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BREEDING AND GENETICS

Breeding for Fusarium Wilt Race 4 Resistance in Cotton under Field and Greenhouse Conditions

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ABSTRACT

***Fusarium oxysporum* f. sp. *vasinfectum* (FOV) Atk. Sny & Hans is a continuing threat to cotton production in the United States that warrants attention in plant breeding programs. Several developments concerning this pathogen (e.g. newly-recognized Australian FOV races and identification of FOV race 4 in California) highlight the need for additional research to provide improved host plant resistance to this pathogen. In order to assess the U.S. cotton gene pool and to study host plant resistance to FOV race 4, field and greenhouse evaluations were conducted in 2003, 2004, and 2005 on more than 150 Pima (*Gossypium barbadense* L.) and Acala and non-Acala Upland (*G. hirsutum* L.) entries. A population of 32 Pima recombinant inbred lines (RILs) and six F_1 combinations of hybrids developed between susceptible and resistant entries were also evaluated. Pima cottons inoculated with race 4 developed more severe symptoms than the Acala and non-Acala Upland cottons, but some Acala and Upland cottons were severely infected. Germplasm with high levels of resistant to FOV race 4 was identified in some Pima entries. Resistance against FOV race 4 in Pima cottons was more complete than expected and may be determined by a single dominant major gene and one or more modifying minor genes, which may explain the trangressive segregation observed in some RILs. FOV resistance in Acala and non-Acala Upland cottons may be more complex and may be inherited in a quantitative manner by several major genes and minor modifying genes. This research identified new, improved resistant germplasm for release in the future for public incorporation into California cotton cultivars. In addition, this research provided information about the susceptibility of commercial cotton and improved germplasm that will allow growers to make informed decisions regarding which cotton cultivar to plant in fields infested with race 4. Development of highly resistant germplasm and genetic mapping of populations to develop and/or identify molecular markers to assist in breeding for FOV resistance in cotton are continuing.**

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Cotton is the most important renewable natural textile fiber and the sixth largest source of vegetable oil in the world. Cotton belongs to the genus *Gossypium,* which consists of at least 45 diploid and five allotetraploid species (Percival et al., 1999; Ulloa et al., 2005; 2006). *G. hirsutum* L. (*Gh*, AD_1 genome) and *G. barbadense* L. (*Gb,* AD_2 genome) are modern allotetraploid (2n = 4*x* = 52) cottons that together represent the most extensively cultivated species worldwide. A-genome diploid cottons produce spinnable fibers, while D-genome species produce short, appressed fibers (Ulloa et al., 2005; 2006). Significant progress has been accomplished in breeding for agronomic traits, fiber characteristics, and pest resistance over the last 70 yr. Modern cotton cultivars produce higher yields than older cultivars, are day-length neutral in flowering, are relatively early-maturing, and have fiber with good to excellent quality that is easily ginned. These improved characteristics resulted from human selection from perennial ancestors with shorter, sparser fiber (Fryxell, 1984). Yield, quality, and resistance to pests and diseases continue to be the primary objectives for plant breeders. Elite lines of cotton contain a number of traits that originated in the primary germplasm pool. Among these are the bacterial blight resistance genes from *G. hirsutum* and *G. barbadense* (Endrizzi et al., 1985), the nectariless trait from *G. tomentosum* (Meyer and Meyer, 1961), root-knot nematode resistance from

landraces (Shepherd, 1974; 1982), and resistance to *Fusarium* and *Verticillium* wilt from landraces of *G. hirsutum* and from *G. darwinii* (Bell, 1984).

Recent developments have confirmed that *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) Atk. Sny & Hans is a recurring and potentially expanding threat to cotton production. The vulnerability of cotton production to this pathogen highlights the need for research to protect the cotton industry from virulent populations of FOV that may be introduced and new virulent strains of FOV that may arise within cotton production areas. Within the past 10 yr, a new, highly virulent isolate of FOV has been identified in cotton fields in Australia (Kochman et al., 2002; Wang et al., 2004), which has not been identified in U.S. cotton fields (Kim et al., 2005). Little is known about resistance to this isolate within U.S. cottons and wild cotton germplasm, or how soil types, soil pH, natural antagonists, and interactions with other pathogens (e.g., the root knot and reniform nematodes) affect this pathogen. Within 5 to 7 yr after its original identification in the early 1990s, the Australian FOV has spread to 6 of the 10 major cotton producing areas in that country and caused significant economic damage. Based on our knowledge of the ecological factors that favor the Australian strains of FOV, cotton production in large areas of the U.S. Cotton Belt could be at risk.

Fusarium wilt of cotton in the United States was first described by Atkinson (1892). FOV is a soil-inhabiting organism and can survive for long periods in soils, even in the absence of cotton, which makes it nearly impossible to eradicate from a field. Eight genotypes of FOV, called races, have been described throughout the world. Until recently, only race 1 and race 2 were known to occur in the United States (DeVay, 1986; Smith et al., 1981). In 2005, additional races were identified in California (Kim et al., 2005). Races of FOV were originally classified based on pathogenicity tests on different cotton species, *G. hirsutum*, *G. barbadense*, and *G. arboretum* L. (Armstrong and Armstrong, 1958; 1960; 1978; Ibrahim, 1966), and by their pathogenicity on alfalfa, soybean, and tobacco (Armstrong and Armstrong, 1978). A number of genetic markers are now used to further characterize these races. Based on sequence differences in the translational elongation factor, phosphate permase, and beta-tubulin genes and intergenic spacer (IGS) restriction enzyme digests, worldwide strains of FOV can be classified into five major lineages (Kim et al., 2005). California isolates were observed in all lineages except for the Australian lineage, indicating a wide diversity of strains in California.

During the 1950s and 1960s, California became one of the main producers of cotton in the United States. By the early 1960s, FOV was identified in multiple locations in cotton fields of West Texas and California (Blank, 1962). The disease was first noted in California in 1959 (Garber and Paxman, 1963), and the number of infested sites remained relatively small until the mid-1970s, when the number of sites increased substantially (Hillocks, 1992). By 1974, cotton growers of the San Joaquin Valley were cheered by the release of a new wilt-tolerant cultivar of cotton by the USDA-ARS Cotton Research Station, Shafter, CA (Turner, 1981). Fusarium wilt being evaluated in studies conducted in California and other western states during the 1960s and 1970s was caused by FOV races 1 or 2, and was typically found in sandy or sandy-loam soils with significant root-knot nematode (*Meloidogyne incognita* Kofoid & White, Chitwood) populations (Veech, 1984; Bell, 1984). Susceptibility to Fusarium was substantially increased in the presence of the root-knot nematode (Garber et al., 1979). Cottons developed for resistance to FOV on soils infested with *M. incognita* usually maintained their resistance when simultaneously challenged by both organisms (Sasser, 1972; Heald and Orr, 1982).

Kim et al. (2005) recently identified race 4 of FOV in cotton plants grown in California fields. This race, first identified in India on Asiatic cottons, had not previously been identified in the United States. Within the past few years, race 4 of FOV has caused extensive symptoms in cotton plants grown in clay loam and loam soils in which root knot nematode populations and root damage from nematodes were nonexistent or extremely low. In these field evaluations in California, disease expression of race 4 has been most severe in Pima cotton fields, but the fungus also infects and causes disease in Acala and Upland cottons (Kim et al., 2005; Hutmacher et al., 2005; Ulloa et al., 2005).

Host-plant resistance is the most economic and effective strategy for control of Fusarium wilt in cotton. Little is known about the mechanisms involved in this resistance, but based on the potential for economic losses, the inevitable field-to-field spread of the pathogen, and the potential for long-distance transport via seed, information on possible resistance is needed for long-term disease management. The

original mission of the USDA-ARS Cotton Enhancement Program at Shafter, CA, was to develop and distribute cotton germplasm particularly suited for production in the San Joaquin Valley. This effort was discontinued in the mid-1970s but was reestablished in 2001. This study reports on field and greenhouse evaluations on the susceptibility of Pima, Acala, and non-Acala Upland germplasm to race 4 of FOV.

MATERIALS AND METHODS

Pathogen. Field investigations were conducted in commercial fields naturally infested with *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) race 4. Fields consisted of clay loam and loam soils in which root knot nematode populations and root damage from nematodes in previous cotton crops were low or nonexistent. The identity of FOV race 4 in plants grown in these fields was confirmed (Kim et al., 2005) before these trials were initiated.

The race 4 isolate used for greenhouse evaluations was obtained from a naturally infested field in the San Joaquin Valley where plants exhibited typical symptoms of Fusarium wilt, including stunted growth, vascular discoloration, foliar symptoms, and leaf abscission. Cultures from single spores were stored on filter paper at 4 °C. Inoculum for pathogenicity tests was produced by placing a 25-mm^2 piece of the filter paper on potato dextrose agar (PDA) in 15-ml Petri dishes.

Plant material. More than 150 cotton entries were evaluated during both field and greenhouse investigations. Result for evaluations for commercial cotton entries and selected improved germplasm will be published elsewhere. In this study, 18 entries of Acala/Upland (*Gossypium hirsutum*) cottons were included as follows: cvs Maxxa, NemX (California Planting Cotton Seed Distributors; Shafter, CA), Phy 72 and Phy 78 (PhytoGen Seed Co.; Corcoran, CA), DP 444BG/RR and DP 555 BR (Delta and Pine Land Co.; Scott, MS), FiberMax 960 BR (Bayer CropScience; Research Triangle Park, NC), ST 4575 BR and STX-00C022 (Stoneville Pedigreed Seed Co.; Memphis, TN), Acala 1517-75, Acala 1517-88, Acala 1517-91, Acala 1517-95 (New Mexico State University; Las Cruces, NM), and SJ 2, SJ 5, accession ARS_Nem, accession ARS_FBCX-2 (USDA-ARS Western Integrated Crop Systems Research Unit, Cotton Enhancement Program; Shafter, CA). For Pima (*G. barbadense*) cottons, 46 entries were included as follows: accessions CPCSD_01 and CPCSD_08 (California Planting Cotton Seed Distributors), Phy 800 and Phy 810R (PhytoGen Seed Co.), DP-744 (Delta and Pine Land Co.), OA 353 (Olvey & Associates, Ltd.; Maricopa, AZ) PS 7, PS 6, P 53, P 72, P 73, NMSI 1601, 8810, and thirty-two recombinant inbred lines (RILs) named ARS_01 through ARS_32 that were developed from a cross between NMSI 1601 x 8810 (USDA-ARS). Each of the thirty-two RILs were developed from a single F_2 plant and advanced from F_2 to F_5 by single plant selection. Field and greenhouse evaluations were performed on generations F_5-derived F_6 and F_6-derived F_7, respectively. In addition, one interspecific hybrid, HA 195, from the Hazera Co. (Davis, CA) was included. NMSI 8810 was developed from a cross between P 73 x P 72, and P 73 was developed from a cross P 53 x PS 6. Three entries of *G. herbaceum* (A_{1_9}, A_{1_18}, and A_{1_52}) and four of *G. arboreum* (A_{2_31}, A_{2_72}, A_{2_80}, and A_{2_241}) from the ancestral diploids of the A genomes, and one each from *G. lobatum* (D_{7_8a}) and *G. trilobum* (D_{8_6}) from the D diploid genomes were also included in this study.

Field evaluations. Field evaluations were conducted in 2003 and 2004. Fifty cotton cultivars (26 entries from *G. barbadense* and 24 entries from *G. hirsutum*), which included most commercial Pima, Acala, and non-Acala Upland cotton cultivars grown in California, and some improved germplasms were planted on 3 July 2003, and grown in single row plots. Plots were 10 m long with a 1-m row spacing and arranged in a randomized complete block design with four replications. On 24 May 2004, 100 entries were planted and grown in single row plots that were 5m long with 1-m row spacing arranged in a randomized complete block design with three replications.

To determine the level of tolerance/resistance for each entry in the field, five plants from each replication were evaluated for foliar symptoms, vascular discoloration, number of nodes, and plant height on 28 Aug. 2003 and on 23 July 2004. In 2004, plant populations were also counted for each entry at 1 wk and 4 wk after complete emergence, and at crop maturity to determine the percentage of plants killed by the disease by the end of the season, and the percentage of plant survival, which included any infected plants. The percentage of surviving plants was calculated by dividing the total number of surviving plants at the end of the study by the initial plant count after plant establishment and multiplied by a hundred.

For the 2003 and 2004 evaluations, individual plants were rated for disease severity based on a 0 to 5 scale, where 0 = no foliar symptoms, 1 = chlorosis and/or wilt restricted to cotyledons or first leaf, 2= chlorosis and/or wilt extending beyond the first leaf, 3 = moderate to severe foliar symptoms usually with some abscised leaves, 4 = severe foliar symptoms on the entire plant, and 5 = dead plant. Plants were also rated for vascular discoloration using the scale as follows: 0 = no discoloration, 1 = light discoloration evident as spotty areas in the cross-section of the stem, 2 = more continuous discoloration covering an area between one quarter and one half of the cross-section of the stem but light in color, 3 = vascular discoloration (moderate in color) evident in a band encircling almost the entire stem cross-section, 4 = vascular discoloration darker in color than in 1 or 2, and evident across most of the vascular tissue in a cross section of the stem, and 5 = plant severely damaged, vascular discoloration evident throughout cross-section of the stem.

Greenhouse evaluations. The pathogenicity of isolates of FOV race 4 from California was determined by inoculating cotton cultivars. Cotton entries were seeded in non-inoculated composite medium of vermiculite and peat moss. Two- to 3-wk-old seedlings were removed from the medium and dipped in water (uninoculated plants) or a conidial suspension of FOV at 1×10^5 conidia per ml for 2 min (treated plants). After inoculation, the plants were replanted in individual pots filled with the composite medium. Spore suspensions were prepared by flooding colonized 2-wk-old cultures grown on PDA with water, scraping-off the spores, and filtering the spore suspension through four layers of cheesecloth.

The first and second greenhouse trials were conducted at the University of California, Kearney Research and Extension Center near Parlier, CA. The climate control for the heating system for these tests was set at 13 °C and the fans and evaporative cooling pad system were set to activate at 28 °C. During the first greenhouse experiment (2004), actual air temperatures never went below 12 °C at any time, and peak greenhouse temperatures never exceeded 30 °C at any time during the experiment. During the second greenhouse experiment (2005), actual air temperatures never went below 13 °C at any time, and peak greenhouse temperatures never exceeded 34 °C at any time during the experiment.

The first greenhouse evaluation was performed on 50 cotton entries in the fall of 2004. Planting began the last week of September and evaluations were conducted in early December. The second greenhouse evaluation was performed on 60 cotton entries in the spring of 2005. Plants were inoculated in late March and evaluated in late May. Each treatment was replicated three times in 2004 and four times in 2005. Treatments were arranged in a randomized complete block design with five plants per block, one plant per pot. To determine the level of tolerance/resistance for each entry, nine inoculated and five uninoculated plants were assayed for foliar damage, vascular discoloration, number of nodes, and plant height.

The third greenhouse evaluation was performed with 60 cotton entries from May to July in 2005 at the University of California at Davis. Each treatment was replicated three times in a randomized complete block design with five plants per block, one plant per pot. To determine the level of tolerance/resistance for each entry, 12 inoculated and three uninoculated plants were assayed for foliar symptoms, vascular discoloration, numbers of nodes, and plant height. Greenhouse temperatures ranged between 22 and 34 °C.

Disease severity for foliar symptoms and vascular discoloration were measured approximately 4 to 5 wk after inoculation, since it was noted in preliminary tests that visible leaf symptoms in some resistant plants became harder to recognize with continued plant growth at more than 5 wk after inoculation (Kim et al., 2005). Individual plants were rated for disease severity based on very similar scales used in field evaluations for foliar damage and vascular discoloration.

Data were analyzed with PROC GLM and the correlations among traits were analyzed using the CORR procedure of the SAS statistical software (ver. 8.1, SAS Institute; Cary, NC). Chi-square analysis was conducted to test goodness-of-fit between expected and observed segregation ratios of resistant to susceptible plants and/or RILs (Weir, 1996). Generation means were used to assess the possibility that actions of more than one gene may control the resistance of this disease. Deviation between mean of the parents and mean of the F_1 were tested using a paired *t* test (Ramey, 1963). In addition, deviation of transgressive segregant mean values from their parent (resistance or susceptible) were also tested using a paired *t* test (McClave and Dietrich, 1988). Heritability estimates for the agronomic and fiber traits were calculated by using the variance components from the analyses of variance, one-way layout-interclass correlation (Robertson, 1977; Ponzoni and James, 1978).

RESULTS

Plant disease symptoms. Based on foliar and vascular symptoms, plant survival, and plant height, most of the commercial Pima cultivars (*G. barbadense*) were more susceptible to FOV race 4 than the Acala and non-Acala Upland cottons (*G. hirsutum*) (Tables 1 and 2; Fig. 1). Some Acala and non-Acala Upland cotton cultivars used in this study, however, were severely affected by FOV race 4 based on vascular discoloration. There was a wide range of symptoms, including chlorosis and abscission of cotyledons, chlorosis and necrosis of individual or multiple leaves, plant wilting, and death of plants, observed across the entries. On older plants, foliar symptoms usually began on the margins of lower leaves (Fig. 2). As veins darkened, leaves became partly chlorotic and sometimes abscised. When infection was severe, vascular discoloration usually occurred in the tap root and in the lower part of the stem approximately 2 to 5 cm above the soil (Fig. 2). In the field, plants started to die within a few days after emergence and in the greenhouse death occurred between 2 and 4 wk after inoculation. Even though Pima cultivars were more susceptible, highly resistant Pima germplasm to FOV race 4 was identified both in the field and greenhouse (Table 1 and Fig. 1). In addition, race 4 of FOV was able to infect species accessions from the ancestral diploid A-genomes, *G. herbaceum* and *G. arboreum*, and to a lesser extent to the D-genomes, *G. lobatum* and *G. trilobum* (Fig. 1).

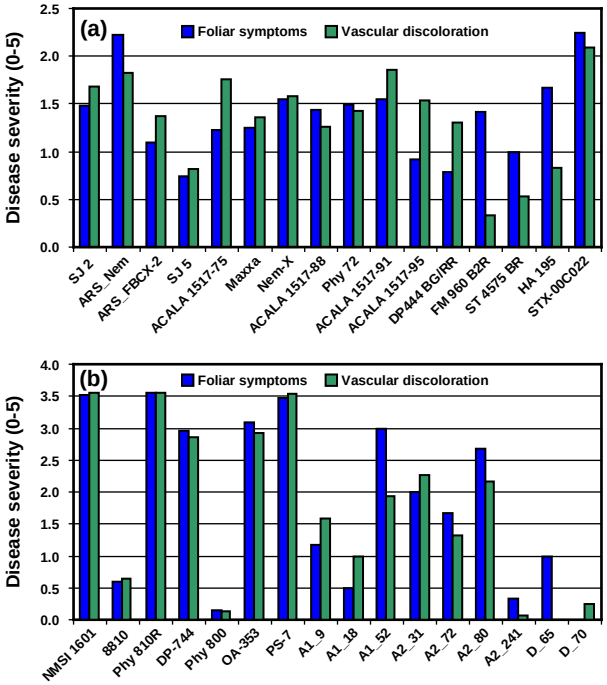

Figure 1. Average mean values from field and greenhouse evaluations for foliar symptoms and vascular discoloration caused by *Fusarium oxysporum* f.sp. *vasinfectum* race 4 on (a) Acala and non-Acala Upland cottons (*Gossypium hirsutum* L.) and (b) Pima cottons (*Gossypium barbadense* L.), *G. herbaceum* (A_{1_9}, A_{1_18}, and A_{1_52}), *G. arboreum* (A_{2_31}, A_{2_72}, A_{2_80}, and A_{2_241}) from the ancestral diploids of the A genomes, and *G. lobatum* (D_{7_8a}) (D_65) and *G. trilobum* (D_{8_6}) (D_70) from the D diploid genomes. Foliar symptoms were rated on a scale of 0-5, where 0 = no symptoms and 5 = dead plant. Vascular discoloration was rated on a scale of 0-5, where 0 = no symptoms and 5 = vascular discoloration evident throughout cross-section of the stem.

Figure 2. Foliar symptoms (a) and vascular discoloration (b) for cotton plants infected with *Fusarium oxysporum* f.sp. *vasinfectum* race 4 (photos: R.B. Hutmacher).

Under infested field conditions, seed germination was difficult to determine with certainty. Based on the percentage of infected dead plants calculated across all entries, race 4 killed 20% of the SJ 5 Acala plants and about 45% of DP-744 Pima plants within 6 wk. The average total plant survival at crop maturity in 2004 was 42% for SJ 5 Acala and 25% for DP-744 Pima cottons (Fig. 3).

Evaluation sites and trait correlations. Significant variation was observed for tolerance/resistance to FOV race 4 across entries within test sites. Field and greenhouse evaluations were variable for foliar symptoms, vascular discoloration, number of nodes,

Table 1. Mean values with standard errors (SE) for foliar symptoms, vascular discoloration, number of nodes, and plant height for Pima cottons grown in a field infested with *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) race 4 or inoculated with FOV in the greenhouse

Cultivar	Location -year[x]	Foliar damage[y]	Vascular discoloration[z]	Number of nodes	Plant height (cm)
PS 7	Field – 2004	2.3 (1.3)	2.6 (0.7)	12.7 (0.3)	19.7 (1.1)
	Kearney – 2004	2.9 (0.5)	2.2 (0.4)	2.4 (0.2)	9.3 (0.7)
	Kearney – 2005	3.6 (0.5)	3.6 (0.5)	4.6 (0.5)	13.8 (1.6)
	UC Davis – 2005	4.6 (0.8)	4.4 (0.9)	3.6 (0.8)	11.8 (2.2)
DP744	Field – 2004	3.8 (1.2)	3.3 (0.7)	10.0 (0.0)	22.5 (0.0)
	Kearney – 2004	2.8 (0.4)	2.4 (0.4)	2.4 (0.2)	9.8 (0.7)
	Kearney – 2005	4.1 (0.4)	4.4 (0.3)	3.6 (0.5)	9.3 (1.3)
	UC Davis – 2005	4.8 (0.4)	4.7 (0.5)	2.8 (0.4)	13.0 (1.7)
NMSI 1601	Field – 2004	2.3 (0.9)	1.8 (0.4)	10.7 (0.3)	15.5 (1.3)
	Kearney – 2004	2.4 (0.5)	2.3 (0.3)	2.6 (0.3)	11.5 (0.6)
	Kearney – 2005	4.1 (0.5)	4.3 (0.4)	3.8 (0.4)	11.8 (2.3)
	UC Davis – 2005	4.2 (2.0)	4.5 (1.2)	3.7 (1.2)	12.0 (5.2)
P53	Field – 2004	2.8 (2.3)	2.4 (1.6)	12.4 (0.6)	29.8 (1.1)
	Kearney – 2004	2.4 (0.4)	2.0 (0.4)	3.0 (0.3)	10.1 (0.6)
	Kearney – 2005	2.5 (0.6)	2.7 (0.6)	4.3 (0.3)	12.7 (2.5)
	UC Davis - 2005	4.2 (1.6)	3.9 (1.7)	4.3 (1.2)	19.2 (21.7)
ARS_10	Field – 2004	0.3 (0.3)	0.5 (0.1)	13.9 (0.2)	35.2 (0.8)
	Kearney – 2004	1.6 (0.2)	1.1 (0.1)	3.1 (0.3)	8.6 (0.9)
	Kearney – 2005	0.3 (0.2)	0.8 (0.4)	6.4 (0.3)	28.3 (1.9)
	UC Davis - 2005	1.4 (1.3)	1.2 (1.6)	6.4 (1.8)	29.6 (9.5)
ARS_12	Field – 2004	4.0 (1.0)	3.2 (0.8)	14.3 (0.0)	32.5 (0.0)
	Kearney – 2004	2.8 (0.3)	2.0 (0.3)	3.1 (0.3)	11.6 (0.3)
	Kearney – 2005	4.3 (0.4)	4.6 (0.2)	4.0 (0.5)	9.8 (0.8)
	UC Davis - 2005	4.8 (0.6)	4.9 (0.3)	3.2 (0.4)	9.7 (1.6)
ARS_28	Field – 2004	3.0 (2.0)	2.8 (1.2)	13.3 (0.0)	18.8 (0.0)
	Kearney – 2004	1.5 (0.2)	1.5 (0.3)	3.3 (0.3)	10.4 (0.8)
	Kearney – 2005	2.4 (0.6)	2.8 (0.6)	5.3 (0.4)	20.0 (3.6)
	UC Davis - 2005	3.3 (1.7)	2.8 (2.0)	4.6 (0.9)	12.5 (2.6)
Phyto 800	Field – 2004	0.0 (0.0)	0.0 (0.0)	14.3 (0.1)	40.7 (1.1)
	Kearney – 2004	0.3 (0.1)	0.0 (0.0)	3.1 (0.2)	10.6 (0.5)
	Kearney – 2005	0.0 (0.0)	0.1 (0.1)	6.9 (0.3)	30.8 (2.4)
	UC Davis - 2005	0.8 (1.4)	0.6 (1.2)	6.3 (1.4)	27.4 (6.8)
PS 6	Field – 2004	0.0 (0.0)	0.1 (0.1)	14.2 (0.5)	40.0 (0.8)
	Kearney – 2004	0.6 (0.1)	0.0 (0.0)	3.1 (0.3)	9.9 (0.9)
	Kearney – 2005	0.1 (0.1)	0.8 (0.4)	7.2 (0.2)	32.8 (1.9)
	UC Davis - 2005	0.8 (1.5)	0.7 (1.5)	5.7 (1.9)	24.8 (8.6)

[x] The field site in 2003 and 2004 was infested with FOV race 4. Kearney greenhouses located at the Kearney Research and Extension Center near Parlier, CA, and the UC Davis greenhouse is located on the University of California campus in Davis, CA.

[y] Foliar damage based on a 0 to 5 scale, where 0 = no foliar symptoms, 1 = chlorosis and/or wilt restricted to cotyledons or first leaf, 2 = chlorosis and/or wilt extending beyond the first leaf, 3 = moderate to severe foliar symptoms usually with some abscised leaves, 4 = severe symptoms on the entire plant, and 5 = dead plant.

[z] Vascular discoloration based on a 0 to 5 scale, where 0 = no discoloration, 1 = light discoloration evident as spotty areas of the cross-section of the stem, 2 = more continuous discoloration (light in color) covering an area between one quarter and one half of the cross-section of the stem, 3 = vascular discoloration (moderate in color) evident in a band encircling almost the entire stem cross-section, 4 = vascular discoloration darker in color than in 1 or 2, and evident across most of the vascular tissue in a cross section of the stem, and 5 = plant severely damaged, vascular discoloration evident throughout cross-section of the stem.

and plant height. For the Pima evaluations in the greenhouse, symptoms expression, especially for the more highly susceptible entries, tended to be greater during the warmer conditions in the summer than during the fall or spring (Table 1). The cultivar (genotype) by location interaction was not significant ($P > 0.05$) for foliar damage and vascular discoloration, but highly significant ($P \leq 0.001$) for number of nodes and plant height.

Correlations between disease symptoms in the Pima RIL population were highly significant at the three locations (Table 3). High correlations between the evaluation sites for foliar damage and vascular discoloration suggest that these two traits are inheritable and that data from the four evaluations can be combined in evaluating Pima cottons. The positive correlations between foliar damage and vascular discoloration also indicate that either trait would be effective in measuring disease severity. In all evaluations, foliar damage and vascular discoloration were negatively correlated with node number and plant height, indicating a causal relationship between severity of disease symptoms and plant height. For Acala and non-Acala Upland cottons, significant

Table 2. Mean values with standard errors (SE) for foliar symptoms, vascular discoloration, number of nodes, and plant height for Acala and non-Acala Upland cottons (*Gossypium hirsutum* L.) grown in fields infested with *Fusarium oxysporum* f.sp. *vasinfectum* (FOV) race 4 or inoculated with FOV in the greenhouse

Cultivar	Location – year[x]	Foliar damage[y]	Vascular discoloration[z]	No. nodes	Plant height (cm)
Maxxa	Field – 2003	1.1 (0.4)	1.6 (0.2)	18.2 (0.2)	28.7 (1.0)
	Field – 2004	1.3 (0.3)	1.9 (0.3)	12.2 (0.4)	31.5 (2.0)
SJ 2	Field – 2004	1.3 (0.7)	1.5 (0.5)	13.1 (0.9)	40.5 (2.8)
	Kearney – 2004	1.3 (0.2)	1.4 (0.2)	2.3 (0.2)	7.6 (0.6)
	Kearney – 2005	1.8 (0.5)	2.9 (0.4)	4.5 (0.3)	11.9 (1.8)
	Davis – 2005	1.5 (1.5)	0.8 (0.7)	4.3 (2.1)	15.1 (6.8)
SJ 5	Field – 2004	1.5 (0.3)	1.9 (0.2)	12.9 (0.6)	29.0 (1.1)
	Kearney – 2004	1.6 (0.2)	1.8 (0.3)	2.2 (0.2)	6.9 (0.5)
	Kearney – 2005	0.8 (0.4)	1.3 (0.4)	5.2 (0.4)	20.1 (3.0)
	Davis – 2005	4.0 (1.5)	2.4 (2.2)	2.6 (1.8)	10.2 (4.4)
NemX	Field – 2003	1.3 (0.4)	1.4 (0.4)	18.5 (0.4)	34.3 (1.8)
ARS_Nem	Field – 2004	1.1 (0.1)	1.2 (0.2)	2.7 (0.2)	8.7 (0.4)
	Kearney – 2004	0.7 (0.3)	1.3 (0.4)	4.8 (0.3)	17.4 (1.7)
	Kearney - 2005	1.5 (1.7)	1.6 (1.6)	4.6 (1.2)	16.1 (4.3)
ARS_FBCX-2	Field – 2004	0.5 (0.3)	1.6 (0.1)	13.9 (0.6)	33.5 (1.7)
	Kearney – 2004	1.6 (0.2)	1.4 (0.2)	2.4 (0.2)	6.8 (0.6)
	Kearney - 2005	0.6 (0.4)	1.3 (0.4)	4.9 (0.3)	17.5 (2.6)
	Davis – 2005	1.4 (1.5)	0.6 (0.8)	4.8 (1.4)	16.3 (5.7)
Phyto 72	Field – 2003	2.0 (0.2)	1.6 (0.2)	19.2 (0.2)	33.6 (1.7)
	Field – 2004	1.8 (0.4)	1.9 (0.3)	13.0 (0.3)	31.7 (1.2)
	Kearney – 2004	1.7 (0.3)	1.9 (0.3)	2.3 (0.2)	7.3 (0.5)
	Kearney - 2005	1.1 (0.4)	1.8 (0.4)	4.8 (0.4)	18.1 (2.9)
	Davis – 2005	0.8 (1.5)	0.7 (0.9)	3.5 (1.6)	10.2 (5.3)

[x] The field site in 1003 and 2004 was infested with FOV race 4. Kearney greenhouse is located at the Kearney Research and Extension Center near Parlier, CA, and the UC Davis greenhouse is located on the University of California campus in Davis, CA.

[y] Foliar damage based on a 0 to 5 scale, where 0 = no foliar symptoms, 1 = chlorosis and/or wilt restricted to cotyledons or first leaf, 2 = chlorosis and/or wilt extending beyond the first leaf, 3 = moderate to severe foliar symptoms usually with abscised leaves, 4 = severe symptoms on the entire plant, and 5 = dead plant.

[z] Vascular discoloration based on a 0 to 5 scale, where 0 = no discoloration, 1 = light discoloration evident as spotty areas of the cross-section of the stem, 2 = more continuous discoloration (light in color) covering an area between one quarter and one half of the cross-section of the stem, 3 = vascular discoloration (moderate in color) evident in a band encircling almost the entire stem cross-section, 4 = vascular discoloration darker in color than in 1 or 2, and evident across most of the vascular tissue in a cross section of the stem, and 5 = plant severely damaged, vascular discoloration evident throughout cross-section of the stem.

correlations ($P \leq 0.05$) were observed for number of nodes and plant height (Table 4). In Acala and non-Acala Upland entries, the lack of correlation among the evaluation sites for foliar damage or vascular discoloration demonstrates differences in the development of symptoms. For some entries, vascular discoloration scores were high (>3.0), but foliar symptoms were non-existent, which made it difficult to diagnose this disease. The occasional significant correlations of foliar damage or vascular discoloration with plant height and node number indicated that the reduction in plant growth was related to symptoms (Table 4).

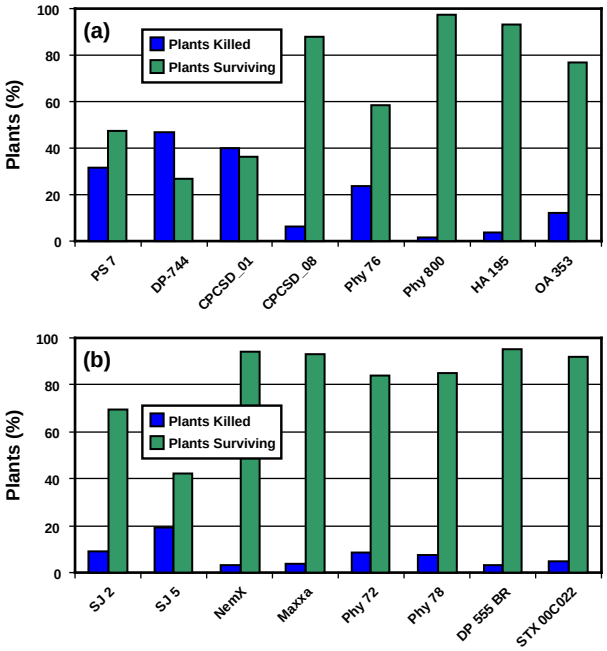

Figure 3. Percentage of plants killed by Fusarium wilt, and the percentage of surviving plants for (a) Acala and non-Acala Upland (*Gossypium hirsutum* L.) and (b) Pima cottons (*Gossypium barbadense* L.). The percentage of plants killed by FOV was determined by counting plant populations at stand establishment and at crop maturity. The percentage of surviving plants was calculated by dividing the total number of plants surviving at the end of the study by the initial plant count at stand establishment and multiplying by 100.

Host-plant resistance. Variability for resistance to FOV race 4 occurred in both Acala/Upland (*G. hirsutum*) and Pima (*G. barbadense*) cottons, but resistance was more complete in Pima at the inoculum levels used (Fig. 1, and Tables 1 and 2). The resistance in Pima cottons to FOV race 4 seems to be determined by a single dominant gene (Fig. 4 and 5) and possibly one or more minor genes (Fig. 6) that boost the resistance of the host plant. Foliar symptoms and vascular discoloration were significantly different ($t > 0.001$) between F_1 hybrids and the mean of the parents, providing evidence for dominant gene effect for resistance to FOV race 4 in Pima cottons (Fig. 4). The bimodal distribution of symptoms in the histogram observed in the RIL population (Fig. 5) is indicative of a single gene. In order to fit a single gene model of the 32 RIL populations into a Mendelian segregation ratio (1:1 ; 50%:50%), the averages of the highest standard errors (SE) for foliar damage and vascular discoloration from one of the parents (NMSI1601 = 0.98) was used to separate resistant and susceptible groups. The ARS_17 RIL was assumed to maintain the lowest (highest score) level of resistance to FOV race 4 with a segregation rate of 15 RILs (47%) : 17 RILs (53%) between resistant and susceptible RILs, respectively (Fig. 6). The goodness-of-fit test was performed in this RIL population, indicating that the resistance to FOV race 4 may be conditioned by a single dominant gene.

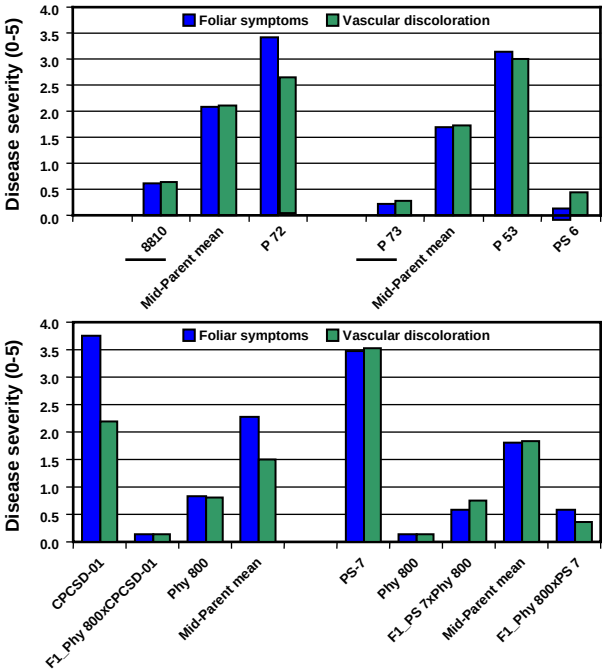

Figure 4. Mean values from three different evaluation sites (one field and two greenhouses) for foliar symptoms and vascular discoloration caused by *Fusarium oxysporum* f.sp. *vasinfectum* race 4 on the parents and hybrid combinations of Pima (*Gossypium barbadense* L.). Mid-parent mean is mean disease severity between the two parents. Foliar symptoms were rated on a scale of 0-5, where 0 = no symptoms and 5 = dead plant. Vascular discoloration was rated on a scale of 0-5, where 0 = no symptoms and 5 = vascular discoloration evident throughout cross-section of the stem.

Table 3. Correlations of foliar symptoms, vascular discoloration, number of nodes, and plant height on 32 Pima (*Gossypium barbadense* L.) recombinant inbred lines (RILs) developed from a cross between NMSI 1601 x 8810 grown at three evaluation sites (one field and two greenhouses)

Traits[y]	Field[z]				Kearney greenhouse[z]				UC Davis greenhouse[z]		
	Foliar symptoms	Vascular discoloration	Number of nodes	Plant height	Foliar symptoms	Vascular discoloration	Number of nodes	Plant height	Foliar symptoms	Vascular discoloration	Number of nodes
Field, 2004											
Vascular discoloration	0.96										
Number of nodes	-0.42	-0.39**									
Plant height	-0.70	-0.72	0.81								
Kearney greenhouse, 2005											
Foliar symptoms	0.72	0.79	-0.48**	-0.74							
Vascular discoloration	0.71	0.78	-0.50**	-0.74	0.99						
Number of nodes	-0.63	-0.71	0.51**	0.71	-0.92	-0.90					
Plant height	-0.62	-0.70	0.45**	0.69	-0.94	-0.93	0.95				
Davis greenhouse, 2005											
Foliar symptoms	0.81	0.80	-0.58	0.77	0.88	0.88	-0.77	0.80			
Vascular discoloration	0.79	0.80	-0.55	0.74	0.88	0.88	-0.77	0.81	0.98		
Number of nodes	-0.78	-0.77	0.58	0.75	-0.83	-0.82	0.77	0.79	-0.89	-0.89	
Plant height	-0.75	-0.76	0.61	0.81	-0.84	-0.84	0.76	0.78	-0.88	-0.87	0.91

[y] The field site in 2004 was infested with FOV race 4. Kearney greenhouse is located at the Kearney Research and Extension Center near Parlier, CA, and the UC Davis greenhouse is located on the University of California campus in Davis, CA.

[z] Values followed by ** are significantly different at $P \leq 0.05$. All other values are significant at $P \leq 0.001$.

Table 4. Correlations of foliar symptoms, vascular discoloration, number of nodes, and plant height on 18 Acala and non-Acala Upland (*Gossypium hirsutum* L.) commercial cottons and improved germplasm grown at three evaluation sites (one field and two greenhouses)

Traits[y]	Field[z]				Kearney greenhouse[z]				UC Davis greenhouse[z]		
	Foliar symptoms	Vascular discoloration	Number of nodes	Plant height	Foliar symptoms	Vascular discoloration	Number of nodes	Plant height	Foliar symptoms	Vascular discoloration	Number of nodes
Field, 2004											
Vascular discoloration	0.68										
Number of nodes	-0.51	-0.90**									
Plant height	-0.41	-0.90**	0.98***								
Kearney greenhouse, 2005											
Foliar symptoms	0.62	0.56	-0.67	-0.60							
Vascular discoloration	0.35	0.11	-0.41	-0.28	0.56						
Number of nodes	0.30	0.76*	-0.58	-0.63	0.28	-0.45					
Plant height	0.36	0.69	-0.41	-0.45	0.08	-0.58	0.95**				
Davis greenhouse, 2005											
Foliar symptoms	0.24	0.43	-0.39	-0.40	0.65	-0.19	0.72	0.54			
Vascular discoloration	0.21	0.03	0.15	0.16	0.37	-0.40	0.43	0.39	0.81**		
Number of nodes	-0.77*	-0.84**	0.67	0.65	-0.75*	-0.01	-0.74*	-0.66	-0.73*	-0.53	
Plant height	-0.83**	-0.96**	0.81**	0.78*	-0.64	-0.12	-0.73	-0.69	-0.50	-0.21	0.93**

[y] The field site in 2004 was infested with FOV race 4. Kearney greenhouse is located at the Kearney Research and Extension Center near Parlier, CA, and the UC Davis greenhouse is located on the University of California campus in Davis, CA.

[z] Values followed by *, **, *** are significantly different at $P \leq 0.1$, $P \leq 0.05$, $P \leq 0.001$, respectively.

The RILs from ARS also showed lower and higher scores for foliar damage and vascular discoloration than the parents, suggesting the presence of one or more minor modifying genes for this transgressive segregation effect in some RILs (Fig. 6). Differences detected ($t > 0.05$) between outlying RIL lines, and resistant and susceptible parents indicated that approximately 12% of the 8810 x NMSI1601 lines were transgressive segregates for resistance and 10% of the lines were transgressive segregates for susceptibility (Fig. 6) If the resistance was treated as being quantitatively inherited (assuming that more than one gene was involved), heritability estimates based on one-way layout-interclass correlation for these traits ranged from 0.64 to 0.95. This result indicates that selection for resistance to FOV race 4 could be accomplished in progeny as early as the F_2 generation and/or advanced generations if one of the contributing parents used to develop this population carries the dominant resistance gene in Pima cottons (Fig. 4).

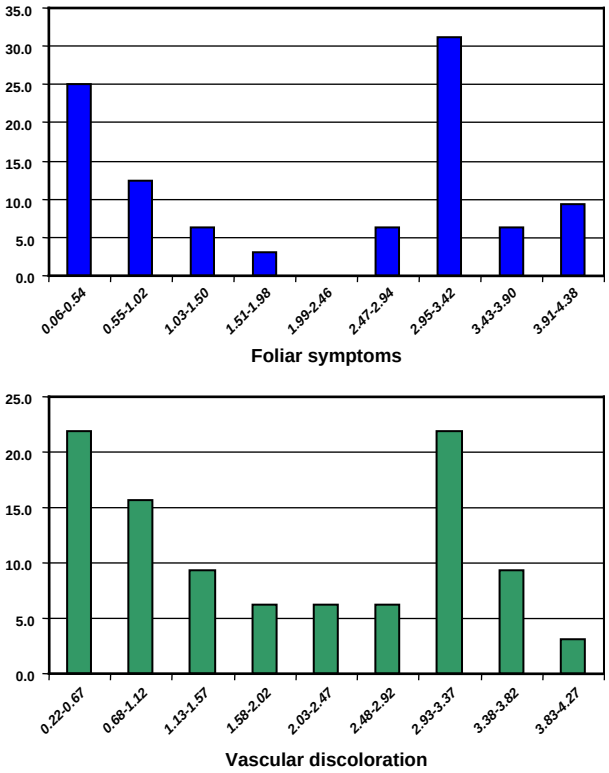

Figure 5. Histogram developed from the average mean values from three different evaluation sites (one field and two greenhouses) of foliar symptoms and vascular discoloration caused by *Fusarium oxysporum* f.sp. *vasinfectum* race 4 on the parents and 32 Pima (*Gossypium barbadense* L.) recombinant inbred lines (RILs) developed from a cross between NMSI 1601 x 8810. The horizontal axis is the disease severity and the vertical axis gives the proportion (%) of RILs that occur in that disease severity interval. . Foliar symptoms were rated on a scale of 0-5, where 0 = no symptoms and 5 = dead plant. Vascular discoloration was rated on a scale of 0-5, where 0 = no symptoms and 5 = vascular discoloration evident throughout cross-section of the stem.

Contrary to Pima cottons, where resistance for FOV race 4 seems to be more complete at the plant-host level, it was difficult to produce and/or identify Acala and non-Acala Upland cotton plants that did not display severe symptoms in order to further investigate the resistance to this pathogen. Resistance levels to race 4 in Acala and non-Acala Upland cultivars were higher than those observed in susceptible Pima cultivars, but did not attain the levels of resistance found in resistant Pima cultivars (Fig. 1). Far less variation in resistance was observed among Acala and non-Acala Upland cultivars than among Pima cultivars. With few exceptions, ratings in Upland entries ranged between 0.75 and 1.50 for foliar damage and vascular damage, while ratings among Pima entries ranged between 0.25 and 3.50 for foliar symptoms and vascular damage (Fig. 1). Similarly, plant survival among Acala and non-

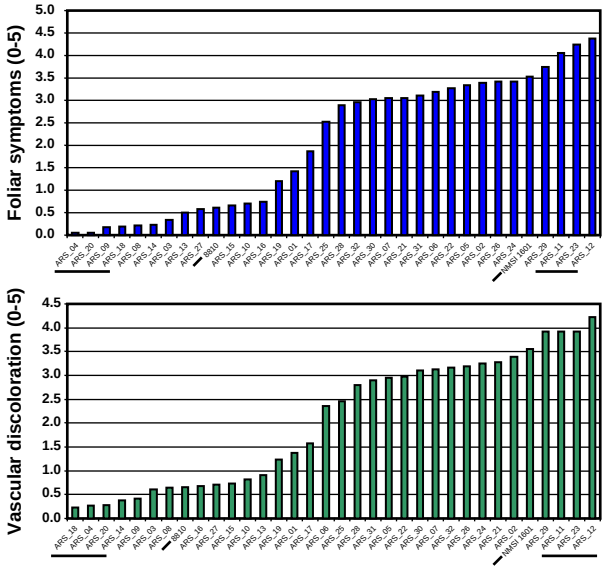

Figure 6. Mean values from three different evaluation sites (one field and two greenhouses) for foliar symptoms and vascular discoloration caused by *Fusarium oxysporum* f.sp. *vasinfectum* race on the parents and 32 Pima (*Gossypium barbadense* L.) recombinant inbred lines (RILs) developed from a cross between NMSI 1601 x 88104. . Foliar symptoms were rated on a scale of 0-5, where 0 = no symptoms and 5 = dead plant. Vascular discoloration was rated on a scale of 0-5, where 0 = no symptoms and 5 = vascular discoloration evident throughout cross-section of the stem.

Acala Upland cottons was generally higher than Pima, ranging between 42% and 95% in Upland entries versus 25% and 95% among Pima entries (Fig. 3). Although Upland cultivars may have higher levels of resistance, the lack of correlation among the three evaluation sites for foliar damage or vascular discoloration indicates that there was far less genetic variation for resistance among the Upland cultivars in the investigation and/or differences were difficult to detect by the disease severity scales used because of asymptomatic foliage and overall agronomic performance of plants in most cases. The inheritance of resistance to FOV race 4 in Acala/Upland cottons is not known. Variation for level of tolerance/resistance to FOV race 4 ranged from 0.58 to 0.99 (R^2), indicating that selection to further improve resistance to Fusarium wilt may be possible within the *G. hirsutum* gene pool.

DISCUSSION

FOV race 4 was first confirmed in California-grown Pima cotton in 2003, followed by two additional sites (in both Pima and Acala/Upland cottons) in 2004. At the time of preparation of this report, at least 15 infested cotton fields have been confirmed in California. The field and greenhouse evaluations conducted in 2003 through 2005 identified sites for Fusarium wilt investigations in San Joaquin Valley, assessed the U.S. cotton gene pool for resistance against race 4, and studied the resistance inheritance in cotton. Preliminary results from this and previous research (field, fungus genetics, greenhouse, and host plant resistance) show the following: a) none of the fields were infested with the two highly virulent Australian strains of FOV (Kim et al., 2005), b) a wide range of FOV strains were identified across different soil types and production regions within the San Joaquin Valley (Kim et al., 2005), and c) FOV race 4 causes foliar disease symptoms and stunting even without damaging root-knot nematode populations.

Most commercial Pima (*Gossypium barbadense*) cultivars grown in California were more susceptible to FOV race 4 (stand loss, stunting, etc.) than Acala and non-Acala Upland cottons (*G. hirsutum*). Some Acala and non-Acala Upland cotton cultivars, however, were still infected by FOV race 4 at levels where the fungus was probably able to reproduce and increase soil inoculum densities. In greenhouse studies, the incidence and severity of Fusarium wilt increased when infected plant residue were incorporated into the soil. Similar observations (the disease became more severe) were observed when cottons were replanted in soil of plots previously planted with the most susceptible cotton cultivar (Wang et al., 1999).

No significant differences for the comparison between field and greenhouse evaluations sites were observed in Pima (Table 4). Both sites were reliable for discriminating susceptible and resistant Pima cultivars (Fig. 4 and 5), but variation in symptom expression was observed for cotton cultivars that showed moderate resistance to FOV race 4, which made it difficult to determine with certainty the type of resistance/tolerance in these cottons, especially for Acala and non-Acala Upland cultivars (Fig. 1 and Table 3). Several researchers (Pfleger and Vaughan, 1971; Del Cid, 1988; Ulloa, 1990) have reported that increasing the inoculum concentration has a direct effect on disease index of susceptible cultivars in seedling screening techniques. The ability of FOV race 4 to cause symptoms varied with its cotton host. One suggestion to permit a better differentiation between moderate (low/high) tolerance/resistance and highly resistant cultivars could be to increase the inoculum levels in order to more closely resemble conditions that would be found in infested fields. In addition, plant host pathogen interactions could be increased or decreased by optimizing environmental factors, such as optimal temperature for fungal growth and host responses (Garber et al., 1979; Ulloa, 1990). Based on this study, different prevailing temperatures and perhaps levels of lighting associated with spring or fall versus summer greenhouse evaluations seemed to affect susceptibility to the disease and relative severity of symptom development (Table 2).

Host-plant resistance is the most economic and effective strategy for Fusarium wilt control in cotton. Based on this study, the resistance of FOV race 4 in Pima cottons is more complete than determined in previous research (Ulloa et al., 2005). This resistance may be determined by single dominant major genes (Fig. 4 and 5) and one or more minor genes that may provide the trangressive segregation observed in the ARS RILs (Fig. 6). Differences detected between F_1 hybrids and the mean of the parents in foliar symptoms and vascular discoloration (Fig. 4) and the bimodal distribution observed on the RIL population (Fig. 5) provide strong evidence for a dominant gene effect. Similar results

were reported by Fahmy (1927) for Fusarium wilt disease in certain Egyptian Sea Island cultivars, and by Mohamed (1963) in cultivars Ashmouni and Menoufi. Two major dominant genes with inter-locus additivity provide a high degree of resistance to Sea Island cotton (Smith and Dick, 1960). Conflicting results have been reported for Acala and non-Acala Upland cottons for resistance to the Fusarium wilt (Smith and Dick, 1960; Kappelman, 1971). It has been difficult to identify/develop highly resistant Acala/Upland cottons to FOV race 4, which suggests that resistance in the *G. hirsutum* gene pool may be more complex than Pima (*G. barbadense*) cottons. The inheritance of the resistance for Upland cottons to Fusarium wilt has been reported as being due to dominant genes with minor modifying genes (Smith and Dick, 1960) or quantitatively inherited and controlled by several major genes and minor modifying genes (Kappleman, 1971). Sources with some level of tolerance/resistance exist in tetraploid cottons and diploid A and D genome-species (Fig. 1). Germplasm in Pima with high levels of resistance to FOV race 4 was identified when certain inoculum levels were tested (field and greenhouse) (Ulloa et al., 2005). After almost a century of breeding for wilt resistance in the United States, it has not been possible to produce a commercially acceptable *G. hirsutum* wilt-immune cultivar (Hillocks, 1992). Even highly tolerant Acala cottons in which growth and yield were not affected, vascular brown discoloration in the plant's xylem was observed that did not produce visible wilting (Fig. 1). Differences were difficult to detect by the disease severity scales used because of asymptomatic foliage and plant overall agronomic performance in most cases. Resistance for Fusarium wilt in Acala and non-Acala Upland cottons may be more complex, and possibly inherited in a quantitative manner by several major genes and minor modifying genes (Kappelman, 1971).

The current genetic base of resistance for commercial Pima cultivars in California is limited. This research has provided information about the status of commercial and improved germplasm of cottons, allowing growers to make informed decisions regarding the choice of cotton cultivar to plant. In addition, this research has identified potentially new improved tolerance/resistance germplasm for release in the near future for public incorporation into California cotton cultivars. Even though disease expression to FOV race 4 has been the most severe in Pima cotton fields, FOV race 4 has the ability to infect Acala and Upland cottons. The ability of FOV race 4 to infect Acala cotton under large-scale field conditions was confirmed in 2003, 2004, and 2005. Several Acala cultivars planted in fields infested with FOV race 4 following a susceptible Pima cultivar displayed significant plant death, stunting, and symptoms (unpublished data). Evaluations of cotton germplasm under field and greenhouse conditions will continue. Development of highly resistant germplasm and the genetic mapping of populations are ongoing in order to develop and/or identify molecular markers to assist in the breeding process for FOV resistance in cotton.

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