Journal of Nematology 36(2):160–166. 2004.

Interaction of *Rotylenchulus reniformis* with Seedling Disease Pathogens of Cotton[1]

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Abstract: The impact of 10 *Fusarium* species in concomitant association with *Rotylenchulus reniformis* on cotton seedling disease was examined under greenhouse conditions. In experiment 1, fungal treatments consisted of *Fusarium chlamydosporum, F. equiseti, F. lateritium, F. moniliforme, F. oxysporum, F. oxysporum* f.sp. *vasinfectum, F. proliferatum, F. semitectum, F. solani,* and *F. sporotrichioides; Rhizoctonia solani;* and *Thielaviopsis basicola.* The experimental design was a 2 × 14 factorial consisting of the presence or absence of *R. reniformis* and the 12 fungal treatments plus two controls in autoclaved field soil. In experiment 2, the same fungal and nematode treatments were examined in autoclaved or non-autoclaved soil. This experimental design was a 2 × 2 × 14 factorial consisting of field or autoclaved soil, presence or absence of *R. reniformis,* and the 12 fungal treatments plus two controls. In both tests, *Fusarium oxysporum* f. sp. *vasinfectum, F. solani, R. solani,* and *T. basicola* consistently displayed extensive root and hypocotyl necrosis that was more severe ($P \leq 0.05$) in the presence of *R. reniformis.* Soil treatment (autoclaved vs. non-autoclaved) influenced the impact of the *Fusarium* species on cotton seedling disease, with disease being more severe in the autoclaved soil. *Rotylenchulus reniformis* reproduction on cotton seedlings was greater in field soil compared to autoclaved soil ($P \leq 0.05$). This study suggests the importance of *Fusarium* species and *R. reniformis* in cotton seedling disease.

Key words: Cotton seedling disease, *Fusarium* species, *Gossypium hirsutum, Rhizoctonia solani, Rotylenchulus reniformis, Thielaviopsis basicola.*

The reniform nematode, *Rotylenchulus reniformis,* is an economically important pathogen on cotton (Lawrence and McLean, 2001). In Alabama, *R. reniformis* is reported to be the most economically damaging nematode on cotton, accounting for 8% of the total yield loss in 2002 (Gazaway and McLean, 2003). *Rotylenchulus reniformis* has spread rapidly, infesting cotton hectares across the state. Since it was first reported on cotton in Georgia in 1940, *R. reniformis* has been detected in every cotton-producing state from North Carolina to Texas and north to Missouri (McLean and Lawrence, 2000; Robinson et al., 1997).

Several studies have concluded that *Fusarium* species are the most common fungi colonizing healthy and diseased cotton tissues in Mississippi, Louisiana, and Texas (Colyer, 1988; Roy and Bourland 1982; Zhang et al., 1996;). *Fusarium solani, F. oxysporum, F. moniliforme, F. semitectum, F. gramenearum,* and *F. equiseti* are reported to be active participants in the cotton seedling disease complex with varying degrees of virulence (Colyer, 2001). A recent survey of cotton in Alabama reported that 10 species of *Fusarium* colonize tissues of cotton, including several species associated with seedlings (Palmateer et al., 2003). These fungi were often isolated from fields infested with *R. reniformis.*

Plant-pathogenic nematodes and fungi often synergistically interact (Walker and Rothrock, 1999). A classic example of such interaction on cotton is that of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *vasinfectum,* where wilt symptoms are more severe in the presence than in the absence of *M. incognita. Rotylenchulus reniformis* also increased the incidence of *Fusarium* wilt in greenhouse experiments with wilt-susceptible, but not wilt-resistant, cotton varieties (Neal, 1954). Brodie and Cooper (1964) reported that cotton seedlings grown in soil infested with *R. reniformis* were susceptible to *Rhizoctonia solani* for a greater length of time than were seedlings grown in nematode-free soil. In contrast, Sankaralingham and McGawley (1994) indicated the severity of cotton seedling blight caused by *R. solani* was not influenced by *R. reniformis;* however, nematode reproduction was enhanced in the presence of the fungus.

Several *Fusarium* species are now being recognized as pathogens of cotton seedlings. Due to the high incidence of multiple *Fusarium* species associated with cotton in *R. reniformis*-infested fields, the relationship between these pathogens needs to be assessed. The first objective of this study was to determine the impact of selected *Fusarium* species alone and in concomitant association with *R. reniformis* on cotton seedlings, plant growth, and *R. reniformis* populations on cotton in the greenhouse. The second objective was to determine if autoclaving the soil influenced the severity of cotton seedling disease caused by *Fusarium* spp. and *R. reniformis.*

Materials and Methods

Experiment 1. The impact of *R. reniformis* and 10 *Fusarium* species on cotton seedling disease and plant development was examined under controlled conditions in the greenhouse in autoclaved soil. Fungal treatments consisted of *Fusarium chlamydosporum, F. equiseti, F. lateritium, F. moniliforme, F. oxysporum, F. oxysporum* f. sp. *vasinfectum, F. proliferatum, F. semitectum, F. solani,* and *F. sporotrichioides, Rhizoctonia solani,* and *Thielaviopsis basicola.* All fungal isolates were isolated from cotton in Ala-

Received for publication 11 August, 2003.

[1] Portion of a PhD dissertation submitted by the senior author as partial fulfillment for the PhD.

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The authors thank E. Sikora and G. W. Lawrence for manuscript review.

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This paper was edited by J. L. Starr.

bama. Pathogenicity of all fungal isolates was tested alone and in combination with *R. reniformis*. Two non-treated controls and a non-treated control with sterile millet, the growth medium for the fungal isolates, were also included. The treatment design was a 2 × 14 factorial consisting of *R. reniformis* (present or absent) and the 12 fungal treatments with two controls.

Experiment 2. To address the second objective, the same fungal and nematode treatments were evaluated in autoclaved and non-autoclaved field soil. The treatment design was a 2 × 2 × 14 factorial with two soils (field and autoclaved), *R. reniformis* (present or absent), and the 12 fungal treatments with two controls.

All experiments were conducted in the greenhouse at the Plant Science Research Center of the Alabama Agricultural Experiment Station, Auburn University, Alabama, between April and June in both 2001 and 2002. The physical layout in the greenhouse for both tests was an RCBD with five replicates, and the experiments were repeated two times. The ambient temperatures in all experiments ranged from 24 °C to 30 °C.

The soil, a Marvyn sandy loam (86% sand, 11% silt, 3% clay; pH 6.2), was collected in bulk from the Cullers rotation on the Auburn University campus. The soil was sterilized by autoclaving at 121 °C and 103.4 kPa for 2 hours on 2 consecutive days. For experiment 2, soil was collected and half was not autoclaved for the natural field soil treatments.

Populations of *R. reniformis* were maintained and increased on cotton cultivar PM 1218 BR in 10-cm-diam. polystyrene pots containing approximately 1.0 kg of soil. Nematode juveniles and vermiform adults were extracted from the soil by combined gravity screening and sucrose (specific gravity = 1.13) centrifugal flotation (Jenkins, 1964) and enumerated with a dissecting microscope.

Fungal isolates were increased on sterile millet seed in 250-ml flasks. Flasks were infested by adding a 5-mm-diam. plug from the periphery of 1-week-old cultures growing on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI). All flasks were incubated for 7 days at 25 °C under cool-light fluorescent illumination. Each flask was shaken daily to allow for thorough mixing of fungal propagules.

Five-gram portions of fungal inoculum were thoroughly mixed with 500 g of soil to obtain an inoculum concentration of 1% (w/w). Cotton seeds were surface sterilized in 70% ethanol for 10 seconds followed by 1.05% sodium hypochlorite for 5 minutes and then placed on sterile germination paper for 2 days. Polystyrene pots (10-cm diam.) were filled with infested soil, and PM 1218 BR cotton seedlings (radical length ~1 to 2 cm) were planted. A suspension of 2,000 *R. reniformis* juveniles and vermiform adults in 5 ml tap water was pipeted into two depressions, 2.5 cm deep and 1 cm wide, and then covered with soil to prevent dehydration.

Plant height was recorded at 14, 28, and 42 days after planting (DAP), whereas seedling survival, disease incidence, plant dry weight, and *R. reniformis* numbers were determined at 42 DAP. Plants with soil intact were carefully removed from pots and placed individually in 4-liter buckets containing 1,000 ml tap water to separate the roots from the soil. *Rotylenchulus reniformis* was extracted from the soil as previously described. Fresh weight of the cotton seedlings was recorded, and plants were scored for root and hypocotyl discoloration. The root and hypocotyl disease index (RHDI) was 0 = no necrosis, 1 < 33% seedling necrosis; 2 = 33% to 66% seedling necrosis; 3 ≥ 66% seedling necrosis; 4 = seedling taproot dead, proliferation of adventitious lateral roots above the dead area; and 5 = seedling dead (Colyer, 1988). A 5-mm section of root tissue was taken from each plant and aseptically plated according to previous methods (Palmateer et al., 2003) to confirm the identity of the fungi isolated from the lesions. Eggs of *R. reniformis* were then extracted from the root systems using NaOCl (Hussey and Barker, 1973). Following egg extraction, seedlings were placed in an oven at 55 °C for 48 hours and dry weights were recorded.

Seedling survival, height, disease severity, dry weight, and *R. reniformis* numbers were analyzed according to analysis of variance via the mixed model procedure of statistical analysis software (SAS Institute, Inc., Cary, NC). Blocks and associated interactions with treatment factors were considered to be random effects in the model. Interactions can complicate data interpretation in two ways: i) if they are truly significant and recognized as such, they force the experimenter to interpret data in terms of simple effects rather than main effects; or ii) if they are important but are not recognized as such, data interpretation will suffer. To guard against the latter, significance of interactions was tested at $P = 0.10$. This higher Type I error rate is an extension of the ideas put forth by Carmer (1976) and Carmer and Walker (1988) regarding risk assessment for means comparisons in crop performance trials. Least-square means for treatments were separated using Fisher's protected least significance difference and were calculated when appropriate.

Results

Experiment 1: Rotylenchulus reniformis differentially affected the cotton seedlings, seedling height, and dry weight as indicated by the interactions ($P \leq 0.05$) between select fungal species and *R. reniformis* (Table 1). The concomitant association of *R. reniformis* increased the RHDI severity ratings for 58% of the 12 fungal species examined. Cotton seedlings grown in soil infested with *R. reniformis* and *F. chlamydosporum, F. equiseti, F. oxysporum* f. sp. *vasinfectum, F. semitictum, F. solani, R. solani,* or *T. basicola* displayed greater RHDI values ($P \leq 0.05$) than seedlings grown in nematode-free soil

(Table 2). However, *F. moniliforme, F. oxysporum,* and *F. proliferatum* produced greater RHDI ratings ($P \leq 0.05$) in the absence of *R. reniformis* than when *R. reniformis* was present. The association of *Rhizoctonia solani* and *R. reniformis* produced the greatest RHDI ($P < 0.05$) compared with all other treatment combinations.

Cotton seedling height was affected by the association of selected fungal species and *R. reniformis* (Table 2). The presence of *R. reniformis* reduced plant height in both control treatments. *Fusarium lateritium, F. moniliforme, F. oxysporum* f. sp. *vasinfectum, F. solani, R. solani,* and *Thielaviopsis basicola* reduced plant height in the presence of *R. reniformis* as compared to the fungal treatment without *R. reniformis*. *Fusarium oxysporum* was the only fungus that reduced cotton seedling height ($P \leq 0.05$) when *R. reniformis* was absent.

Cotton seedling dry weight was reduced by eight of the 12 fungal species when combined with *R. reniformis*. *Fusarium chlamydosporum, F. oxysporum* f. sp. *vasinfectum, F. proliferatum, F. semitectum, F. solani, F. sporotrichioides, R. solani,* and *T. basicola* in association with *R. reniformis* reduced seedling dry weight ($P \leq 0.05$) as compared to the same fungal species without *R. reniformis* (Table 2).

Cotton seedling survival was not affected by *R. reniformis* (Table 1). Fewer cotton seedlings survived in all the fungal treatments ($P \leq 0.05$) when compared to the control plus millet except for the *F. oxysporum* treatment (Table 2). These fungi reduced seedling stand 17% compared to the controls.

Numbers of *R. reniformis* ranged from a high of 42,846 to a low of 1,987/500 cm^3 of soil in the control without millet and *T. basicola* treatments, respectively (Table 3). Plants in soil infested with *F. moniliforme, F. oxysporum* f. sp. *vasinfectum, R. solani,* and *T. basicola* supported fewer ($P \leq 0.05$) *R. reniformis* than the control with millet. Treatments with *T. basicola* reduced the *R. reniformis* RI value to 1, indicating the nematode was not increasing in numbers.

Experiment 2: The soil condition, autoclaved or natural, influenced RHDI, seedling height, dry weight, and survival. An interaction between selected fungi and *R. reniformis* was observed for RHDI and seedling height, as also observed in the first experiment (Table 1). Root hypocotyls disease index ratings were more severe for the same fungal species in association with *R. reniformis* as was observed in experiment 1 (Table 4). Soil condition affected the fungus-soil interaction and was observed for RHDI. In autoclaved soil, the RHDI ratings were greater ($P \leq 0.05$) for seedlings growing in the *F. moniliforme, R. solani,* and *T. basicola* treatments compared to the natural soil infested with the same fungi. However, *F. chlamydosporum, F. equiseti, and F. solani* produced greater RHDI ratings in natural soil as compared to autoclaved soil. In autoclaved soil, all fungal isolates reduced plant height ($P \leq 0.05$) as compared to natural soil infested with the same fungi.

Seedling dry weight was affected by a fungus × nematode × soil three-way interaction (Table 1). *Rotylenchulus reniformis* reduced seedling dry weight ($P \leq 0.05$) for control treatments in both autoclaved and natural soils (Table 5). In autoclaved soil, four and eight of the fungal species in *R. reniformis* infested and non-infested soil, respectively, reduced seedling dry weight ($P \leq 0.05$) as compared to the control plus millet. However, in field soil 2% and 11% of the fungal species in *R. reniformis* infested and non-infested soil, respectively, reduced seedling dry weight ($P \leq 0.05$) as compared to the control plus millet. *Thielaviopsis basicola* and *R. solani* were the only fungal treatments that resulted in lower ($P \leq 0.05$) seedling dry weight than the control

TABLE 1. Main and interaction effects (*P* values) for 'Paymaster 1218 BG/RR' cotton grown in pots infested with *Fusarium* species, *Rhizoctonia solani, Thielaviopsis basicola,* with or without *Rotylenchulus reniformis*.

Experiments 1[a]					
Source	Survival[b]	RHDI[c]	Height[d]	Dry weight[c]	*R. reniformis*[f]
Fungus	0.0001	0.0001	0.0001	0.0006	0.0001
Nematode	0.8402	0.8283	0.5084	0.0001	—
Fungus × Nematode	0.7779	0.0520	0.0555	0.0012	—
Experiments 2					
Source	Survival[b]	RHDI[c]	Height[d]	Dry weight[e]	*R. reniformis*[f]
Fungus	0.0001	0.0001	0.0001	0.0001	0.0046
Soil	0.1162	0.7243	0.0001	0.0001	0.0196
Nematode	0.8702	0.0419	0.0034	0.0006	—
Fungus × soil	0.0603	0.0577	0.0001	0.0002	0.8470
Fungus × Nematode	0.7449	0.0820	0.0122	0.0210	—
Soil × Nematode	0.2875	0.8232	0.1978	0.0030	—
Fungus × Soil × Nematode	0.1605	0.4114	0.4798	0.0930	—

[a] Experiment 1 and 2 analysis based on five replications in three and two trials, respectively; $P \leq 0.05$ are significant.
[b] Seedling survival at 42 days after planting.
[c] Mean root and hypocotyl disease index of 3 seedlings per species on a 0–5 scale, where 0 = no necrosis, 1 = <33%, 2 = ≥33% to <66%, 3 = >66% to ≤100% necrosis on roots or hypocotyls, 4 = dead taproot with proliferation of adventitious lateral roots above dead area, and 5 = dead plant.
[d] Seedling height in centimeters.
[e] Seedling dry weight in grams.
[f] Number of *R. reniformis* eggs, juveniles, and vermiform adults extracted at 42 days planting.

TABLE 2. Seedling height and root hypocotyl disease index of 'Paymaster 1218 BG/RR' cotton as influenced by *Fusarium* species, *Rhizoctonia,* and *Thielaviopsis basicola* with or without *Rotylenchulus reniformis (Rr)* on seedling disease of cotton.

Species	Seedling height (cm)		RHDI a		Dry weight		
	−*R. r*	+*R. r*	−*R. r*	+*R. r*	−*R. r*	+*R. r*	% Survival
Control	23.4	20.6	0.3	0.2	5.07	3.78	94.2
Control + millet	18.8	16.5	0.4	0.4	4.83	3.47	89.6
Fusarium chlamydosporum	21.1	21.3	0.3	0.8	4.12	3.87	77.5
Fusarium equiseti	19.7	20.2	0.6	0.8	4.11	4.29	78.7
Fusarium lateritium	18.8	17.6	0.8	0.7	3.77	4.00	69.2
Fusarium moniliforme	19.7	17.5	1.6	1.3	3.24	3.71	64.2
Fusarium oxysporum	16.9	18.8	1.0	0.8	4.35	4.32	88.4
Fusarium oxysporum f.sp. *vasinfectum*	20.8	17.7	1.0	1.5	3.77	3.49	80.0
Fusarium proliferatum	21.1	20.7	0.8	0.5	4.27	3.88	78.3
Fusarium semitectum	19.8	20.1	0.7	1.0	4.67	4.39	80.8
Fusarium solani	21.9	19.4	0.7	1.2	4.49	3.98	82.1
Fusarium sporotrichioides	20.8	20.8	0.8	0.9	4.25	3.98	76.7
Rhizoctonia solani	18.5	16.6	1.7	2.6	2.95	2.43	57.1
Thielaviopsis basicola	20.6	15.1	0.9	1.6	3.24	2.84	63.7
FLSD ($P \leq 0.05$)	0.5	0.5	0.2	0.2	0.2	0.2	7.5

Data are least square means of five replications in three trials. Least square means compared using Fisher's protected least significant difference (FLSD).
[a] Mean root and hypocotyl disease index of 3 seedlings per species on a 0–5 scale, where 0 = no necrosis, 1 < 33%, 2 = 33% to 66%, 3 = >66% necrosis on roots or hypocotyls, 4 = dead taproot with proliferation of adventitious lateral roots above dead area, and 5 = dead plant.

plus millet in *R. reniformis*-infested and non-infested natural soil. In field soil infested with *R. reniformis,* none of the *Fusarium* species resulted in lower ($P \leq 0.05$) seedling dry weight than the control.

Soil condition affected seedling survival; however, as in experiment 1 the presence of *R. reniformis* did not. Seedling survival was reduced by all fungal species ($P \leq 0.05$) when compared to both controls in field and autoclaved soils (Table 6). Autoclaved soil infested with *F. oxysporum* f. sp. *vasinfectum, F. semitectum, R. solani,* and *T. basicola* had fewer ($P \leq 0.05$) seedlings surviving than in field soil infested with these fungi. Only *F. moniliforme* killed more seedlings in field soil than autoclaved soil.

Results for nematode reproduction followed a similar trend, as observed in the first experiment (Table 3). Nematode numbers of *R. reniformis* ranged from a high of 44,648 for the control to a low of 3,445/500 cm^3 of soil also infested with *T. basicola.* Numbers of *R. reniformis* ($P \leq 0.05$) were lower in soil infested with all fungal species when compared to the non-infested soil. Soil infested with *F. moniliforme, R. solani,* and *T. basicola* supported fewer ($P \leq 0.05$) numbers of *R. reniformis* than the control with millet. Single degree-of-freedom

TABLE 3. Reproduction of *Rotylenchulus reniformis* on 'Paymaster 1218 BG/RR' cotton as influenced by *Fusarium* spp., *Rhizoctonia solani,* and *Thielaviopsis basicola.*

Species	Experiment 1[a]		Experiment 2[b]	
	Total *R. reniformis*/pot[c]	RI[d]	Total *R. reniformis*/pot[c]	RI[d]
Control	42846	21.4	44648	22.3
Control + millet	16361	8.2	11870	5.9
Fusarium chlamydosporum	35829	17.9	37744	18.9
Fusarium equiseti	22961	11.5	23169	11.6
Fusarium lateritium	31994	16.0	35909	18.0
Fusarium moniliforme	8741	4.4	6714	3.4
Fusarium oxysporum	28456	14.2	30658	15.3
Fusarium oxysporum f. sp. *vasinfectum*	12389	6.2	15763	7.9
Fusarium proliferatum	21457	10.7	20804	10.4
Fusarium semitectum	18201	9.1	18563	9.3
Fusarium solani	29412	14.7	27127	13.6
Fusarium sporotrichioides	21478	10.7	24283	12.1
Rhizoctonia solani	5587	2.8	3958	2.0
Thielaviopsis basicola	1987	1.0	3445	1.7
FLSD ($P \leq 0.05$)	1814		1052	

[a] Data are least square means of five replications in three trials.
[b] Data are least square means of five replications in two trials.
[c] Total eggs + vermiform *R. reniformis* per pot.
[d] RI (reproductive index) = final *R. reniformis* population/initial *R. reniformis* population.
Least square means compared using Fisher's protected least significant difference (FLSD).

TABLE 4. Seedling height and root hypocotyl disease index of 'Paymaster 1218 BG/RR' cotton as influenced by *Fusarium* species, *Rhizoctonia solani,* and *Thielaviopsis basicola* with or without *Rotylenchulus reniformis (Rr)* in autoclaved (A) and natural (N) soil on seedling disease of cotton.

Species	Seedling height (cm)				RHDI[a]			
	−R.r	*+Rr*	A	N	*−R.r*	*+Rr*	A	N
Control	20.3	19.5	22.0	22.1	0.1	0.3	0.0	0.5
Control + millet	17.5	17.7	15.9	19.4	0.1	0.1	0.3	0.5
Fusarium chlamydosporum	18.0	17.0	18.0	24.4	0.3	0.7	0.4	0.8
Fusarium equiseti	18.1	17.7	18.0	21.9	0.5	0.5	0.6	0.9
Fusarium lateritium	18.7	16.9	16.5	20.0	0.5	0.4	0.8	0.7
Fusarium moniliforme	18.3	16.8	14.0	23.2	1.3	0.9	1.8	1.1
Fusarium oxysporum	17.1	16.2	17.0	18.7	0.8	0.7	0.8	1.0
Fusarium oxysporum f.sp. *vasinfection*	17.8	17.0	14.5	24.0	1.0	1.5	1.3	1.2
Fusarium proliferatum	19.5	17.7	19.2	22.5	1.1	0.8	0.6	0.8
Fusarium semitectum	19.2	18.5	16.5	23.4	0.6	0.8	0.8	0.9
Fusarium solani	18.2	16.2	19.2	22.0	0.6	1.3	0.8	1.1
Fusarium sporotrichioides	12.6	11.2	19.7	21.9	0.5	0.6	0.7	0.9
Rhizoctonia solani	18.4	15.9	14.9	20.2	2.9	3.5	2.3	2.0
Thielaviopsis basicola	19.4	14.6	17.0	18.8	1.0	1.7	1.6	0.9
FLSD ($P \leq 0.05$)	0.4	0.4	0.5	0.5	0.2	0.2	0.2	0.2

Data are least square means of five replications in two trials. Least square means compared using Fisher's protected least significant difference (FLSD).

[a] Mean root and hypocotyl disease index of 3 seedlings per species on a 0–5 scale, where 0 = no necrosis, 1 < 33%, 2 = 33 to 66%, 3 = >66% necrosis on roots or hypocoytls, 4 = dead taproot with proliferation of adventitious lateral roots above dead area, and 5 = dead.

contrast indicated cotton seedlings supported greater numbers ($P \leq 0.05$) of *R. reniformis* in field soil than autoclaved soil, with mean *Rotylenchulus reniformis* numbers of 23,062 and 12,725/500 cm^3 of soil in field and autoclaved soil, respectively.

DISCUSSION

Rotylenchulus reniformis affected cotton seedling disease in both experiments. *Fusarium moniliforme, F. oxysporum* f. sp. *vasinfectum, F. solani, R. solani,* and *T. basicola* consistently induced extensive root and hypocotyl necrosis of cotton seedlings that was more severe and resulted in greater RHDI values in the presence of *R. reniformis*. These symptoms were most extensive with the combination of *R. solani* and *R. reniformis* and consisted of decayed taproots, where proliferation of lateral roots occurred above the necrotic area. These observations are consistent with a previous report that *R. reniformis* increased the incidence and severity of Fusarium wilt (Neal, 1954). In a previous study isolates of *F. oxysporum* f. sp. *vasinfectum* were shown to infect cotton seedlings, causing wilt and seedling death (Colyer, 2001).

Cotton seedling height and dry weight were reduced by *R. reniformis;* however, the presence of select seedling

TABLE 5. Seedling dry weight of 'Paymaster 1218 BG/RR' cotton as influenced by *Fusarium* spp., *Rhizoctonia solari,* and *Thielaviopsis basicola* with or without *Rotylenchulus reniformis* in autoclaved and natural soil on seedling disease of cotton.

Species	Autoclaved soil		Natural soil	
	− *R. reniformis*	+ *R. reniformis*	− *R. reniformis*	+ *R. reniformis*
Control	4.89	4.35	5.23	3.23
Control + millet	4.39	3.05	5.28	3.87
Fusarium chlamydosporum	4.06	3.23	4.15	4.49
Fusarium equiseti	4.31	3.70	3.90	4.91
Fusarium lateritium	3.14	3.07	4.40	4.93
Fusarium moniliforme	2.17	3.01	4.29	4.38
Fusarium oxysporum	3.65	3.73	5.05	4.93
Fusarium oxysporum f.sp. *vasinfectum*	3.31	2.47	4.21	4.50
Fusarium proliferatum	4.47	3.30	4.04	4.48
Fusarium semitectum	4.20	3.37	5.15	5.41
Fusarium solani	4.76	3.59	4.20	4.33
Fusarium sporotrichioides	3.79	2.95	4.70	5.02
Rhizoctonia solani	2.54	1.86	3.36	2.97
Thielaviopsis basicola	3.10	2.38	3.39	3.29
FLSD ($P \leq 0.05$)	0.2	0.2	0.2	0.2

Data are least square means of five replications in two trials. Least square means compared using Fisher's protected least significant difference (FLSD).

TABLE 6. Seedling survival of 'Paymaster 1218 BG/RR' cotton as influenced by *Fusarium* spp., *Rhizoctonia solari,* and *Thielaviopsis basicola* in autoclaved and natural soil in seedling disease of cotton.

Species	Autoclaved	Natural
Control	96.7	93.3
Control and millet	90.0	90.0
Fusarium chlamydosporum	76.7	73.3
Fusarium equiseti	78.3	76.7
Fusarium lateritium	70.0	70.0
Fusarium moniliforme	63.3	58.3
Fusarium oxysporum	89.2	88.3
Fusarium oxysporum f.sp. *vasinfectum*	77.5	85.0
Fusarium proliferatum	77.5	76.7
Fusarium semitectum	80.8	86.7
Fusarium solani	76.7	78.3
Fusarium sporotrichioides	81.7	83.3
Rhizoctonia solani	57.5	70.0
Thielaviopsis basicola	63.3	73.3
FLSD ($P \leq 0.05$)	3.5	3.5

Data are least square means of five replications in two trials. Least square means compared using Fisher's protected least significant difference (FLSD).

disease fungi enhanced the growth reduction. *Fusarium lateritium, F. moniliforme, F. solani, R. solani,* and *T. basicola* consistently reduced plant height in both field and autoclaved soils. *Rhizoctonia solani* further reduced seedling dry weight in the presence of *R. reniformis.* Similar results were reported in a previous study in which cotton seedlings grown in *R. reniformis*-infested soil were susceptible to *R. solani* far a longer period of time than when they were grown in nematode-free soil (Brodie et al., 1964). Prolonged susceptibility to *R. solani* was associated with reduction in seedling growth and susceptibility to *R. solani* was correlated with numbers of *R. reniformis* (Brodie and Cooper, 1964).

Several *Fusarium* species reduced seedling dry weight; however, soil condition influenced the effect of the *Fusarium* species. *Fusarium* species increased disease ratings and reduced seedling growth more in autoclaved soil. It is probable the microorganisms in field soil were competing with *Fusarium* species, consequently influencing their ability to elicit disease on cotton seedlings. This was most obvious for the fungus * soil interaction on seedling height in which all seedlings growing in soil infested with fungi were shorter in autoclaved soil than in field soil.

Cotton seedlings supported greater numbers of *R. reniformis* in field soil than autoclaved soil. The soil used in this study was obtained from a cotton field that was not infested with *R. reniformis.* The higher population density of *R. reniformis* in the field soil may be a result of minimal competition from other microorganisms since this soil was not naturally colonized by *R. reniformis.* A recent greenhouse study found soils of similar texture, autoclaved or field, varied in their ability to support populations of *R. reniformis* in a greenhouse (McLean and Lawrence, 2003). Populations of *R. reniformis* increased to greater numbers in field soil when compared to autoclaved soil in 25% of the soils tested.

A previous study involving *R. reniformis* on cotton reported that nematode reproduction was enhanced in the presence of *R. solani* (Sankaralingham and McGawley, 1994). Further, higher *R. reniformis* population densities were associated with cotton that was infected with *Verticillium dahlia* than when cotton was not infected with the wilt pathogen (Prasad and Padeganur, 1980). Our current findings show that seedling disease fungi had a negative impact on the reproduction of *R. reniformis.* The decrease in *R. reniformis* population density with seedling disease fungi present may be attributed to the availability of healthy root tissue, specifically as feeding sites for the nematode. Starr et al. (1989) examined the effects of *M. incognita* and *F. oxysporum* f. sp. *vasinfectum* on cotton and found *M. incognita* had no apparent influence on the fungus population in the soil, whereas the presence of the fungus did affect populations of the nematode. Walker et al. (2000) reported similar results in a study involving *M. incognita* and *T. basicola,* where reduced nematode populations were observed in the presence of the *T. basicola.* Our findings with select *Fusarium* species, *R. solani,* and *T. basicola* seem to parallel interaction studies with *M. incognita* on cotton and contradict previous reports involving *R. reniformis.*

In summary, the interaction of *Fusarium* species and *R. reniformis* on cotton has provided a greater understanding of soilborne pathogens involved in the seedling disease complex. *Rotylenchulus reniformis* increased cotton seedling disease severity caused by several *Fusarium* species, *R. solani,* and *T. basicola.* Natural field soil as a comparison to autoclaved soil provided a greater understanding of the interaction effect on cotton seedling disease, plant growth, and *R. reniformis* population densities than could be obtained with only autoclaved soil. Future studies should focus on the long-term effects of damage to the cotton plant caused by this pathogen interaction and the potential implications on yield in the field.

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