Characterization of California Isolates of *Fusarium oxysporum* f. sp. *vasinfectum*

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ABSTRACT

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Thirty isolates of *Fusarium oxysporum* f. sp. *vasinfectum* from California, Australia, China, and the American Type Culture Collection were characterized by partial sequences of translational elongation factor (EF-1 α), phosphate permase (PHO), and beta-tubulin (BT) genes, restriction digests of the intergenic spacer (IGS) region of nuclear rDNA, and pathogenicity tests. Based on phylogenetic analysis of combined sequences of EF-1 α , PHO, and BT genes, California isolates represented four lineages. Lineage I contained race 3, lineage II contained races 1, 2, and 6, lineage III contained race 8, and lineage IV contained race 4. The Australian isolates formed a strongly supported independent clade. There were nine haplotypes based on restriction digests of the IGS region. In greenhouse pathogenicity tests with California isolates, those from the race 4 lineage were highly aggressive on certain Pima cotton (*Gossypium barbadense*) cultivars and less aggressive on Upland cotton (*Gossypium hirsutum*) cultivars. All isolates belonging to the other lineages caused relatively mild symptoms on both Pima and Upland cultivars. This is the first report of the occurrence of races 3, 4, and 8 in California.

Additional keywords: Fusarium wilt

Fusarium wilt of cotton, caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *vasinfectum* (Atk.) W.C. Snyder & H.N. Hans, is a widespread disease in the United States and most cotton-growing areas of the world. The disease was first identified in California in 1960 (9), where until recently, it caused mild symptoms on widely grown Upland cotton (*Gossypium hirsutum* L.) cultivars except when plants were also infected with root-knot nematodes (*Meloidogyne incognita* (Kofoid & White) Chitwood). Coinfected plants were stunted more than those exposed to the root-knot nematode or *F. oxysporum* f. sp. *vasinfectum* alone, and suffered greater yield losses. Growers successfully managed the disease with root-knot nematode tolerant cultivars, crop rotation, and fumigation for nematode control.

Recently, two developments have raised concerns about Fusarium wilt of cotton in the San Joaquin Valley of California. The first was the emergence of extremely virulent strains of *F. oxysporum* f. sp. *vasinfectum* in Australia. These strains caused extensive damage to Australian cotton

despite the absence of root-knot nematodes (6). When the California dairy industry began importing hundreds of thousands of metric tons of Australian cotton seed for feed in the late 1990s, cotton growers worried about the possible introduction of these strains into local fields. Because of this concern, the California Cotton Ginners and Growers Association asked the California Department of Food and Agriculture to halt the importation of cotton seed from Australia. In the decision to allow seed to continue to enter California, it was noted that *F. oxysporum* f. sp. *vasinfectum* isolates resident in California had not been characterized. In the last few years, severe economic losses to Fusarium wilt in some Pima cotton (*Gossypium barbadense* L.) fields in the San Joaquin Valley, even in the absence of root-knot nematodes, also raised concerns. Because of these issues, an investigation was needed to determine the diversity and locality of California strains of *F. oxysporum* f. sp. *vasinfectum*.

Although eight races have been described throughout the world, only race 1 and race 2 are known to occur in the United States (7,16). These races are classified based on pathogenicity tests on different cotton species, *G. hirsutum*, *G. barbadense*, and *G. arboreum* L. (2,3,4,11), and by their pathogenicity on alfalfa, soybean, and tobacco (3). However, pathogenicity of *F. oxysporum* f. sp. *vasinfectum* on noncotton hosts is difficult to consistently reproduce (Y. Kim, unpublished). Variations in environmental conditions, inoculation levels, and subject-

tive evaluation methods may lead to variable results.

Skovgaard et al. (15) studied *F. oxysporum* f. sp. *vasinfectum* using multi-gene genealogies and determined that the eight races were nonmonophyletic with two independent evolutionary origins. Races fell into four lineages that also correlated with geographic origins. Fernandez et al. (8) used rDNA and mtDNA restriction fragment length polymorphism (RFLP) to separate *F. oxysporum* f. sp. *vasinfectum* isolates into four rDNA haplotypes and seven mtDNA haplotypes.

The objective of this study was to characterize isolates of *F. oxysporum* f. sp. *vasinfectum* using both molecular markers and pathogenicity tests with emphasis on the diversity of the organism's genotypes in the San Joaquin Valley of California.

MATERIALS AND METHODS

Isolates. Thirty isolates of *F. oxysporum* f. sp. *vasinfectum* from California, Australia, China, and the American Type Culture Collection (ATCC) were characterized (Table 1). Twenty-two isolates were obtained from individual fields in the San Joaquin Valley of California where plants exhibited symptoms typical of Fusarium wilt, including stunted growth, vascular discoloration, and leaf wilt, chlorosis, necrosis, and ultimately abscission. Cultures from single spores were stored on filter paper at 4°C.

DNA extraction, amplification, purification, and sequencing. In order to obtain DNA from cultures, *F. oxysporum* f. sp. *vasinfectum* isolates were grown for 4 to 5 days at room temperature on a shaker in 250-ml Erlenmeyer flasks containing 100 ml of sterile medium consisting of (per liter) D-glucose (20.7 g), DL-asparagine (1.2 g), K₂HPO₄·3H₂O (1.2 g), MgSO₄·7H₂O (0.5 g), yeast extract (0.5 g), and NaCl (0.1 g). The mycelium was harvested by filtration through Miracloth and washed with sterile distilled water. To obtain total genomic DNA, approximately 200 mg of mycelia was ground into a powder in liquid nitrogen. DNA extraction was performed with a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) using the manufacturer's protocol. DNA was stored at -20°C prior to use.

Fragments of three nuclear genes, the translation elongation factor (EF-1 α), phosphate permase (PHO), and beta-tubulin (BT) genes, were sequenced (see Table 2 for primers). Amplification was carried out in 50- μ l reactions containing 3

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µl of DNA, 50 mM KCl, 10 mM Tris-HCl (pH 9), 1% Triton X-100, 2.5 mM each of dATP, dCTP, dGTP, and dTTP, 25 mM MgCl₂, 50 mM of each primer, 1 unit of *Taq* polymerase, and 32.6 µl of milliQ water. Amplification was carried out in a thermocycler (PTC-100, MJ Research, Watertown, MA) as follows: 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min for 40 cycles, and a final extension of 5 min at 72°C for EF-1α; 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min 30 s for 35 cycles for BT; and 97°C for 1 min of initial denaturing followed by 35 cycles of 96°C for 30 s, 50°C for 1 min, and 72°C for 1 min followed by a final extension time of 10 min at 72°C for PHO. Negative controls (no template DNA) were included in every assay. Amplicons were visualized on 1.5% agarose gels by staining with ethidium bromide. The remaining product was purified using QIAquick polymerase chain reaction (PCR) Purification Kits according to the manufacturer's instructions (Qiagen). The purified DNA fragment was then sequenced in both directions using automated sequencing at the UC Davis DBS Automated DNA Sequencing Facility. Primers used for sequencing were the same as those used in amplification.

DNA sequences were aligned using the pileup option in Accelrys Seqweb (version 2.1, Accelrys, Madison, WI) and were manually adjusted (MacClade Phylogenetic Software 3.0, Sinauer Associates Inc., Sunderland, MA). Phylogenetic trees were constructed using maximum parsimony methods (PAUP 4.0b10, Sinauer Associates). All characters were run unordered with equal weight, and gaps were treated as missing data. In all phylogenetic trees, a saprophytic isolate of *Fusarium oxysporum* from the rhizosphere of melons growing in the San Joaquin Valley was included as an outgroup. Statistical support for the most parsimonious tree was derived by running 1,000 bootstrap replicates. The final alignment and the tree obtained are deposited in TreeBASE (accession number SN1988).

Restriction digestion analysis of the intergenic spacer (IGS) region of nuclear rDNA. The IGS region of nuclear rDNA was amplified with the conserved primers LR12R and CNS1, the complement of primer NS1, with priming sites at the 3' end of the 28S gene and the 5' end of the 18S gene, respectively (1,18). Cycling conditions were 94°C for 1 min, 55°C for 2 min, and 72°C for 2.5 min for 40 cycles followed by an extension time at 72°C for 10 min. The amplified product was then digested by the restriction enzymes *Sac*II (New England BioLabs, Beverly, MA) or *Rsa*I (Promega, Madison, WI) at 37°C for 2 h. Digested products were separated by electrophoresis at 60 volts for 2.5 to 3 h on a 2% agarose gel. Gels were stained by ethidium bromide and examined with a UV transillu-

minator. A 100-bp ladder (Promega) was included in the gels to estimate the size of restriction fragments.

Greenhouse pathogenicity tests. Pathogenicity of California *F. oxysporum* f. sp. *vasinfectum* isolates was determined by inoculating cotton cultivars PhytoGen-72 (*G. hirsutum*) and S-7 (*G. barbadense*). Roots of 2-week-old seedlings were dipped in water or a spore suspension of 1×10^5 conidia per ml for 2 min. Spore suspensions were prepared by flooding colonized 2-week-old cultures on potato dextrose agar with water, scraping off the

spores, and filtering the spore suspension through four layers of cheesecloth.

Two Upland cultivars, PhytoGen-72 and CPCSD Maxxa, and two Pima cultivars, S-7 and DP-744, were inoculated with California *F. oxysporum* f. sp. *vasinfectum* isolates and representative ATCC race 1, 2, 3, or 4 isolates to compare the pathogen's aggressiveness on Upland and Pima cotton. Disease severity and vascular discoloration were measured approximately 4 weeks after inoculation since it was noted in preliminary tests that visible symptoms caused by relatively less aggressive iso-

Table 1. Race, lineage, and source of isolates of *Fusarium oxysporum* f. sp. *vasinfectum* included in this study

Isolate	Lineage ^a	Geographic origin	Source ^b
CC-612-3, race 3 (ATCC 16612)	I	Egypt	G. M. Armstrong
CA-3	I	Tulare Co., CA	S. N. Smith/J. E. DeVay
CA-11	I	Tulare Co., CA	This study
CC-421-1, race 1 ^c (ATCC 16421)	II	United States	G. M. Armstrong
CC-611-2, race 2 ^c (ATCC 16611)	II	United States	G. M. Armstrong
CC-198-6, race 6 ^c (ATCC 36198)	II	Brazil	G. M. Armstrong
CA-2	II	Tulare Co., CA	S. N. Smith/J. E. DeVay
CA-4	II	Tulare Co., CA	S. N. Smith/J. E. DeVay
CA-5	II	Kern Co., CA	S. N. Smith/J. E. DeVay
CA-6	II	Kern Co., CA	S. N. Smith/J. E. DeVay
CA-8	II	Kings Co., CA	S. N. Smith/J. E. DeVay
CA-10	II	Kern Co., CA	This study
31665, race 8	III	China	K. O'Donnell
CA-1	III	Tulare Co., CA	S. N. Smith/J. E. DeVay
CA-7	III	Kings Co., CA	S. N. Smith/J. E. DeVay
CC-613-4, race 4 (ATCC 16613)	IV	India	G. M. Armstrong
CA-9	IV	Fresno Co., CA	This study
CA-12	IV	Fresno Co., CA	This study
CA-13	IV	Fresno Co., CA	This study
CA-14	IV	Fresno Co., CA	This study
CA-15	IV	Fresno Co., CA	This study
CA-100	IV	Fresno Co., CA	This study
CA-104	IV	Fresno Co., CA	This study
CA-S6	IV	Tulare Co., CA	This study
CA-S7	IV	Tulare Co., CA	This study
CA-S8	IV	Fresno Co., CA	This study
CA-S9	IV	Fresno Co., CA	This study
CA-S12	IV	Fresno Co., CA	This study
Aust16	V	Cecil Plains, Australia	S. Bentley
Aust 19	V	Boggabilla, Australia	S. Bentley

^a Lineage was determined from combined translational elongation factor (EF-1α), phosphate permease (PHO), and beta-tubulin (BT) sequence data.

^b Isolates from S. N. Smith/J. E. DeVay were collected from 1975 to 1988.

^c Races 1, 2, and 6 were placed in the same lineage according to sequence analysis.

Table 2. Primers used in this study

Locus	Primer sequence	Reference
Translational elongation factor (EF-1α)		
EF-1	ATGGGTAAGGAAGACAAGAC	13
EF-2	GGAAGTACCAGTGATCATGTT	13
Beta-tubulin (BT)		
BT 3	CGTCTAGAGGTACCCATACCGGCA	17
BT 5	GCTCTAGACTGCTTTCTGGCAGACC	17
Phosphate permease (PHO)		
PHO 1	ATCTTCTGGCGTGTTATCATG	14
PHO 6	GATGTGGTTGTAAGCAAAGCCC	14
Intergenic spacer (IGS) region		
LR12R	CTGAACGCCTCTAAGTCAGAA	10
CNS1	GAGACAAGCATATGACTAC	1,18

lates started to diminish about 5 weeks after inoculation. Some plants of certain cultivars inoculated with aggressive isolates started to die about the same time. Individual plants were rated for disease severity based on the following scale: 1 = no symptoms, 2 = chlorosis and/or wilt restricted to cotyledons or first leaf, 3 = similar symptoms extended beyond the first leaf, 4 = severe symptoms with the whole plant affected, 5 = plant dead (12). Stems above the soil line were cut in cross section and longitudinally to note vascular discoloration. Vascular discoloration ratings were graded on the following scale: 1 = no symptoms, 2 = light brown, streaky vascular discoloration, 3 = dark brown vascular discoloration, 4 = plant dead. Shoot and root dry weights were measured after they were dried in an oven at 50°C for 3 to 4 days.

Each treatment was replicated four times in a random complete block design with one plant per pot, and each experiment was repeated once. Greenhouse tem-

peratures ranged between 18 and 24°C. Australian *F. oxysporum* f. sp. *vasinfectum* isolates could not be used in pathogenicity tests due to quarantine regulations.

Data were analyzed with PROC GLM and CONTRAST statements in SAS version 8.1 (SAS Institute, Cary, NC).

RESULTS

Sequence analysis. Amplification of the EF-1 α gene yielded a single PCR product with an approximate length of 700 bp. A total of 654 nucleotides was analyzed with 71 variable sites; 17 were phylogenetically informative. Unweighted maximum parsimony analysis of the EF-1 α gene data yielded two most parsimonious trees with five clades each.

Amplification of the BT gene yielded a single PCR product with an approximate length of 600 bp. A total of 406 nucleotides was analyzed with 10 variable sites; two were phylogenetically informative. Unweighted maximum parsimony analysis of the BT gene data yielded one most par-

simonious tree with four clades (data not presented).

Initial amplifications of the PHO gene yielded multiple PCR products with approximate lengths of 950, 750, and 450 bp. The annealing temperature was then increased from 50 to 53°C in order to obtain a single product of 950 bp. A total of 514 nucleotides was analyzed with 29 variable sites; five were phylogenetically informative. Unweighted maximum parsimony analysis of the PHO gene data yielded one most parsimonious tree with three clades (data not presented). When all three genes were analyzed as a combined data set, parsimony analysis revealed one most parsimonious tree with five distinct clades (Fig. 1). Isolates from California with identical sequences are represented by a single specimen.

Restriction enzyme digestion analysis.

RsaI digestion of the amplified IGS PCR product revealed approximately nine bands larger than 300 bp that ranged in size from approximately 358 to 1,358 bp (Table 3). Seven unique banding patterns revealed polymorphisms useful in classifying isolates (Fig. 2). *ScrfI* digests of the amplified IGS PCR product revealed six bands that ranged in size from approximately 230 to 670 bp. Four unique banding patterns revealed polymorphisms also useful in distinguishing isolates (Fig. 3). On the basis of the restriction patterns observed with the enzymes *RsaI* and *ScrfI* (Table 3), nine IGS haplotypes were identified (Table 4).

Greenhouse pathogenicity tests. All Californian *F. oxysporum* f. sp. *vasinfectum* isolates were pathogenic on either 'Phytogen-72' or 'S-7', although there was a range of symptoms and level of aggressiveness. Symptoms included cotyledon drop, leaf chlorosis and necrosis, plant wilt, and sometimes death. Aggressive isolates, such as CA-14, typically produced symptoms within 10 days of inoculation and caused a cessation of growth. Cotyledons became flaccid, turned yellow, then brown, and abscised. Plants started to die about 3 to 4 weeks after inoculation. On older plants, symptoms usually began on the margins of lower leaves. As veins darkened, leaves became partly chlorotic and sometimes abscised. Vascular discoloration usually occurred in the tap root and in the lower part of the stem approximately 2 to 5 cm above the soil level. Relatively less aggressive isolates often caused a slight yellowing of cotyledons and light vascular discoloration, but these symptoms became less apparent as the plants aged. *F. oxysporum* f. sp. *vasinfectum* was reisolated from all plants exhibiting wilt symptoms.

Most isolates used in this study caused mild symptoms on the Pima and Upland cultivars included in the pathogenicity tests (Table 5). Isolates from lineage IV (race 4), however, caused symptoms on both Pima cultivars that were significantly more

Fig. 1. Single most parsimonious tree generated from combined analysis of translational elongation factor, beta tubulin, and phosphate permease gene sequences (CI = 0.9640, RI = 0.9216). The outgroup is a saprophytic *Fusarium oxysporum* (isolate 1502). Five lineages (I to V) of *Fusarium oxysporum* f. sp. *vasinfectum* races are identified by brackets. Numbers of changes are noted above branches. Bootstrap frequencies from 1,000 replications are noted below branches.

severe than symptoms caused by other isolates. In contrast, isolates from lineage IV and all other isolates caused similar symptoms on the Upland cultivars (except on one Upland cultivar in one of the two trials, where isolates in lineage IV caused less intense symptoms). In general, there were few differences among varietal reactions caused by isolates in lineages I, II, and III. Regardless of cotton cultivar, plant weights were generally reduced by all isolates according to single degree orthogonal contrasts (Table 6). Lineage IV isolates consistently caused greater growth reductions of both Pima cultivars than other isolates, but did not cause greater growth reductions in the Upland cultivars. Isolates in lineage II caused significantly smaller plant weights than isolates in lineages I and III in two of the four tests with Upland cultivars. Because the degree of vascular discoloration caused by the various isolates was almost identical to the extent of symptom expression by the same isolates, the data are not included here.

The ATCC race 4 isolate did not cause *Fusarium* wilt symptoms in any of the experiments, nor did it colonize any of the four cotton cultivars (it was not recovered from the asymptomatic plants). Therefore, data from this isolate were not included in greenhouse pathogenicity test results. The replicated trials were analyzed separately due to significant differences in variance between the trials and significant interactions among trials, cultivars, and isolates.

DISCUSSION

Several distinct lineages, generally corresponding to known races of *F. oxysporum* f. sp. *vasinfectum*, were identified by molecular markers and gene sequencing. The complete collection of California and Australian isolates was classified into five major lineages based on sequence differences in EF-1 α , PHO, and BT genes and into nine IGS haplotypes. California isolates were observed in all lineages except for the Australian lineage, indicating a wide diversity of strains in California. This is the first report of the occurrence of races 3, 4, and 8 in California.

Our conclusion that *F. oxysporum* f. sp. *vasinfectum* is polyphyletic is consistent with those of Skovgaard et al. (15), who found four lineages within the pathogen

based on partial sequences of EF-1 α , mtSSU rDNA, PHO, and NIR genes of eight races (excluding any Australian strains). The Australian isolates included in

Fig. 2. *RsaI* restriction patterns of amplified intergenic spacer (IGS) polymerase chain reaction (PCR) product of *Fusarium oxysporum* f. sp. *vasinfectum* isolates. Lanes 1 to 11: 31665 (race 8), ATCC 36198 (race 6), ATCC 16613 (race 4), ATCC 16612 (race 3), ATCC 16611 (race 2), ATCC 16421 (race 1); California isolates 3, 11, 14; Australian isolates 19 and 16. Fragment sizes in kb. L = 100-bp DNA ladder.

Fig. 3. *ScrFI* restriction patterns of amplified intergenic spacer (IGS) polymerase chain reaction (PCR) product of *Fusarium oxysporum* f. sp. *vasinfectum* isolates. Lanes 1 to 11: AUST-19 and 16, CA-3, 14, 11, 31665 (race 8), ATCC 36198 (race 6), ATCC 16613 (race 4), ATCC 16612 (race 3), ATCC 16611 (race 2), and ATCC 16421 (race 1). Fragment sizes in kb. L = 100-bp DNA ladder.

Table 3. Restriction enzyme (*RsaI* and *ScrFI*) band patterns of the intergenic gap spacer (IGS) region from *Fusarium oxysporum* f. sp. *vasinfectum* (fragments are estimates of number of base pairs)

	Restriction enzyme patterns										
	<i>RsaI</i>							<i>ScrFI</i>			
	A	B	C	D	E	F	G	A	B	C	D
Total length	1,358		1,358					669			
		797	871	871					580	580	580
	579	579		579	579	579	579	482	482	482	482
				563	563	573	563			268	291
		553				563	563	228	228	228	228
	391	391	391	391	391	391	391				
						358					
	2,328	2,320	2,620	2,404	1,533	2,106	1,891	1,379	1,290	1,558	1,581

our study were distinct from all other isolates, which suggests a fifth separate lineage.

In this study, lineage I consisted of two isolates from Tulare County and the ATCC race 3 isolate. Phylogenetic analysis revealed that these isolates evolved independently of the other races. Fernandez et al. (8) discovered that race 3 isolates formed a single homogenous group with regard to RFLP haplotypes. Skovgaard et al. (15) also detected no DNA polymorphism from isolates within race 3. However, there was variation in the EF-1 α gene sequences and in the IGS restriction pattern of the two California race 3 isolates; hence, each isolate had a unique haplotype. The ATCC race 3 isolate and both California race 3 isolates caused few symptoms on both Pima and Upland cotton in the greenhouse experiment. We conclude that

race 3 isolates are mildly pathogenic to cotton, and damage in commercial fields is probably limited.

ATCC representatives of races 1, 2, and 6, along with six California isolates, represented lineage II. Races 1, 2, and 6 have been reported in the United States, South America, and Africa (2,4). California isolates from this lineage were collected in Kern, Kings, and Tulare counties. Except for a single base difference in each of the EF-1 α and PHO genes in the ATCC race 2 isolate, the ATCC race 1, 2, and 6 isolates and the California isolates in this lineage were identical in gene sequence. Assibetse et al. (5) regrouped these races into a single race based on pathogenicity and random amplified polymorphic DNA analyses. We support this reclassification, as these races were almost identical in gene sequence and, more importantly, are

indistinguishable based on their pathogenicity on cotton. They can be separated by symptom expression in secondary host plants (2,3), but this separation is difficult to reproduce (unreported in this study) and is of no relevance to cotton. In any case, the designation of race in *F. oxysporum* f. sp. *vasinfectum* is not used in the traditional sense since there are no clear resistance genes in cotton yet reported, although we have initiated a study to investigate this possibility. In a worldwide collection of isolates of races 1, 2, and 6, three rDNA haplotypes were identified in a previous study (8). Our results also revealed three IGS haplotypes. The ATCC race 6 isolate had its own unique restriction pattern, and the ATCC isolates of race 1 and 2 and all of the California isolates from this lineage, except isolate CA-8, shared a different pattern. Isolate CA-8, from this lineage, possessed an IGS pattern shared with isolates from lineage IV. Shared rDNA haplotypes between different races were also observed in the study by Fernandez et al. (8). In our study, classification of race based exclusively on restriction pattern analysis was inadequate. Races 1, 2, and 6 are reported to be pathogenic on *G. barbadense* cotton ('Ashmouni 106' and 'Sakel') and *G. hirsutum* cotton ('Isa 205' and 'Acala S.J.') (5). The California isolates from lineage II were also pathogenic on both species and were slightly more virulent on *G. hirsutum* cultivars than isolates from other lineages. The interrelationships among isolates of races 1, 2, and 6 warrant further investigation. In commercial fields in California, isolates from

Table 4. *Fusarium oxysporum* f. sp. *vasinfectum* intergenic gap spacer (IGS) region haplotypes based on *RsaI* and *ScrFI* restriction patterns

Isolates	Lineage	IGS haplotypes	IGS pattern	
			<i>RsaI</i>	<i>ScrFI</i>
ATCC 16612 (race 3)	I	1	B	B
CA-3	I	2	C	B
CA-11	I	3	F	A
ATCC 16421 (race 1) and ATCC 16611 (race 2), and CA-2, 4, 5, 6, and 10	II	4	A	A
ATCC 36198 (race 6)	II	5	G	B
CA-8	II	7	E	C
31665 (race 8) and CA-1 & 7	III	6	E	A
ATCC 16613 (race 4) and CA- 9, 12, 13, 14, 15, 100, 104, S6, S7, S8, S9, and S12	IV	7	E	C
AUST-16	V	8	D	B
AUST-19	V	9	A	D

Table 5. Effect of *Fusarium oxysporum* f. sp. *vasinfectum* on symptom expression^a of select cotton cultivars

Isolate	Trial 1				Trial 2			
	Pima cultivars		Upland cultivars		Pima cultivars		Upland cultivars	
	DP-744	S-7	Maxxa	Phy-72	DP-744	S-7	Maxxa	Phy-72
None	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lineage I								
CA-3	1.00	1.50	2.50	1.50	1.00	1.00	1.25	1.50
CA-11	1.00	1.50	1.00	2.00	1.25	1.25	1.00	1.25
ATCC 16612	2.25	1.75	1.00	1.00	3.50	1.75	1.25	1.25
Lineage II								
CA-4	1.25	1.50	2.25	4.25	1.25	1.25	1.25	2.25
CA-8	2.25	1.75	2.50	2.75	1.00	1.25	1.25	1.50
CA-10	2.00	1.50	3.00	4.75	1.25	1.00	2.00	1.25
ATCC 16421	2.00	1.00	2.50	2.00	1.50	1.50	2.00	2.25
ATCC 16611	2.00	1.75	1.50	2.00	1.00	1.00	1.50	1.00
Lineage III								
CA-1	1.50	1.50	2.25	2.25	1.25	1.25	1.00	1.25
CA-7	1.50	1.25	4.00	2.50	1.00	1.50	1.25	1.50
Lineage IV								
CA-9	5.00	3.75	1.75	2.00	5.00	2.50	1.50	1.25
CA-14	5.00	4.50	1.50	2.00	5.00	3.00	3.00	2.75
Contrast	Sums of squares ^b							
None vs. all lineages	50.6753*	18.2326*	26.2775*	38.9553*	50.0244*	5.7348*	7.7450*	11.1337*
Lineage IV vs. I, II, + III	306.7666*	34.2608*	2.6549*	1.4086	782.8547*	4.8260*	1.7515	0.9216
Lineage II vs. I + III	1.5601	0.9517	0.0466	8.7440*	3.8768	0.5439	3.0749	1.4213
Lineage I vs. III	0.0340	0.9815	5.9578	1.9992	5.6181*	2.4530	0.0826	0.0231

^a Disease severity ratings were made 28 days after inoculation using a scale based on external symptoms where 1 = no symptoms, 2 = vein discoloration, chlorosis, and/or wilt, restricted to cotyledons or first leaf, 3 = similar symptoms extended beyond the first leaf, 4 = severe symptoms with the whole plant affected, and 5 = plant dead. Values are means of four replications.

^b Single degree of freedom contrasts. * = significant at $P \leq 0.05$.

this lineage cause economic damage only in plants infected with root-knot nematodes (7,16).

Two California isolates and the race 8 isolate that originated in China comprised lineage III. All three isolates were indistinguishable based on IGS restriction pattern and gene sequences. Race 8 was first described in China and has not been reported in other countries. One of the California race 8 isolates caused mild symptoms on both Upland and Pima cultivars.

The majority of the California isolates included in this study belonged to lineage IV, which also included the ATCC race 4 isolate. Isolates included in this lineage were recovered from Fresno (10 isolates) and Tulare counties (3 isolates). Race 4 isolates in California have caused serious economic losses in the last few years in several fields planted with 'DP-744' (in the absence of nematodes). The overrepresentation of race 4 isolates in this study is due to its ability to cause severe economic damage, not because it was necessarily more common than isolates from lineage II (races 1, 2, and 6). In regard to the IGS restriction pattern, all isolates belonging to race 4 were homogenous and produced only one pattern. Fernandez et al. (8) also found only one rDNA haplotype among race 4 isolates.

Isolates from lineage IV, which were identical to the ATCC isolate of race 4 according to genetic characteristics used in this study (three gene sequences and IGS restriction patterns), were highly aggressive on Pima cotton and less aggressive on Upland cultivars. The ATCC race 4 culture, however, was nonpathogenic to either type

of cotton. Other methods may need to be employed to explore possible genetic differences between California isolates and the ATCC isolate. While it is possible that pathogenicity of the ATCC isolate was lost during storage, it was reported to be pathogenic in earlier studies on certain cotton cultivars (3,5). It is interesting that this isolate did not have the ability to colonize any of the four cotton cultivars used here. Although most isolates in three of the four lineages caused very minor symptoms, all isolates except the ATCC culture successfully colonized all four cotton cultivars. In earlier studies, the ability of race 4 to cause disease varied with its cotton host, which may be responsible for our results. For example, it was nonpathogenic on 'Ashmouni 106' and 'Sakel' (*G. barbadense*) and 'Acala S.J.' and 'Isa 205' (*G. hirsutum*) (5), but was pathogenic on 'K-1' and 'Rozi' (*G. arboreum*) and 'Coastland' (*G. barbadense*) (3). Apparently, good resistance exists in some Pima (*G. barbadense*) lines, and this has been confirmed in our field observations in California. Little is currently known about the mechanisms involved in this resistance, but based on the potential for economic losses and the inevitable field-to-field spread of the pathogen and its long-distance transport via seed, information on possible resistance genes is needed for long-term disease management.

The Australian isolates comprised a fifth independent lineage and were distinctly different from all the California isolates based on a three-base deletion and at least nine single base differences in the EF-1 α gene. In addition, there was a single base

difference in the BT gene and two unique restriction patterns, which allowed differentiation between the two genotypes from Australia. Based on all available data, the Australian strains currently do not exist in California.

F. oxysporum f. sp. *vasinfectum* was possibly introduced into California from several areas of the world, which created a population that is diverse in genotype and pathogenicity. Australian strains appear distinct from anything currently found in California, and regulatory measures to avoid the introduction of these strains into California are warranted. Our research has immediate utility for the California industry because the identification of *F. oxysporum* f. sp. *vasinfectum* strains allows growers to make informed decisions regarding choice of cotton cultivars to plant. For example, only Upland cultivars or Pima cultivars with high levels of resistance should be grown in fields infested with race 4 isolates.

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Table 6. Effect of *Fusarium oxysporum* f. sp. *vasinfectum* on total dry weights^a of select cotton cultivars

Isolate	Trial 1				Trial 2			
	Pima cultivars		Upland cultivars		Pima cultivars		Upland cultivars	
	DP-744	S-7	Maxxa	Phy-72	DP-744	S-7	Maxxa	Phy-72
None	5.55	4.46	5.19	4.89	8.79	8.74	7.52	7.56
Lineage I								
CA-3	4.94	3.65	2.82	4.56	9.36	9.26	7.45	5.60
CA-11	3.62	3.46	3.85	3.78	8.81	4.50	7.64	5.03
ATCC 16612	3.64	3.67	4.15	4.37	4.66	5.57	6.53	6.27
Lineage II								
CA-4	5.33	4.32	2.73	1.39	8.49	7.19	6.52	4.43
CA-8	2.78	3.87	2.56	2.02	8.19	7.12	5.23	4.24
CA-10	3.34	4.52	2.45	0.48	9.16	7.55	5.04	6.40
ATCC 16421	3.95	4.12	2.59	3.43	7.10	6.34	2.81	3.96
ATCC 16611	2.80	4.18	6.07	3.27	9.05	8.32	2.17	7.52
Lineage III								
CA-1	4.51	5.19	3.14	2.75	8.28	6.84	7.97	3.98
CA-7	3.35	3.66	1.30	2.45	8.93	8.26	6.52	4.48
Lineage IV								
CA-9	0.88	2.38	2.30	2.55	1.14	3.76	7.53	6.50
CA-14	0.52	1.05	4.21	2.10	1.09	4.14	4.02	4.17
Contrast	Sums of squares ^b							
None vs. all lineages	17.1645*	7.6900*	25.2194*	28.8964*	2.2879	9.3220*	3.2603*	14.6318*
Lineage IV vs. I, II, + III	113.0298*	26.5408*	0.0258	0.7183	356.0596*	9.1679*	0.0004	0.0138
Lineage II vs. I + III	0.4984	0.4498	0.1170	5.6709*	0.7816	0.5719	16.2095*	0.1572
Lineage I vs. III	0.5666	1.2868	2.1204	2.8032	0.5674	5.3997*	0.0016	5.3900*

^a Dry weights (in grams) of shoots and roots harvested 28 days after inoculation. Values are means of four replications.

^b Single degree of freedom contrasts. * = significant at $P \leq 0.05$.

LITERATURE CITED

1. Appel, D. J., and Gordon, T. R. 1996. Relationships among pathogenic and nonpathogenic isolates of *Fusarium oxysporum* based on the partial sequence of the intergenic spacer region of the ribosomal DNA. *Mol. Plant-Microbe Interact.* 9:125-139.
2. Armstrong, G. M., and Armstrong, J. K. 1960. American, Egyptian, and Indian cotton-wilt Fusaria: Their pathogenicity and relationship to other wilt fusaria. *U.S. Dep. Agric. Tech. Bull.* 219.
3. Armstrong, G. M., and Armstrong, J. K. 1978. A new race (race 6) of the cotton-wilt *Fusarium* from Brazil. *Plant Dis. Rep.* 62:421-423.
4. Armstrong, J. K., and Armstrong, G. M. 1958. A race of the cotton wilt *Fusarium* causing wilt of Yelredo soybean and flue-cured tobacco. *Plant Dis. Rep.* 42:147-151.
5. Assigbetse, K. B., Fernandez, D., Dubois, M. P., and Geiger, J.-P. 1994. Differentiation of *Fusarium oxysporum* f. sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. *Phytopathology* 84:622-626.
6. Davis, R. D., Moore, N. Y., and Kochman, J. K. 1996. Characterisation of a population of *Fusarium oxysporum* f. sp. *vasinfectum* causing wilt of cotton in Australia. *Aust. J. Agric. Res.* 47:1143-1156.
7. DeVay, J. E. 1986. Half a century dynamics and control of cotton diseases: Fusarium and Verticillium wilts. Pages 35-41 in: 1986 Proc. Beltwide Cotton Conf. J. Brown, ed. National Cotton Council of America, Memphis, TN.
8. Fernandez, D., Assigbetse, K., Dubois, M., and Geiger, J. P. 1994. Molecular characterization of races and vegetative compatibility groups in *Fusarium oxysporum* f. sp. *vasinfectum*. *Appl. Environ. Microbiol.* 60:4039-4046.
9. Garber, R. H., and Paxman, G. A. 1963. Fusarium wilt of cotton in California. *Plant Dis. Rep.* 47:398-400.
10. Harrington, T. C., and Wingfield, B. D. 1995. A PCR-based identification method for species of *Armillaria*. *Mycologia* 87:280-288.
11. Ibrahim, F. M. 1966. A new race of the cotton-wilt *Fusarium* in the Sudan Gezira. *Emp. Cotton Grow. Rev.* 43:296-299.
12. Ibrahim, G., and Nirenberg, H. I. 1993. Response of some Sudanese cotton cultivars to race 1 and 5 of *Fusarium oxysporum* f. sp. *vasinfectum*. *Z. Pflanzenkrankh. Pflanzenschutz* 100:645-651.
13. O'Donnell, K., Kistler, H. C., Cigelnik, E., and Ploetz, R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. *Proc. Natl. Acad. Sci. USA* 95:2044-2049.
14. O'Donnell, K., Kistler, H. C., Tacke, B. K., and Casper, H. H. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. *Proc. Natl. Acad. Sci. USA* 97:7905-7910.
15. Skovgaard, K., Nirenberg, H. I., O'Donnell, K., and Rosendahl, S. 2001. Evolution of *Fusarium oxysporum* f. sp. *vasinfectum* races inferred from multigene genealogies. *Phytopathology* 91:1231-1237.
16. Smith, S. N., Ebbels, D. L., Garger, R. H., and Kappelman, A. J. 1981. Fusarium wilt of cotton. Pages 29-38 in: *Fusarium: Diseases, Biology, and Taxonomy*. P. E. Nelson, T. A. Tossoun, and R. J. Cook, eds. Pennsylvania State University, University Park.
17. Tooley, P. W., Goley, E. D., Carras, M. M., Frederick, R. D., Weber, E. L., and Kuldau, G. A. 2001. Characterization of *Claviceps* species pathogenic on sorghum by sequence analysis of the β -tubulin gene intron 3 region and EF-1 α gene intron 4. *Mycologia* 93:541-551.
18. White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: *PCR Protocols: A Guide to Methods and Applications*. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and J. W. White, eds. Academic Press, New York.