P = 0.02) but not by seed treatments. Aldicarb effect on yield was dependent on cultivar, whereas affect of seed treatment on root health was consistent and independent of cultivar and aldicarb. No conditions were identified when use of triadimenol + mefenoxam was detrimental. *Key words:* aldicarb, cotton, fungicides, *Meloidogyne incognita*, root-knot nematode, seedling disease, *Thielaviopsis basicola*.

Cotton seedling diseases caused greater losses (2.8% average) than other cotton diseases in the United States from 1952 to 2001 (Cotton Pest Loss Database, 2002). The pathogen complex associated with cotton seedling diseases includes Pythium spp., particularly P. ultimum, Rhizoctonia solani, and Thielaviopsis basicola. Pythium spp. and R. solani are associated with seed rot and pre- and post-emergence damping-off (Minton and Garber, 1983). Because Thielaviopsis basicola rots and blackens roots and portions of the hypocotyl below the soil line, the disease name "black root-rot" is used. Cool soil temperatures increase the severity of black root-rot (Blank et al., 1953; Rothrock, 1992). The southern root-knot nematode, Meloidogyne incognita, can act synergistically with cotton seedling pathogens, such as *R. solani* and *T.* basicola, to increase disease (Carter, 1981; Reynolds and Hansen, 1957; Starr et al., 1989; Walker et al., 1999, 2000).

The approaches for management of cotton seedling disease complexes involving *M. incognita* include fungicides (seed treatments and in-furrow applications at planting), in-furrow nematicide application, and the use of cultivars with resistance. While there have been numerous studies on individual approaches for management of seedling disease complexes, there is limited information on the efficacy of combinations of these individual tactics, particularly for the combination of *T. basicola* and *M. incognita*.

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The efficacy of seed treatment fungicides for improvement of plant stand and root health has been demonstrated in field trials (Hillocks et al., 1988; Kaufman et al., 1998; Minton et al., 1982; Wang and Davis, 1997; Wheeler et al., 1997). Systemic fungicides with efficacy against *R. solani* include azoxystrobin, carboxin (Borum and Sinclair, 1968), fludioxonil, myclobutanil (Butler et al., 1996), and triadimenol (Arthur, 1996). Metalaxyl and its *R*-enantiomer, mefenoxam, are systemic fungicides with efficacy against *Pythium* (Davis, 1997; Howell, 2002). Systemic fungicides with some efficacy against *T. basicola* are myclobutanil and triadimenol (Arthur, 1996; Butler et al., 1996; Kaufman et al., 1998).

Soil-applied nematicides can be used to control the southern root-knot nematode. Aldicarb is a nonfumigant nematicide that affects *Meloidogyne* hatch and movement (Hough et al., 1975; Hough and Thomason, 1975). Experiments between *M. incognita* (+/-) and certain soilborne fungi (*Fusarium oxysporum* f. sp. vasinfectum, R. solani, T. basicola) (+/-) demonstrated that less fungal disease occurred on cotton when *M. incognita* was absent than when it was present (Carter, 1981; Starr et al., 1989; Walker et al., 2000). Even partial control of *M. incognita* with a non-fumigant nematicide increased cotton yield by more than 20% in the presence of the wilt pathogen, *F. oxysporum* f. sp. vasinfectum (Colyer et al., 1997).

Cotton production areas where *M. incognita* and *T. basicola* occur together include the southern High Plains of Texas (Wheeler et al., 2000) and Arkansas (Rothrock, 1997; Rothrock et al., 2002). Both pathogens can be found in California, New Mexico, and Arizona, but their coexistence in cotton fields has not been documented. In the southern High Plains, approximately 73% of the irrigated cotton acreage was infested with *T. basicola*, and more than 30% of the irrigated cotton acreage was infested with both *M. in*-

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cognita and *T. basicola* (Wheeler et al., 2000). In Ashley County, Arkansas, 75% of the surveyed fields contained *T. basicola* (Rothrock et al., 2002). Management of these disease complexes may require more than a single tactic. It is unknown whether the interaction between *M. incognita* and *T. basicola* can be modified by using a combination of control practices, particularly when control is only partially effective for each pathogen. The objective of this research was to evaluate a combination of control practices for management of these pathogens.

MATERIALS AND METHODS

Commercial cotton fields in the High Plains of Texas were selected for tests in 1999 and 2000 based on seedling disease and nematode history, isolations from or examination of cotton seedlings collected from the field, or seedlings grown in a growth chamber using soil collected from the field.

All sites had coarsely textured soils with a history of black root-rot and southern root-knot nematode. All sites were planted in cotton in the year previous to the test except for site 3. Treatments were evaluated in 2000, with one exception. Sites 1 and 4 were the same field but tested in 1999 and 2000, respectively. This site, located in Terry County, had a history of both severe black root-rot and a high population density of M. incognita. Cotton roots were examined microscopically in 1998 to confirm the presence of T. basicola-like clamydospores and galls typical for root-knot nematode. The soil was a Tokio fine sandy loam (fine-loamy, mixed, active, thermic Calcidic Haplustalfs). Site 2, in Terry County, was a Patricia loamy fine sand (fineloamy, mixed, superactive, thermic Aridic Paleustalfs) with a history of black root-rot symptoms. Both Pythium and R. solani were isolated at low frequency from seedlings at this site in 1998, and the presence of T. basicola was confirmed on root samples microscopically. Site 3, in Hockley County, was an Amarillo fine sandy loam. Site 3 had a history of severe stand problems under wet conditions. Both R. solani and Pythium spp. were isolated from diseased seedlings in 1997, and T. basicola was identified microscopically. Meloidogyne incognita was found at moderate-to-high population densities in 1997. In 1999, this site was planted to peanut, which is not a host for M. incognita (Johnson et al., 2000; Kirkpatrick and Sasser, 1984). Site 5, in Gaines County, was a Brownfield fine sand (loamy, mixed, superactive, thermic Arenic Aridic Paleustalfs). The history of this field was unknown at the time of the study. However, 120 soil samples taken during January 2000 and planted with fungicide-free cotton seed had stand problems under wet, cool conditions. Plants that emerged were colonized with T. basicola (determined microscopically). Galls were also present on roots from the cotton stalks still present in the field.

The treatments evaluated at these sites included seed treatment fungicides, cultivars, and soil treatment with and without aldicarb at planting. Two fungicide combinations were evaluated, thiram + mefenoxam + triadimenol (31 + 8 + 10 g a.i./100 kg seed, respectively) and $\operatorname{carboxin} + \operatorname{PCNB} + \operatorname{mefenoxam} (66 + 66 + 8 \text{ g a.i.}/100$ kg seed, respectively). Fungicides were obtained from Gustafson LLC (Plano, TX) and applied to non-treated seed obtained from Delta Pine & Land Co. (Lubbock, TX) using a Hege 11 Seed Treater (Hege Maschinen GmbH, Waldenburg, Germany). The cultivars were 'PM 2200 RR', whose recurrent parent, 'PM HS-200', supports a rapid population increase of M. incognita, and 'PM 2326 RR', whose recurrent parent, 'PM HS-26', supports a relatively slower population increase of M. incognita (Robinson et al., 1999; Wheeler and Gannaway, 1998). The nematicide, aldicarb, was applied at 0.0 or 0.83 kg a.i./ha at planting. The producers supplied nematicide applicators for each test. Applicators were calibrated using an electric motor attached to the drive shaft of the planter (AG Products, Davis Junction, IL). Plots were 2 rows wide (0.9 to 1-m spacing), 11.3 m long, and planted at 16 seeds/m row. All treatments were arranged as a complete factorial in a randomized complete block design with four replications, under center pivot irrigation. Acephate was applied at 204 g a.i./ha for thrips control, once plant stand was established (approximately 21 days after planting).

Four weeks after planting, plant stand was evaluated in one row and six plants were removed from three locations along the other row of each plot. Roots were washed and rated for severity of hypocotyl lesions and percentage root necrosis. The hypocotyl rating scale was: 0 = no lesion, 1 = superficial lesion, 2 = sunken lesion, and 3 = lesion killing the plant. A composite soil sample, consisting of five cores taken to a depth of 15 cm with a narrow-bladed shovel, was removed from plots at midseason and assayed for plant-parasitic nematodes. Site 1 was sampled on 20 July 1999. Sites 2 through 5 were sampled on 19, 14, 18, and 28 July 2000, respectively. Second-stage juveniles (J2) of M. incognita were assayed using a modified Baermann funnel (Thistlethwayte, 1970). Eggs were extracted from sieved organic matter with 0.525% NaOCl (Hussey and Barker, 1973) from soil prepared as described in Wheeler and Kaufman (2003). Yield was recorded for each plot using a two-row cotton stripper (John Deere 482) modified to have a small cage to catch the cotton (lint, seed, and trash) and equipped with load cells (Rice Lake Weighing Systems, Model RL20000A-100, Rice Lake, WI 54868). A sample of harvested cotton from two of the four replications for each treatment was ginned to determine the percentage of lint.

Data were analyzed with SAS (SAS Institute, Cary, NC), using the general linear model (PROC GLM). The independent variables were replication, seed treatment, nematicide, cultivar, and site. All interactions

were included in the model statement. A variable was considered significant when the *F*-test for that particular variable was significant at $P \leq 0.05$.

RESULTS

There was no interaction between site and any other independent variable for plant stand, hypocotyl rating, root-knot nematode population density at midseason, and yield; therefore, data from all sites were combined for further analysis (Table 1). However, there was an interaction (P = 0.05) between aldicarb application and site for root necrosis; thus, each site was analyzed individually for this parameter. There was also an interaction (P = 0.02) between aldicarb and cultivar with respect to yield. For all other combinations involving field, aldicarb, seed treatment, and cultivar, only the main effects were significant. There was no evidence of seed rot or post-emergence damping-off at any of the sites. Plant stands, hypocotyl ratings, root necrosis, rootknot nematode reproduction, and yield differed significantly (P = 0.0001) between sites (Tables 1,2). Thielaviopis basicola was observed on necrotic portions of roots sampled from all sites, although site 3 had only low levels of root necrosis (Table 2). Sites 2 and 5 had moderate levels of root necrosis (20% to 26%, Table 2), and sites 1 and 4 had the greatest level of root necrosis (35% to 67%). Root-knot nematode population density at midseason was high for sites 1, 3, and 4 (8,048 to $20,503 \text{ eggs}/500 \text{ cm}^3$ soil) and moderate for sites 2 and 5 (1,376 to 1,483 eggs/500 cm³ soil) (Table 1). Site 3 had the highest midseason density of M. incognita, yet had been planted in peanut the previous season (Table TABLE 2. Effect of the interaction between aldicarb and site, and aldicarb and cultivar on root necrosis and yield (kg lint/ha).

Variable ^a	Root necrosis (kg a.i	Yield aldicarb (kg a.i./ha)		
	0	0.83	0	0.83
Site 1	35 c ^b	48 b		
Site 2	26 d	24 d		
Site 3	4 e	5 e		
Site 4	$56 \mathrm{b}$	67 a		
Site 5	20 d	20 d		
PM 2326 RR			$569 \mathrm{b}$	672 a
PM 2200 RR			636 a	660 a

^a PM = Paymaster.

 $^{\rm b}$ Mean separations were based on the Waller-Duncan k-ratio t-test (P=0.05).

1). A wheat cover crop was planted after the peanuts were harvested. There was no evidence of leaf damage by thrips at any site.

Aldicarb: Plant stand averaged over all sites after 4 weeks was higher (P=0.02) with aldicarb present (77%, 12.8 plants/m row) than when aldicarb was absent (74%, 12.3 plants/m row). Root hypocotyl symptoms were not affected by aldicarb application. There was an interaction between site and effect of aldicarb on root necrosis (Table 2). At sites 2, 3, and 5 aldicarb application had no effect on root necrosis. At sites 1 and 4 (which was the same site tested in 1999 and 2000), there was greater root necrosis (P=0.0007) in the presence of aldicarb (58%) than in its absence (46%). There was no effect of aldicarb treatment on density of eggs or J2 at midseason. There was an aldicarb × cultivar effect on yield (Table 2). PM 2326 RR yielded less (569 kg lint/ha) than PM 2200RR (636 kg lint/ha) in

TABLE 1. Influence of fungicide seed treatment, aldicarb, cultivar, and site on plant stand, root health, *Meloidogyne incognita* population density, and yield (kg lint/ha).

Variable ^a	Plant stand (%)	Hypocotyl rating ^b	Root necrosis (%)	Root-knot nematode ^c		
				J2	Eggs	Yield
Fungicide 1	76 a ^d	0.53 b	27 b	680 a	9,322 a	558 a
Fungicide 2	75 a	0.93 a	34 a	574 a	7,960 a	575 a
P =	ns	0.001	0.002	ns	ns	ns
Aldicarb-	$74 \mathrm{b}$	0.73 a	inter ^e	576 a	8,861 a	inter
Aldicarb +	77 a	0.73 a	inter	678 a	8,410 a	inter
P =	0.02	ns	0.05	ns	ns	0.02
PM 2326RR	$74 \mathrm{b}$	0.68 a	28 b	411 b	6,442 b	inter
PM 2200RR	77 a	0.78 a	33 a	845 a	10,860 a	inter
P =	0.04	ns	0.03	0.005	0.005	0.02
Site 1	86 a	1.4 a	inter	825 b	11,552 b	972 a
Site 2	65 c	0.8 b	inter	19 c	1,376 d	274 e
Site 3	76 b	0.4 d	inter	1,244 a	20,503 a	530 d
Site 4	68 c	0.6 c	inter	947 ab	8,048 c	800 b
Site 5	83 a	0.4 d	inter	81 c	1,483 d	594 c
P =	0.0001	0.0001	0.05	0.0001	0.0001	0.000

^a Variable included two fungicide treatments: 1 = triadimenol + mefenoxam + thiram at 31 + 8 + 10 g a.i./100 kg seed and 2 = carboxin + PCNB + mefenoxam at 66 + 66 + 8 g a.i./100 kg seed); aldicarb (0 vs. 0.83 kg a.i./ha); cultivar (PM = Paymaster), and site.

^b The hypocotyl disease rating scale was: 0 = no lesion; 1 = superficial lesion; 2 = sunken lesion; and 3 = lesion killing the plant.

^c Plots were sampled at midseason and densities are per 500 cm³ soil. J2 = second-stage juveniles.

^d Different letters indicate significant differences (*P*=0.05) among treatment means, within a variable group. For site, the Waller-Duncan k-ratio t-test was used. ^e Inter = interaction. Aldicarb rate × site had a significant affect on root necrosis, so individual treatment means are not presented and aldicarb rate × cultivar had a significant affect on yield. the absence of aldicarb (P = 0.004). In the presence of aldicarb, yield of these two cultivars was not significantly different (672 vs. 660 kg lint/ha, respectively) (Table 2).

Cultivar: Plant stand of PM 2200 RR after 4 weeks was higher (P = 0.04) (77%, 12.8 plants/m row) than that of PM 2326 RR (74%, 12.3 plants/m row). Hypocotyl ratings were similar between the two cultivars. Mean root necrosis of PM 2200 RR (33%) was greater (P = 0.03) than that of PM 2326 RR (28%). There was a greater ($P \le 0.005$) population density of *M. incognita* associated with PM 2200 RR (10,860 eggs and 845 J2/ 500 cm³ soil) than with PM 2326 RR (6,442 eggs and 411 J2/500 cm³ soil). The effect of cultivar on yield was described in the aldicarb section.

Seed-applied fungicides: There was no difference between the two seed treatment fungicides on emergence after 4 weeks. Hypocotyl damage was less (P = 0.0001) with the triadimenol + thiram + mefenoxam seed treatment, which resulted in an average rating of 0.53, than with the carboxin + PCNB + mefenoxam seed treatment, which resulted in an average rating of 0.93. Root necrosis with the triadimenol + thiram + mefenoxam seed treatment was 27%, which was less (P = 0.002) than with the carboxin + PCNB + mefenoxam seed treatment, which was 34%. Root-knot nematode population density and yield were not affected by seed treatment.

DISCUSSION

The goal of this research was to test an integrated approach to managing cotton fields with both T. basicola and M. incognita. The most influential factor affecting yield was aldicarb, when applied to PM 2326 RR. Since thrip injury was not observed at any of the sites, the yield increase is mainly attributed to nematode control. PM 2326 RR was more resistant to M. incognita reproduction than PM 2200 RR; however, PM 2326 RR also appeared to be more sensitive to yield loss by M. incognita. Yield increased by an average of 103 kg of lint/ha, or by 18% for PM 2326 RR, by the addition of aldicarb. However, PM 2200 RR which allowed greater reproduction of M. incognita, did not have any significant yield response to aldicarb treatment. Neither cultivar used in this study had resistance to M. incognita similar to that of 'Stoneville LA887' or 'Acala NemX' (Garber and Oakley, 1996; Jones et al., 1990; Zhou et al., 2000). Tolerance to *M. incognita* and resistance are not equivalent (Barker, 1993), and in this study the cultivar supporting the most reproduction, PM 2200 RR, was also more tolerant of nematode damage.

A seed treatment combination with triadimenol, which is active against *T. basicola*, reduced severity of root and hypocotyl symptoms caused by this pathogen but did not have an effect on yield. The improvement in root health was small for both hypocotyl ratings (a difference of 0.4 on a 0-to-3 scale) and root necrosis

(7% difference). This difference, while statistically significant, may be too trivial for plant growth differences. When seed treatments have significantly impacted yield in the presence of *T. basicola* (Kaufman et al., 1998), root necrosis differences between treatments were greater. The better seed treatment for root health in the presence of *T. basicola* was triadimenol + thiram + mefenoxam. Since seed treatment effects were additive with aldicarb or cultivar, there were no conditions identified where use of this seed treatment was detrimental.

Aldicarb at planting was shown to reduce the severity of another disease, Fusarium wilt, in soil where M. incognita was present (Colyer et al., 1997). Fusarium wilt also interacts with M. incognita (Starr et al., 1989). In our study, aldicarb increased yields by an average of 18% for one cultivar in the presence of both T. basicola and M. incognita. However, no reduction in root necrosis was associated with this yield increase. There is no evidence that aldicarb reduced the severity of root necrosis. At one site in both years, aldicarb presence was associated with an increase in root necrosis. The improvement in yield for cotton infected with both M. incognita and T. basicola is probably due to effects on M. incognita and may have no impact on the interaction between T. basicola and M. incognita. Management of T. basicola and M. incognita using partial control methods like aldicarb and seed treatment appeared to work in an additive manner. This is in contrast to the effect of aldicarb and cultivar, where there was a negative interaction (aldicarb plus PM 2200 RR resulted in no additional control). The following recommendation was developed as a result of this work for cotton fields infested with both T. basicola and M. incognita: When using a cultivar tolerant to M. incognita, a seed treatment active against T. basicola is recommended, but aldicarb is not recommended for nematode control. When a root-knot nematode sensitive cultivar is grown, then both a seed treatment active against T. basicola and aldicarb is recommended. One outcome of this work is basing aldicarb recommendations on cultivar sensitivity to M. incognita rather than resistance (i.e., nematode reproductive ability).

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