The Texas Journal of Agriculture and Natural Resources 25:46-56 (2012)

Effect of *Fusarium oxysporum* f. sp. *vasinfectum* Inoculum Density, *Meloidogyne incognita* and Cotton Cultivar on Fusarium Wilt Development

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ABSTRACT

A greenhouse experiment was conducted to evaluate the interactive effects of different *Fusarium oxysporum* f. sp. *vasinfectum* isolates at increasing inoculum densities with *Meloidogyne incognita* on partially resistant (Stoneville (ST) 4554B2RF) and susceptible (FiberMAX (FM) 9058F) cotton cultivars. Disease incidence and area under the disease progress curve (AUDPC) were significantly higher for FM 9058F compared to ST 4554B2F for all the *Fov* isolates, densities and *M. incognita* combinations. Differences in pathogenicity were observed among the *Fov* isolates tested, suggesting that variability in aggressiveness may exist in populations of the fungus, though all isolates were within the same Race grouping (Race 1). A total of four isolates showed significantly higher AUDPC in the absence of *M. incognita* at higher inoculum densities of *Fov*; while two isolates had significantly higher AUDPC in the presence of *M. incognita* at low inoculum densities. Plant growth differed between cultivars where FM 9058F plants were shorter, and had decreased root, shoot, and total plant weights compared to ST 4554B2RF. Plants inoculated with *M. incognita* had root galls and were stunted with reduced shoot weight and total plant weight. Management of Fusarium wilt can be substantially improved by using partially resistant cultivars or reducing the inoculum density of root-knot nematode.

KEY WORDS: *Gossypium hirsutum* L., root-knot nematode

INTRODUCTION

The Fusarium wilt – Root-knot nematode complex is an economically important disease of cotton (*Gossypium hirsutum* L.) in most cotton-growing regions of the world (Colyer et al., 1997). The disease complex is caused by a soil-borne fungus, *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *vasinfectum* (Atk.) W. C. Snyder and H. N. Hans (*Fov*), and the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood

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(DeVay et al., 1997). Since the first report of Fusarium wilt of cotton, in Alabama (Atkinson 1892), the disease has increased in importance (Davis et al., 2006) and is responsible for losses averaging $20 million each year across the cotton belt of the United States of America (Blasingame et al., 2008). Under conducive environmental conditions, high losses occur when susceptible cultivars are grown on heavily infested soil. Losses are greatest on sandy soils that are infested with *M. incognita* (DeVay et al., 1997). Losses due to Fusarium wilt of cotton vary depending upon the virulence of *Fov*, host resistance, environmental factors, soil type and fertility, and interactions with nematodes (Hao et al., 2009; Smith and Snyder 1975). Symptoms of Fusarium wilt appear earlier when densities of both *M. incognita* and *Fov* are increased (Garber et al., 1979).

There are eight races of *Fov* that have been described throughout the world, with Race 1 and Race 2 historically being the most prevalent in the United States (Kim et al., 2005). Recent studies have found that Races 1, 3, and 8 are mildly virulent and cause wilt symptoms in the presence of *M. incognita*. However, Race 4 of *Fov*, which was identified in California (Kim et al., 2005), is capable of causing severe wilt symptoms and economic loss in the absence of nematodes.

Fusarium oxysporum f. sp. *vasinfectum* density in soil and wilt incidence are correlated (DeVay et al., 1997; Starr et al., 1989). Development of varieties that have at least some ability to resist Fusarium wilt is important in managing this disease (Hillocks 1992). Understanding the virulence of local *Fov* isolates is critical in the development of a screening program. It is important to understand the effect of *Fov* inoculum density on incidence of wilt for cultivars that differ in their susceptibility to the disease. It is also important to determine how isolates of *Fov* may differ in aggressiveness to different genotypes of cotton, or more importantly, are some genotypes relatively resistant to a collection of isolates, or is there a cultivar × isolate interaction. Ideally, isolates should be selected for a breeding program that are fairly aggressive across both partially resistant and susceptible germplasm, but where the germplasm differences are still obvious. This may ultimately allow for separation between high levels of resistance (which have not been identified in commercial cultivars) and partial resistance in future screening efforts.

Initial symptoms of Fusarium wilt include chlorosis and necrosis of the leaf margins (Fig. 1A). Severely diseased plants can be killed as seedlings, or if they survive, may often remain stunted throughout the season (Fig. 1B). *Fov* invades the host through the taproots behind the root tip. The combined effect of fungal metabolites and the production of lipoidal substances by the host in response to infection may lead to the occlusion of the vascular tissues (Shi et al., 1992). The vascular system of plants exhibits discoloration due to systemic infection of the fungus. In most severely affected plants, leaves wilt and drop and the plants may die (Colyer 2001; Nelson 1981; Fig. 1). Plants that develop symptoms early usually die before producing any bolls, whereas plants that develop symptoms after the onset of flowering often survive but produce fewer bolls.

Chlamydospores are thick-walled specialized resting structures that remain dormant in the soil until exudates or leachates from plant roots stimulate their germination (Mai and Abawi 1987). The germinated chlamydospores produce hyphae that eventually form conidia and new chlamydospores if a suitable host is not found. Once a field is infested with *Fov*, the fungus usually persists indefinitely in the decaying plant tissues and soil as chlamydospores (Nelson 1981; Smith and Snyder 1975). The fungus is capable of surviving for over 10 years in the soil not planted to cotton (Smith et al., 2001). The ability of the pathogen to survive in soils for long periods has important consequences on disease management. Management options available for Fusarium wilt

are typically aimed at control of *M. incognita*, rather than *Fov*. These options include rotations with non-host crops for *M. incognita*, nematicides, and planting of nematode resistant cultivars (DeVay 1986). Use of rotations to reduce *Fov* has been of limited value because of its saprophytic ability (Smith and Snyder 1975). There are few economically viable crops for use by cotton producers in a rotation program due to the broad host range of *M. incognita*. Control of nematodes with nematicides has resulted in considerable decrease in Fusarium wilt and increased yield (Hyer et al., 1979; Jorgenson 1979; Smith 1948).

Figure 1a. Fusarium wilt symptoms. Chlorosis and necrosis of the leaf margin.

Figure 1b. Range of symptoms on field grown cotton.

Field observations indicate that use of a partially nematode resistant cultivar (Stoneville 5599BR) over a period of three years led to a substantial decrease in disease incidence (Wheeler, *personal communication*). A recent survey found that extensive genetic diversity exists in *Fov* populations in West Texas. However, all isolates collected to date were found to be characterized as Race 1 (Woodward, *unpublished*). The purpose of this study was to characterize the effects of *Fov* Race 1 isolates, their interaction with *M. incognita*, and cotton cultivars with varying levels of resistance to *Fov* on Fusarium wilt development.

MATERIALS AND METHODS

An experiment was conducted in the greenhouse during spring and fall of 2009. The experiment was designed as a split-split-split plot with four replications. Twelve isolates of *Fov* Race 1 served as the main plot, *Fov* inoculum densities (0, 2.5×10^3, 9.5×10^4, 6.5×10^5 colony forming units (cfu)/cm^3 of potting mix) served as sub-plots, two cultivars (Stoneville 4554B2RF (partially resistant), and FiberMax 9058F (susceptible)) served as sub-sub-plots and root-knot nematode densities (0 and 1,000 eggs/pot) served as sub-sub-subplots. Plastic containers (Stuewe & Sons, Tangent, OR) (30 cm height and 6 cm diameter at top) were filled with 700 cc soil mix/pot (70% sand, 25% top soil, and 5% peat moss). The number of eggs/pot represents the population of nematode eggs in a typical infested field soil. *Fov* inoculum was prepared from 3-week-old cultures maintained on Komada's Selective Medium (Komada 1975) and maintained at room temperature under continuous light. Petri dishes were flooded with water and conidia were scraped off the culture with a rubber spatula. The conidial suspension was then filtered through four layers of cheesecloth, quantified with the aid of a hemacytometer, and diluted with water to make the desired density for each *Fov* isolate. *Fov* inoculum was delivered using pipette inoculation technique (Latin and Snell 1986) into a potting mixture.

Root-knot nematodes were reared in a greenhouse on a susceptible tomato cultivar 'Homestead' and inoculum was extracted according to methods described by Hussey and Barker (1973). Soil was inoculated with *M. incognita* eggs (1,000/pot) to ensure root-knot nematode infestation and cotton (three seeds per cone) was planted. Plant densities were thinned to two plants per cone after three weeks.

Plant height and disease incidence were measured four weeks after planting. Disease incidence was observed every five days after the appearance of the first disease symptom. The percent disease incidence was rated on the following scale: 0% - no symptoms, 50% - chlorosis, necrosis, and wilting of one plant, and 100% - chlorosis, necrosis, and wilting of both plants. After 12 weeks of planting, plant growth was scored by measuring height, fresh root weight, and fresh shoot weight before termination of the experiment in each season. Root galling was also noted at this time to confirm the presence or absence *M. incognita*. Vascular discoloration was examined from cross and longitudinal section of the stem at the soil line and then positive samples were placed on to Petri dishes containing potato dextrose agar media to confirm *Fov* presence. Area under the disease progress curve (AUDPC) was calculated for quantitative disease assessment using repeated disease incidence as described by Shaner and Finey (1977).

Plant height, AUDPC, root weight, shoot weight, and total plant weight were analyzed using Proc MIXED (SAS Institute Inc., 2008, Ver. 9.2, Cary, NC, USA). The method used to adjust the degrees of freedom (df), to match adjustments in the sums of square, was the Satterthwaite option in the LSMEANS statement. Standard error and LSD values were determined from the PDIFF option.

RESULTS AND DISCUSSION

Interaction of *Fov* Race 1 isolates, their inoculum densities, *M. incognita* densities, and cotton cultivars was significant ($P \leq 0.0001$) for AUDPC. FM 9058F (susceptible cultivar) had significantly ($P \leq 0.05$) higher AUDPC than ST 4554B2RF (partially resistant cultivar) (Fig. 2) which indicated the importance of planting a partially resistant cultivar. *Fov* isolates 3, 4, 5, 6, 7, 10, 11, and 12 had significantly ($P \leq 0.05$) higher AUDPC with FM 9058F than isolates 1, 2, 8, and 9 (Fig. 2) suggesting that variability in aggressiveness occur among *Fov* isolates. Isolates 3, 4, 6, and 7 showed significantly higher AUDPC in the absence of *M. incognita* at higher inoculum densities of *Fov* (6.5×10^5 cfu/cm^3) with cultivar FM 9058F compared to other *Fov* isolates tested (Fig. 2) which implies that disease incidence may be higher even in the absence of *M. incognita* when *Fov* inoculum density in soil is high. Isolates 5 and 11 of *Fov* Race 1 had significantly ($P \leq 0.05$) higher AUDPC in the presence of *M. incognita* at lower inoculum density (2.5×10^3 cfu/cm^3) compared to other *Fov* isolates tested (Fig. 2) indicating the presence of varying levels of virulence within the same *Fov* Race 1 isolates. Isolates 4, 7, 10, and 11 showed significantly higher AUDPC than the rest of *Fov* isolates tested in the presence of *M. incognita* at *Fov* inoculum density of 9.5×10^4 cfu/cm^3. Isolates 2, 4, 6, 7, and 10 resulted in higher AUDPC at highest *Fov* inoculum density used for the study (6.5×10^5 cfu/cm^3) in the presence of *M. incognita* compared to other *Fov* isolates tested (Fig. 2). Isolate 3, for example, resulted in the highest level of symptoms in the absence and presence of *M. incognita* on FM 9058F, as well as ST 4554B2RF, but there was still clear separation between the two cultivars, both in the presence and absence of root-knot nematodes. Both the intermediate and highest density of the fungus resulted in clear differences between susceptible and partially resistant cultivars. This isolate would be a good choice to use in a germplasm screening program.

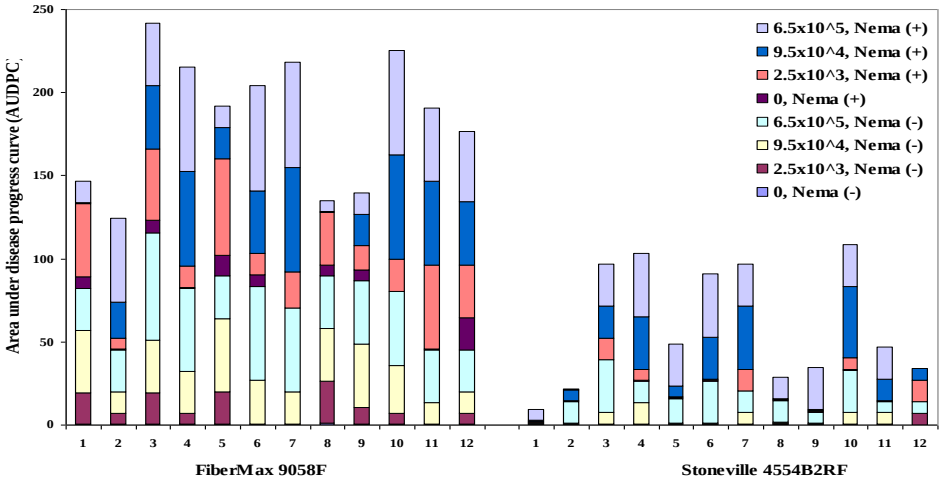

Figure 2. Effect of interaction of twelve *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) isolates, inoculum density, *Meloidogyne incognita* and cotton cultivar on area under the disease progress curve (AUDPC). Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) using mixed model analysis and df were determined using the Satterthwaite option. LSD for comparing means between *Fov* isolates was 20.76, for cultivars was 20.3, for nematode levels was 19.6, and for inoculum densities was 20.4 at $P \leq 0.05$ according to Fisher's Protected LSD. FiberMax 9058F was

susceptible and Stoneville 4554B2RF was partially resistant to *Fov*. Four inoculum densities of *Fov* used in the study were 0, 2.5×10^3, 9.5×10^4, and 6.5×10^5 colony forming units/cubic centimeter of soil. Nema (-) and Nema (+) represent *Meloidogyne incognita* levels 0 and 1,000 eggs/pot, respectively.

The interaction between inoculum densities of *Fov*, *M. incognita*, and cultivar was significant ($P \leq 0.0001$) for shoot weight. ST 4554B2RF had higher shoot weight than FM 9058F with or without root-knot nematode at all four *Fov* inoculum densities tested (Table 1). AUDPC was significantly higher ($P \leq 0.05$) for FM 9058F than ST 4554B2RF for all *Fov* isolates tested with or without *M. incognita* (Fig. 3). Total plant weight was significantly higher for plants not inoculated with *M. incognita* at all four *Fov* inoculum densities tested (Table 2) showing that root-knot nematode affects plant growth and development. The plants inoculated with *M. incognita* showed low total plant weight at higher *Fov* inoculum densities (6.5×10^5 cfu/cm^3 and 9.5×10^4 cfu/cm^3) than low *Fov* inoculum density (2.5×10^3 cfu/cm^3) or non-inoculated plants (Table 2). High *Fov* inoculum density (6.5×10^5 cfu/cm^3) resulted in significantly higher AUDPC with both the cultivars tested and AUDPC was significantly higher for FM 9058F than ST 4554B2RF (Table 3). Interaction between inoculum densities of *Fov* and cultivars tested was significant for plant height ($P \leq 0.05$) and total plant weight ($P \leq 0.0001$). ST 4554B2RF had higher plant height and total plant weight than FM 9058F. High inoculum density of *Fov* had higher plant heights and total plant weight than low inoculum densities (Table 4). Interaction between *Fov* Race 1 isolates and cultivars tested was significant for plant height ($P \leq 0.05$) root and shoot weight ($P \leq 0.0001$). All these parameters were significantly higher for ST 4554B2RF than FM 9058F (Table 5).

Table 1. Effect of interaction between inoculum density of *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) Race 1, *Meloidogyne incognita*, and cotton cultivar on shoot weight[a].

Fov inoculum density (cfu/cm^3)	Shoot weight (g)				
	M. incognita (0 eggs/pot)		*M. incognita* (1,000 eggs/pot)		
	FiberMax	Stoneville	FiberMax	Stoneville	
0	8.2 B[b], a[c]	8.9 A, b[c]	7.3 B[b], a	8.5 A, a[c]	
2.5×10^3	8.2 B, a	9.3 A, ab	7.3 B, a	8.2 A, a	LSD[e]
9.5×10^4	8.5 B, a	9.3 A, ab	6.7 B, b	8.2 A, a	0.6
6.5×10^5	8.0 B, a	9.5 A, a	5.6 B, c	8.5 A, a	
LSD[d]	0.6				

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [b]Upper case letters are for comparing means between cultivar FiberMax and Stoneville (row). [c]Lower case letters are for comparing means between inoculum densities (column). [d]LSD for comparing means between two cultivars. [e]LSD for comparing means between four *Fov* inoculum densities. FiberMax 9058F is susceptible and Stoneville 4554B2RF is partially resistant to *Fov*.

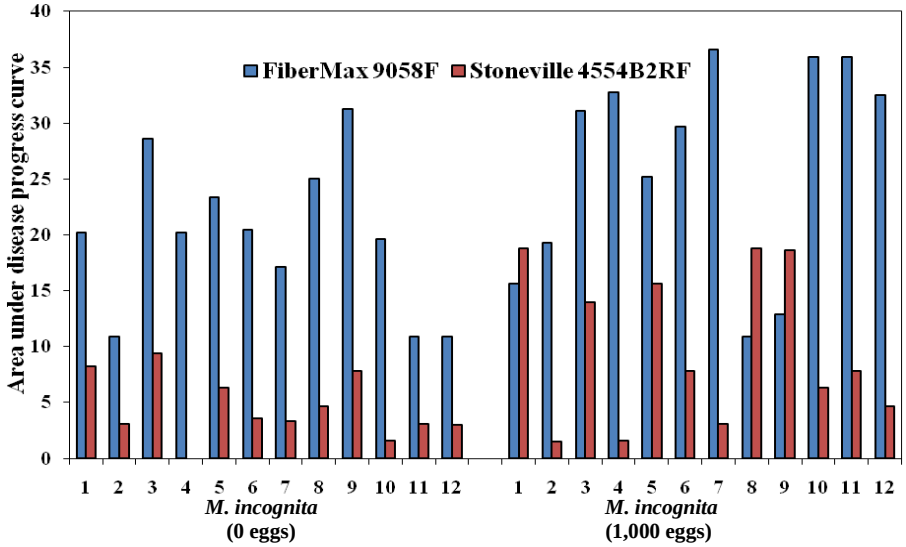

Figure 3. Effect of 12 *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) isolates, *Meloidogyne incogita*, and cotton cultivar on area under the disease progress curve (AUDPC). Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) using mixed model analysis and df were determined using the Satterthwaite option. Level of significance was determined at $P \leq 0.05$ according to Fisher's Protected LSD. LSD = 16.0 for comparing means between cultivars FiberMax and Stoneville at each nematode level for each *Fov* isolate. LSD = 17.7 for comparing means between *Fov* isolates of each cultivar at each nematode level. FiberMax 9058F was susceptible and Stoneville 4554B2RF was partially resistant to *Fov*. N = 31.

Table 2. Effect of interaction between inoculum density of *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) Race 1 and *Meloidogyne incognita* on total plant weight[a].

	Total plant weight (g)		
Fov inoculum density (cfu/cm^3)	*M. incognita* (0 eggs/pot)	*M. incognita* (1,000 eggs/pot)	
0	10.8 A[b], a[c]	10.2 B, a[c]	
2.5×10^3	11.1 A, a	10.1 B, a	LSD[e]
9.5×10^4	11.2 A, a	9.7 B, ab	0.6
6.5×10^5	11.1 A, a	9.3 B, b	
LSD[d]	0.50		

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [b]Upper case letters are for comparing means between nematode densities (row). [c]Lower case letters are for comparing means between inoculum densities (column). [d]LSD for comparing means between nematode levels. [e]LSD for comparing means between inoculum densities. N = 188.

Root galling was found to be associated with *M. incognita* presence and plants that exhibited galls were stunted, had reduced shoot and total plant weight; whereas, root weight was not affected by the presence of root galls (Table 6), because roots infested with *M. incognita* were smaller and less fibrous than roots without galls. Plant growth and symptoms expressions were affected by the susceptibility of cultivar. FM 9058F had significantly stunted plants, decreased root weight, shoot weight, and total plant weight

compared to ST 4554B2RF (Table 7) suggesting the importance of planting a resistant cultivar in infested fields.

Table 3. Effect of inoculum density of *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) and cotton cultivar on area under the disease progress curve (AUDPC)[a].

Fov inoculum density (cfu/cm^3)	AUDPC		
	FiberMax	Stoneville	
0	3.4 A[b], d[c]	0.3 A, c[c]	
2.5×10^3	20.7 A, c	2.3 B, c	LSD[e]
9.5×10^4	29.9 A, b	9.4, B, b	7.0
6.5×10^5	38.8 A, a	16.7 B, a	
LSD[d]	6.5		

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [b]Upper case letters are for comparing means between cultivar FiberMax and Stoneville(row). [c]Lower case letters are for comparing means between inoculum densities (column). FiberMax 9058F is susceptible and Stoneville 4554B2RF is partially resistant to *Fov*. N = 188.

Table 4. Effect of interaction between inoculum density of *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) and cultivar on plant height (35 DAP) and total plant weight[a].

Fov inoculum density (cfu/cm^3)	Plant height (cm)		Total plant weight (g)	
	FiberMax	Stoneville	FiberMax	Stoneville
0	15.9 A[b], ab[c]	16.2 A[b], ab[c]	10.0 B[b], a[c]	11.0 A[b], a[c]
2.5×10^3	15.7 B, b	16.8 A, a	10.0 B, a	11.2 A, a
9.5×10^4	16.2 B, a	16.8 A, a	9.7 B, a	11.1 A, a
6.5×10^5	16.0 A, ab	16.4 A, ab	8.9 B, b	11.4 A, a
LSD[d]	0.4		0.5	
LSD[e]	0.4		0.6	

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [b]Upper case letters are for comparing means between cultivars FiberMax and Stoneville for plant height and total plant weight (row). [c]Lower case letters are for comparing means between *Fov* inoculum densities (column). [d]LSD for comparing means between cultivars for plant height and total plant weight. [e]LSD for comparing means between *Fov* inoculum densities. FiberMax 9058F is susceptible and Stoneville 4554B2RF is partially resistant to *Fov*. N = 189.

There is a positive correlation between *Fov* inoculum density and disease incidence (DeVay et al., 1997; Hao et al., 2009; Starr et al., 1989). *Fov* Race 1, which resulted in significant damage when plants were co-infected with *M. incognita*, caused significant symptom expression with isolates 3, 4, 6, and 7 in the absence of nematodes on FiberMax 9058F at 6.5×10^5 cfu/cm^3 inoculum density (data not shown). This suggests that variability exists in aggressiveness among *Fov* Race 1 isolates at high inoculum density especially with planting a susceptible cultivar. Garber et al. (1979) found that Fusarium wilt occurs with *Fov* alone at high spore numbers (≥ 77,000 propagules per g soil) or in combination with root-knot nematodes at much lower densities (< 650 propagules per g soil with 50 second-stage juveniles) on a susceptible cultivar (Acala SJ-2). Kim et al. (2005) found that virulent Australian *Fov* isolates in controlled experiments, which, like *Fov* Race 4, do not require damage from nematodes

to cause disease, and caused increasingly severe symptoms at higher aqueous suspensions of conidia. However, the effect of *Fov* inoculum density in soil varied with the resistance of the cotton cultivar.

Table 5. Effect of interaction between *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) isolates and cultivars on plant growth parameters[a].

Fov isolate	Plant height (cm)		Root weight (g)		Shoot weight (g)	
	FM	ST	FM	ST	FM	ST
1	22.6 B[b],bc[c]	24.1 A,ab[c]	2.0 B[b],b[c]	2.3 A,b[c]	6.7 B[b],b[c]	8.6 A,ab[c]
2	24.8 A,a	25.2 A,a	2.2 B,ab	2.5 A,ab	7.1 B,ab	8.9 A,ab
3	21.9 B,c	24.6 A,ab	2.1 B,ab	2.7 A,a	6.8 B,b	9.6 A,a
4	22.8 B,bc	24.6 A,ab	2.2 B,ab	2.5 A,ab	7.6 B,ab	9.1 A,ab
5	22.9 B,bc	24.5 A,ab	2.2 A,ab	2.4 A,ab	7.4 B,ab	8.8 A,ab
6	22.4 B,bc	23.4 A,b	2.4 A,a	2.1 B,b	7.6 A,ab	8.2 A,b
7	22.9 A,bc	23.5 A,b	2.3 A,ab	2.3 A,b	8.0 B,a	8.7 A,ab
8	22.6 B,bc	24.2 A,ab	2.1 A,ab	2.3 A,b	7.6 A,ab	8.2 A,b
9	23.3 B,b	25.3 A,a	2.0 B,b	2.6 A,ab	7.2 B,ab	9.6 A,a
10	22.4 B,bc	23.7 A,b	2.1 A,ab	2.3 A,b	8.0 B,a	9.5 A,a
11	23.4 A,ab	24.0 A,ab	2.2 A,ab	2.3 A,b	7.8 A,ab	8.2 A,b
12	23.8 A,ab	24.6 A,ab	2.2 A,ab	2.3 A,b	8.1 A,a	8.3 A,b
LSD[d]	0.9		0.6		0.7	
LSD[e]	1.4		0.4		1.1	

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [b]Upper case letters are for comparing means between cultivar FM (FiberMax 9058F) and ST (Stoneville 4554B2RF) (row). [c]Lower case letters are for comparing means between isolates (column). [d]LSD for comparing means between cultivar. [e]LSD for comparing means between *Fov* isolates. N = 63.

Indeed, results of this greenhouse study may not reflect the field responses of cotton cultivars because field grown plants are frequently under environmental or biotic stresses not present in the greenhouse. Another inherent difference between the conditions of this greenhouse study and those of the field is the inoculum itself. *Fov* overwinters in field soil primarily as chlamydospores (Nelson 1981), but micro- and macro-conidia were used in this study to infest the potting mixture. Conidia are not as well suited for long term survival in the soil as are the thick-walled chlamydospores, but may germinate in pot cultures (Nelson 1981). Despite the differences between this experiment and field conditions, disease development in the field likely follows the general trends observed here. In the present study, four isolates of Race 1 showed significantly higher AUDPC in the absence of *M. incognita* at higher inoculum densities of *Fov*; while two isolates of Race 1 showed significantly higher AUDPC in the presence of *M. incognita* at low inoculum densities, indicating the presence of varying levels of *Fov* virulence. Further research is necessary in order to determine the soil inoculum threshold for disease development in the field with varying levels of *Fov* virulence. The relationship between *Fov* inoculum density, *M. incognita*, and severity of Fusarium wilt-Root-knot nematode complex is important for the development of management strategies because populations of *Fov* in the soil may be affected by the cotton cultivar selection.

Table 6. Effect of *Meloidogyne incognita* density on plant growth parameters[a].

M. incognita density (eggs/pot)	Plant height (cm)		Root weight (g)	Shoot weight (g)	Total plant weight (g)
	35 DAP	90 DAP			
0	17.2 a[b]	25.4 a[b]	2.3 a[b]	8.7 a[b]	11.0 a[b]
1,000	15.3 b	21.9 b	2.3 a	7.5 b	9.8 b
LSD[c]	0.2	0.3	ns	0.5	0.5

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. [b]Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [c]LSD for comparing *M. incognita* densities. N = 754.

Table 7. Effect of cultivar selection on plant growth parameters[a].

Cultivar	Plant height (cm)		Root weight (g)	Shoot weight (g)	Total plant weight (g)
	35 DAP	90 DAP			
FiberMax 9058F	15.9 b[b]	23.0 b[b]	2.2 b[b]	7.5 b[b]	9.6 b[b]
Stoneville 4554B2RF	16.5 a	24.3 a	2.4 a	8.8 a	11.2 a
LSD[c]	0.4	0.4	0.1	0.5	0.7

[a]Data were analyzed using Proc MIXED (SAS 2008, Ver. 9.2) and df were determined using the Satterthwaite option. [b]Means followed by the same letter in the same column are not significantly different ($P \leq 0.05$) according to Fisher's Protected LSD. [c]LSD for comparing cotton cultivar. FiberMax 9058F is susceptible and Stoneville 4554B2RF is partially resistant to *Fusarium oxysporum* f. sp. *vasinfectum*. N = 75.

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