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Evaluation of Fusarium Wilt Resistance in Six Upland Cotton Germplasm Lines

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ABSTRACT

Fusarium wilt of cotton, caused by the soilborne fungus *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), is a widespread and devastating disease occurring in most cotton-growing regions of the world. The most successful strategy to manage Fusarium wilt is the use of resistant cultivars. Recently, two sources of germplasm, MD25-26ne and MD25-27, which have superior yield, fiber quality, and resistance against Fusarium wilt and root-knot nematodes (*Meloidogyne* spp.), were released. The purpose of this study was to evaluate the response of eight Upland cotton (including MD25-26ne and MD25-27) and two Pima cultivars (susceptible and resistant controls) against seven genotypes of FOV (races 1, 2, 3, 4, and 8 and genotypes LA108 and LA140) in a greenhouse trial. To inoculate plants, roots of 2-wk-old seedlings of each cultivar were dipped for 4 min in a conidial suspension (10^6 conidia/mL) of each FOV genotype. Based on dry shoot weights and extent of vascular discoloration; Phytogen 800 followed by MD25-26ne and MD25-27 were the most resistant cultivars against multiple genotypes of FOV. FOV race 4 caused mild symptoms on PHY 800, PHY 98M-2983, MD25-26ne, and MD25-27 but caused severe symptoms on DP 744 and Rowden. In addition, Rowden was highly susceptible to FOV races 1, 2, and 8 and LA108 and LA140. Phytogen elite lines PHY x1, PHY x2, and PHY x3 were moderately susceptible to multiple genotypes of FOV. These data indicate that PHY 800, MD25-26ne, and MD25-27 can be recommended for planting in fields with previous histories of Fusarium wilt and can be used in breeding programs as sources of resistance to Fusarium wilt.

Fusarium wilt of cotton, caused by the soilborne fungus *Fusarium oxysporum* Schlechtend.:Fr f. sp. *vasinfectum* (Atk.) W.C. Snyder & H.N. Hans (FOV), is an increasingly important disease of cotton in the United States (U.S.). The disease was first described by Atkinson in the U.S. in 1892 (Atkinson, 1892). Currently, there are six nominal races of *F. oxysporum* f. sp. *vasinfectum*: 1, 2, 3, 4, 6, and 8, as well as many unnamed genotypes worldwide (Holmes et al., 2009; Davis et al., 2006; Kim et al., 2005; DeVay, 1986). Races 1, 2, 3, 6, and 8 are generally mildly virulent but can cause severe symptoms if plants are also infected with root knot nematodes (*Meloidogyne* spp.) (Bennett et al., 2011; Hutmacher et al., 2005; Kim et al., 2005; Ulloa et al., 2006). Many genotypes of FOV are widespread, but in the U.S. race 4, which is highly virulent on many cotton cultivars, is apparently restricted to California (Bennett et al., 2013; Bennett and Colyer, 2010; Bennett et al., 2008; Kim et al., 2005). Race 4 is of great concern to growers because most cotton cultivars lack resistance to this race. In addition to FOV race 4, two virulent genotypes of FOV in Australia and two genotypes (LA108 and LA140) in the southeastern United States cause severe symptoms in cotton in the absence of the root-knot nematode (*Meloidogyne* spp.) (Bennett et al., 2011; Holmes et al., 2009; Ulloa et al., 2006; Hutmacher et al., 2005; Kim et al., 2005; Allen and Lonergan, 2000).

The use of resistant cultivars to manage Fusarium wilt has been the most successful management strategy. For example, Phytogen 800, a Pima cotton cultivar resistant to FOV race 4, is planted in California in fields where the population of FOV race 4 is high. Phytogen 800 is among the few cotton lines (SJ-07P-FR01, SJ-07P-FR02, SJ-07P-FR03, and SJ-07P-FR04) that are sources of resistance against FOV race 4 (Ulloa et al., 2009). Recently, two noncommercial cotton lines, MD25-26ne and MD25-27, which have superior yield, fiber quality, and resistance against Fusarium wilt and root-knot nematodes, were released by the United States Department of Agriculture Agricultural Research Service (USDA-ARS) (Meredith, 2013). These lines were reselections from MD 25 germplasm that, based on the Regional Breeders Testing Network, showed promise against Fusarium wilt in naturally infested soil where genotypes of FOV were not identified (Meredith, 2013). Resistance against Fusarium wilt

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was also documented, based on field observations, in Phytogen Seed Company's (Dow Chemical Company, Midland, MI) elite Upland germplasms PHY 98M-2983, PHY x1, PHY x2, and PHY x3 (T. Anderson, personal communication). However, the genotypes of FOV to which these lines are resistant are unknown. The purpose of this study was to evaluate the response of eight Upland cotton and two Pima cultivars against seven genotypes of FOV (races 1, 2, 3, 4, and 8 and genotypes LA108 and LA140). The susceptibility or resistance of MD25-26ne, MD25-27, PHY 98M-2983, PHY x1, PHY x2, and PHY x3 was evaluated. Phytogen 800 was used as a resistant check against all genotypes of FOV; Rowden was used as a susceptible check against all genotypes of FOV. Delta Pine 744 (DP 744) was used as a susceptible check for FOV race 4. In addition, Phytogen 72 was used as a susceptible control for FOV race 1 and 2 and LA108 and LA140 (Holmes et al., 2009).

MATERIALS AND METHODS

FOV isolates representing races 1, 2, 3, 4, and 8, were CA10, TX34, CA11, CA14, and CA1, respectively (Table 1). All isolates were maintained on 9 cm-diameter petri plates containing acidified potato dextrose agar (APDA) under constant fluorescent light for 7 d at room temperature (22 ± 1°C). Each isolate originated from single spore cultures stored on filter paper at 4°C. Conidial suspensions of FOV were prepared from 1-wk-old APDA plates by flooding with 15 mL autoclaved deionized water, dislodging the conidia with a glass slide and filtering the suspension through four layers of sterile cheesecloth. The concentration of the conidial suspension was quantified using an Improved Neubauer hemacytometer (Hausser Scientific, Horsham, PA), and adjusted to $1x10^6$ conidia per ml with sterile deionized water.

Seeds of eight Upland (*Gossypium hirsutum* L.) cultivars, PHY 72 (Dow AgroSciences, Midland, MI), PHY 98M-2983, PHY x1, PHY x2, PHY x3, Rowden, MD25-26ne (USDA-ARS, Washington, D.C.), and MD25-27, and two Pima cultivars, PHY 800 and DP 744 (Monsanto Company, Creve Coeur, MO), were sown in 128 cell-seedling trays with 3.5cm x 3.5cm x 6.5cm cells containing autoclaved UC potting mix (Hao et al., 2009). To inoculate plants, roots of 2-wk-old cotton seedlings of each cultivar removed from the cells were rinsed with deionized water to remove soil then dipped for four min in a conidial suspension (10^6 conidia per ml) of each FOV genotype or water (non-infected control). Seedlings were then transplanted individually into plastic pots 10 cm in diameter (750ml) containing UC potting mix. The experimental design was a randomized block with ten replications (blocks) in the greenhouse on a 13-h photoperiod provided by high-pressure sodium bulbs with daytime temperatures ranging from 29-32°C and nighttime temperatures ranging from 18-21°C.

Foliar symptoms, vascular discoloration, and dry shoot weight for each plant were measured five weeks post-inoculation. Foliar symptom ratings were based on the following scale: 1 = no symptoms, 2 = chlorosis and/or wilt restricted to cotyledons or first leaf, 3 = chlorosis and/or wilt extended beyond the first leaf, 4 = chlorosis and/or wilt of whole plant, 5 = plant death (Holmes et al., 2009; Kim et al., 2005). For vascular discoloration rating, stems above the soil line were cut in cross section and longitudinally. Vascular discoloration ratings were based on the following scale: 1= no symptoms, 2 = light brown, streaky vascular discoloration, 3 = dark brown vascular discoloration, 4 = plant death (Holmes et al., 2009; Kim et al., 2005). To measure dry weight, individual plants were cut in cross section at the

Table 1.Source of genotypes of *Fusarium oxysporum* f. sp. *vasinfectum* included in this study.

Genotype	Geographic origin	Source
CA10, race 1	Kern County, CA	R. M. Davis (Kim et al., 2005)
TX34, race 2	Texas	J. E. Woodward
CA11, race 3	Tulare County, CA	R. M. Davis (Kim et al., 2005)
CA14, race 4	Fresno County, CA	R. M. Davis (Kim et al., 2005)
CA1, race 8	Tulare County, CA	J. E. DeVay (Kim et al., 2005)
LA108	Georgia	P. Colyer (Holmes et al., 2009)
LA140	Arkansas	P. Colyer (Holmes et al., 2009)

soil line and whole shoots were dried in an oven at 60°C for 7 d. To confirm the presence of FOV, crown tissue below ground from two symptomatic plants of each cultivar was washed with anti-bacterial soap, cut into four ~0.5cm pieces per plant, immersed in 0.6% sodium hypochlorite (10% bleach) for 1 min, and placed on APDA plates. After 5 d, DNA was extracted from *Fusarium*-like colonies, and FOV were identified with PCR using translation elongation factor (EF-1α) primers EF-1 (5′ATGGGTAAGGAAGACAAGAC) and EF-2 (5′GGAAGTACCAGTGATCATGTT) (O'Donnell et al., 1998). The experiment was repeated once.

Data were analyzed using the Statistical Analysis Software 9.3 general linear model (SAS Institute, Cary, NC). Plant shoot dry weight and vascular discoloration data for each plant were evaluated using analysis of variance. Where significant F values were obtained, the significance of mean differences was assessed using Tukey's HSD test. There was no significant difference in vascular discoloration and shoot dry weight between the two independent trials; therefore, data from the two trials were analyzed together. There was a significant interaction in the vascular discoloration and shoot dry weight between cultivars and genotype.

RESULTS AND DISCUSSION

Vascular discoloration ratings for PHY 800 were relatively low for all the FOV genotypes tested (Table 2). The average vascular discoloration rating for PHY 800 was the lowest (1.0), followed by MD25-26ne (1.9) and MD25-27 (1.9). Vascular discoloration ratings for Rowden were the highest for all the FOV genotypes tested except for race 3 (Table 2). Race 8 caused more vascular discoloration (vascular ratings between 3 and 4) in PHY x3 than in MD25-27, MD25-26ne, PHY x1, PHY x2, and PHY 98M-2983. DP 744 was among the most resistant cultivars to race 8. In addition, race 8 caused more vascular discoloration in MD25-26ne (2.6) than in MD25-27 (1.4). Genotypes LA108 and LA140 caused vascular discoloration of greater magnitude in MD25-27 than MD25-26ne. FOV races 1, 2, 4, and 8 and LA108 and LA140 caused relatively high vascular discoloration (ratings between 3 and 4) in Rowden and PHY 72. FOV Race 1 and 2 caused high vascular discoloration (ratings between 3 and 4) in Phytogen elite lines PHY x1, PHY x2, and 98M-2983 but not in PHY x3. In general, FOV race 4 and LA108 caused relatively severe vascular discoloration in DP 744, PHY x1, PHY x2, and PHY x3. LA140 caused high vascular discoloration in PHY x1, PHY x3, and 98M-2983 but not in PHY x2. In general, FOV Race 3 did not cause significant vascular discoloration or reduction in shoot dry weight for any of the cultivars tested (Table 3). Similar results were observed with foliar ratings (data not shown). FOV was re-isolated and confirmed in all sub-samples of FOV colonies transferred to APDA (data not shown).

Table 2. Effect of *Fusarium oxysporum* f. sp. *vasinfectum* on vascular discoloration of select cotton.

Genotype	Cultivar									
	DP 744	Rowden	MD25-26ne	MD25-27	PHY 800	PHY 72	PHY 98M-2983	PHY x1	PHY x2	PHY x3
None	1.0[z] d[y]	1.0 c	1.0 c	1.0 d	1.0 a	1.0 d	1.0 c	1.0 d	1.0 c	1.0 e
Race 1	3.9 a	4.0 a	1.7 b	1.7 b	1.0 a	3.5 b	3.5 a	3.5 ab	3.4 a	2.7 cd
Race 2	2.8 b	4.0 a	1.8 b	1.7 bc	1.0 a	3.5 b	3.7 a	3.1 b	3.4 a	2.6 d
Race 3	1.0 d	1.2 c	1.0 c	1.0 d	1.0 a	1.0 d	1.1 c	1.0 d	1.0 c	1.0 e
Race 4	4.0 a	3.5 b	2.6 a	2.0 b	1.0 a	3.4 bc	2.6 b	3.3 ab	3.3 a	3.1 bc
Race 8	1.3 cd	4.0 a	2.6 ab	1.4 cd	1.1 a	3.0 c	2.6 b	2.1 c	2.1 b	4.0 a
LA108	3.5 a	4.0 a	2.3 a	3.1 a	1.0 a	4.0 a	4.0 a	3.8 a	3.7 a	4.0 a
LA140	1.8 c	4.0 a	1.3 bc	2.7 b	1.1 a	3.5 b	3.8 a	3.1 b	2.0 b	3.5 ab

[z] **Data from two trials were combined. Values represent average vascular discoloration ratings of ten replications in each of two independent trials. Vascular discoloration ratings are based on the following scale: 1= no symptoms, 2 = light brown, streaky vascular discoloration, 3 = dark brown vascular discoloration, 4 = plant death.**

[y] **Means of vascular discoloration ratings followed by a common letter in the same column are not significantly different according to *Tukey's HSD* at *P* = 0.05.**

Dry shoot weights for Phytogen elite lines PHY 72, PHY x1, PHY x2, and PHY x3 and Rowden showed similar patterns of susceptibility (Table 3). The dry shoot weights for these cultivars were significantly reduced relative to the noninoculated control by races 1, 2, 4, and 8, and LA108 and LA140. In addition, dry shoot weights for PHY 98M-2983 were significantly reduced relative to the noninoculated control by races 1, 2, and 8, and LA108 and LA140 (Table 3). However, dry shoot weights of PHY 98M-2983 were not affected by FOV race 4 (Table 3) . Dry shoot weights for MD 25-26ne and MD 25-27 were significantly reduced by races 4 and 8 and LA108 and LA140 relative to the noninoculated control (Table 3). Dry shoot weights for DP 744 were significantly reduced by races 1, 2, and 4, and LA108. Dry shoot weights were moderately reduced for PHY x3 by races 1 and 2, PHY x1 and 98M-2983 by races 4 and 8, and PHY x2 by race 8.

Of the cultivars tested, PHY 800 followed by MD25-26ne and MD25-27 were the most resistant cultivars against multiple genotypes of FOV. The average vascular discoloration ratings for these cultivars were less than 2. Based on these observations and previous research, a vascular discoloration rating between 1 and 2 indicated resistance to FOV, while a rating between 3 and 4 indicated susceptibility (Abd-Elsalam et al., 2014; Holmes et al., 2009; Kim et al., 2005). Thus, MD25-26ne and MD25-27 were moderately resistant against multiple genotypes of FOV. This supports the observation made by the Regional Breeders Testing Network that MD25-26ne and MD25-27 are resistant against Fusarium wilt. Since MD25-26ne and MD25-27 were moderately resistant against multiple genotypes of FOV, they grew well in soil at various locations where the genotypes of FOV were not identified (Meredith, 2013). In addition, FOV race 4 caused mild symptoms on PHY 800, PHY 98M-2983, MD25-26ne, and MD25-27. Resistance between MD25-26ne and MD25-27 to different genotypes of FOV varied. For example, based on vascular discoloration ratings and dry shoot weights, MD25-27 was more susceptible to LA108 and LA140 than MD25-26ne. This study also supports the observation that some Phytogen elite lines are resistant to Fusarium wilt. For example, Phytogen 98M-2983 and PHY x1 were moderately resistant to race 4 and 8, while PHY x3 was moderately resistant to race 1 and 2. Although only one isolate of each FOV genotype was tested, Kim et al. (2005) reported that different isolates of the same genotype caused similar disease severity on a particular cultivar. The knowledge of which cultivars are resistant enables growers to plant the proper cultivar if the identities of FOV in the field are known. In addition, MD25-26ne, MD25-27, and the Phytogen elite lines can be used as sources of resistance in breeding programs for different FOV genotypes. DP 744 and Rowden were the most susceptible cultivars to FOV race 4. Furthermore, Rowden showed extreme susceptibility to FOV race 1, 2, and 8 and LA108 and LA140, suggesting that Rowden is an appropriate check in future studies.

Table 3. Effect of *Fusarium oxysporum* f. sp. *vasinfectum* on plant shoot dry weight of select cotton cultivars.

Genotype	Cultivar									
	DP 744	Rowden	MD25-26ne	MD25-27	PHY 800	PHY 72	PHY 98M-2983	PHY x1	PHY x2	PHY x3
None	2.9[z] a[y]	2.7 b	3.5 a	3.4 a	4.1 a	3.7 a	3.7 a	3.9 a	3.9 a	3.9 a
Race 1	1.1 b	0.1 b	3.0 a	3.2 a	4.0 a	0.4 d	0.2 c	0.3 cd	0.4 e	1.9 b
Race 2	1.8 b	0.2 b	2.9 a	3.2 a	4.1 a	0.3 d	0.3 c	0.6 cd	0.9 de	2.1 b
Race 3	2.9 a	2.6 b	3.5 a	3.3 a	4.2 a	3.5 a	3.5 a	3.8 a	3.7 ab	3.7 a
Race 4	0.2 c	0.2 b	2.1 b	1.9 b	4.1 a	2.8 ab	3.4 a	2.0 b	2.1 c	1.0 c
Race 8	2.8 a	0.1 b	1.6 b	2.8 b	4.0 a	1.9 c	1.2 b	2.5 b	3.0 b	0.2 d
LA108	0.7 c	0.1 b	2.5 b	1.3 b	4.0 a	0.1 d	0.2 c	0.1 d	0.1 e	0.2 d
LA140	2.8 a	0.1 b	2.9 b	2.0 b	4.1 a	0.7 d	0.2 c	1.0 c	2.2 d	0.3 cd

[z] Data from two trials were combined. Values represent average plant shoot dry weights (g) of ten replications in each of two independent trials.

[y] Means of plant shoot dry weights followed by a common letter in the same column are not significantly different according to *Tukey's HSD* at $P = 0.05$.

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DISCLAIMER

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the University of California.

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