The *Journal of Cotton Science 19:328–336 (2015)*
http://journal.cotton.org, © The Cotton Foundation 2015


# PLANT PATHOLOGY AND NEMATOLOGY

## Survey of *Fusarium oxysporum* f. sp. *vasinfectum* in the United States

Amanda N. Cianchetta, Tom W. Allen, Robert B. Hutmacher, Robert C. Kemerait, Terrence L. Kirkpatrick, Gary W. Lawrence, Kathy S. Lawrence, John D. Mueller, Robert L. Nichols, Mary W. Olsen, Charles Overstreet, Jason E. Woodward, and R. M. Davis*

### ABSTRACT

**Fusarium wilt is an important and widespread disease of cotton (*Gossypium hirsutum* L. and *Gossypium barbadense* L.) caused by several races and genotypes of *Fusarium oxysporum* f. sp. *vasinfectum* W.C. Snyder & H.N. Hans (FOV). A two-year survey (2012-2013) was conducted to assess the distribution of the fungus, and in particular FOV race 4, throughout the United States (U.S.) Cotton Belt. Partial sequences of the translation elongation factor (EF-1α) region of FOV cultured from symptomatic cotton plants were compared to a database of sequences of known genotypes. Five nominal races of FOV, races 1, 2, 3, 4, and 8, and four previously recognized southeastern genotypes, LA108, LA110, LA112, and LA127/140, were identified. Representative isolates of each were pathogenic on cotton in greenhouse assays. A unique genotype of FOV, MDS-12, which was characterized with additional sequencing of the phosphate permease-like protein, β-tubulin, and intergenic spacer regions, was identified in Alabama, Arkansas, Mississippi, Louisiana, and Georgia. FOV race 4 was not detected outside of California.**

A.N. Cianchetta, Department of Plant Pathology, University of California, Davis, One Shields Ave., Davis, CA 95616; T.W. Allen, Delta Research and Extension Center, Mississippi State University, 82 Stoneville Road, P.O. Box 197, Stoneville, MS 38776; R.B. Hutmacher, Department of Plant Science, University of California, Davis, WestSide Research and Extension Center, 17353 West Oakland Ave. , Five Points, CA 93624; R.C. Kemerait, Department of Plant Pathology, University of Georgia, 2360 Rainwater Road, Tifton, GA 31793; T.L. Kirkpatrick, Southwest Research and Extension Center, University of Arkansas, 362 Highway 174 N., Hope, AR 71801; G.W. Lawrence, Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology , Mississippi State University, 206 Dorman Hall, Mississippi State, MS 39762; K.S. Lawrence, Department of Entomology & Plant Pathology, Auburn University, 209 Rouse Life Science Building, Auburn, AL 36849; J.D. Mueller, Edisto Research and Education Center, Department of Entomology, Soils, and Plant Sciences, Clemson University, 64 Research Road, Blackville, SC 29817; R.L. Nichols, Cotton, Inc., 6399 Weston Parkway, Cary, North Carolina 27513; M.W. Olsen, Department of Plant Sciences, The University of Arizona, 1140 E. South Campus Dr., Tucson, AZ 85721; C. Overstreet, Department of Plant Pathology and Crop Physiology, Louisiana State University AgCenter, 302 Life Science Building, Baton Rouge, LA 70803; J.E. Woodward, Department of Plant Pathology and Microbiology, Texas A&M AgriLife, Research and Extension Center, 1102 E. FM 1294, Lubbock, TX 79403; and R.M. Davis*, Department of Plant Pathology, University of California, Davis, One Shields Ave., Davis, CA 95616.
*Corresponding author: rmdavis@ucdavis.edu

Fusarium wilt of cotton (*Gossypium hirsutum* L. and *Gossypium barbadense* L.), caused by *Fusarium oxysporum* f. sp. *vasinfectum* W.C. Snyder & H.N. Hans (FOV), is an important and widespread disease affecting nearly all cotton growing regions of the world. In the United States (U.S.), approximately 11.7% of cotton bales are lost to disease and 0.2% of the 11.7% is due to FOV (Lawrence et al., 2014). Cotton is produced in 17 states and the majority, more than 97%, is Upland (*G. hirsutum* L.). The remaining 3% is from Pima (*Gossypium barbadense* L.) cultivars and about 90% of the acreage is in California (Anonymous, USDA-NASS).

FOV is both seedborne and soilborne, and colonizes the roots and vascular system of susceptible cotton cultivars, causing root and vascular discoloration, wilting and sometimes death of the plant. Discrete symptoms vary with pathogen genotype, inoculum density, cotton cultivar, and plant age (Hao et al., 2009). There are six nominal races of FOV, 1, 2, 3, 4, 6, and 8, known to affect cotton worldwide (Armstrong and Armstrong, 1958; Armstrong and Armstrong, 1960; Armstrong and Armstrong, 1978; Atkinson, 1892; Chen et al., 1985; Fahmy, 1927; Holmes et al., 2009; Ibrahim, 1966; Kim et al., 2005; Nirenberg et al., 1994). Although eight races were initially identified, molecular classification and pathogenicity testing has eliminated redundancy between races 4 and 7 and races 3 and 5 (Abd-Elsalam et al., 2004; Hering et al., 1999; Nirenberg et al., 1994; Skovgaard et al., 2001).

Five races, 1, 2, 3, 4, and 8, are known to occur in the U.S. (Armstrong and Armstrong, 1958; Armstrong and Armstrong, 1960; Holmes et al., 2009; Kim et al., 2005). Race 4, a particularly virulent genotype of FOV, first reported in India in 1960, (Armstrong and Armstrong, 1960), was detected in California in 2001 (Kim et al., 2005). Unlike most other genotypes of FOV, this genotype causes economic losses independent of the root-knot nematode (*Meloidogyne incognita* (Kofoid &White) Chitwood).

The term "race" used to distinguish these genotypes does not imply a correlation in host gene resistance in the classical use of the term, but the designation of certain genotypes by race has been maintained by the cotton industry (Davis et al., 2006). *Fusarium oxysporum* f. sp. *vasinfectum* is polyphyletic and isolates belonging to four lineages have been documented in the United States (O'Donnell et al., 2009; Kim et al., 2005; Skovgaard et al., 2001). In addition to the nominal races of FOV, four genotypes from the southeastern United States are prominent pathogens due to their virulence on commercial cotton cultivars (Holmes et al., 2009). Designated LA108, LA110, LA112, and LA127/140, these genotypes have unique partial translation elongation factor (EF-1α) sequences (Holmes et al., 2009). In general, these nominal races and genotypes of FOV can be distinguished from each other by sequence analysis, restriction fragment length polymorphisms of the intergenic spacer region, mating type, vegetative compatibility, and pathogenicity on cotton (Abo et al., 2005; Skovgaard et al., 2001; Armstrong and Armstrong, 1958; Armstrong and Armstrong, 1960; Chen et al., 1985; Fahmy, 1927; Holmes et al., 2009; Ibrahim, 1966; Kim et al., 2005). There are also specific PCR primers for FOV race 4 detection and identification (Doan et al., 2014; Yang et al., 2006).

Of these races and genotypes, FOV race 4 is of most concern to California and, potentially, U.S. cotton growers. Many Pima cultivars are particularly susceptible to FOV race 4 (Kim et al., 2005). Commercial cultivars resistant to race 4 are limited (Hutmacher et al., 2013); Phytogen 800, a Pima cultivar, is the most resistant cultivar to FOV race 4 (Ulloa et al., 2006). Upland cultivars, while affected by FOV race 4, are not as susceptible to the pathogen as are Pima varieties (Ulloa et al., 2006). This survey intended to detect movement of FOV race 4, if any, outside of California and to provide the industry with an updated assessment of the distribution of FOV races and genotypes in the U.S. Cotton Belt.

## MATERIALS AND METHODS

**Isolates.** Symptomatic cotton plants identified by researchers and county Cooperative Extension agents across the Cotton Belt were submitted to R. M. Davis in the Department of Plant Pathology at the University of California, Davis by overnight mail (Table 1). Upon receipt, samples were washed with soap and water and examined for vascular discoloration by cutting lengthwise into the crown and lower stem. Stem sections 0.5 to 1 cm in length were immersed in a 0.5% hypochlorite solution for 2 min and placed onto acidified potato dextrose agar (APDA). *Fusarium*-like cultures were sub-cultured and kept in a clear plastic box on a laboratory bench at room temperature.

**Table 1. Cotton samples with symptoms of Fusarium wilt collected in 2012 and 2013.**

| Origin | Number of Samples[z] | |
|---|---|---|
| | 2012 | 2013 |
| California | 167 | 94 |
| Alabama | 27 | 51 |
| Arizona | 0 | 6 |
| Arkansas | 17 | 13 |
| Georgia | 0 | 5 |
| Louisiana | 16 | 10 |
| Mississippi | 32 | 20 |
| South Carolina | 1 | 0 |
| Texas | 36 | 15 |

[z] More than one cotton plant was submitted in one sample.

**Out-of-California samples: DNA extraction, amplification, purification and sequencing.** DNA from each isolate was extracted from mycelia of putative FOV cultures on APDA. In a laminar flow hood, sterile forceps were used to place two tufts of mycelium, approximately 15 mg, into a 1.5 ml cryogenic tube containing three sterile glass beads and 500 µl of OmniGenX Purespin gDNA MiniPrep Kit (E&K Scientific; Santa Clara, CA) extraction buffer. After the samples were macerated on a Vortexer Genie2 (VWR; Radnor, PA) at maximum speed for 10 min, the manufacturer's directions for solid tissue sample DNA extraction were followed.

All samples were tested for FOV race 4 using specific primers in 25 µl reactions to amplify a 208 bp fragment unique to FOV race 4. Race 4 specific primers were R4F (5' GCT CCG TGT CWG AGC TTC TT 3') and R4R (5' TGC TCA TCG TGG

AGC ATA AC 3') (Yang et al., 2006). Amplification was performed in a thermocycler (PTC-100; MJ Research, Watertown, MA) as follows: 94°C for 3 min, 10 cycles of 94°C for 30 s, 59°C for 30 s, and 72°C for 30 s, 25 cycles of 90°C for 30 s, 59°C for 30 s, and 72°C for 15 s and a final extension at 72°C for 1 min. PCR was also performed using primers for the translation elongation factor (EF-1α) region (O'Donnell et al., 1998) to amplify the 650 bp region commonly used to identify races of FOV (Table 2).

Partial fragments of two other nuclear genes, phosphate permease-like protein (PHO) (O'Donnell et al., 2000) and β-tubulin (BT) (Tooley et al., 2001), were amplified for samples that did not amplify with race 4 specific primer but had EF sequence identity to FOV race 4. The intergenic spacer region (IGS) was also sequenced for these isolates (Appel and Gordon, 1996; Harrington and Wingfield, 1995; White et al., 1990) (Table 2).

Each PCR reaction consisted of 12.5 µl of 2X Mean Green Master Mix (Syzygy Biotech; Grand Rapids, MI), 1 µl of each 10 µM primer, and 2 µl of genomic DNA. Negative controls (no template DNA) were included in each assay. Five µl of each sample were visualized by UV light after electrophoresis on a 1.5% agarose gel and staining with ethidium bromide.

PCR products were purified with a GeneJET PCR Purification Kit (Thermo Fisher Scientific, Inc.; Waltham, MA) following the manufacturer's instructions. A 15-50 ng sample of DNA and 8-12 µl of 3 µM primers were sequenced at the UC DNA Sequencing Facility (Davis, CA) or Quintara Biosciences (Richmond, CA). Sequences were manually scored to eliminate ambiguous bases and aligned to reference sequences obtained from GenBank using MEGA (Tamura et al., 2013) and VectorNTI (Life Technologies; Carlsbad, CA) (Table 3). Each sample was identified as a putative race of FOV by aligning EF sequences, and other sequences where available, to those of the reference strains (Table 3). Samples identified as negative for FOV either failed to produce fungal growth on APDA or were identified as other fungi by sequence analysis.

**California samples: DNA extraction, amplification and AmplifyRP Acceler8 assays for FOV4.** California samples were only identified as negative or positive for FOV race 4 using race 4 specific primers as described above or with AgDia AmplifyRP Acceler8 kits specific for FOV 4 (AgDia; Elkhart, IN) (Doan et al., 2014). In the latter, cotton stem tissues were prepared following manufacturer's instructions and results were read after 20 min.

**Table 2. PCR primers and conditions used in this study.**

| Locus | Primer Sequence | Reference | Thermocycler Settings |
|---|---|---|---|
| **Translation elongation factor (EF-1α) 650 bp** | | | |
| EF1 | ATG GGT AAG GAA GAC AAG AC | O'Donnell et al., 1998 | 40 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 5 min |
| EF2 | GGA AGT ACC AGT GAT CAT GTT | | |
| **Phosphate permease-like protein (PHO) 900 bp** | | | |
| PHO1 | ATC TTC TGG CGT GTT ATC ATG | O'Donnell et al., 2000 | 97°C for 1 min followed by 35 cycles of 96°C for 30 s, 50°C for 1 min, and 72°C for 1 min and a final extension at 72°C for 10 min |
| PHO6 | GAT GTG GTT GTA AGC AAA GCC C | | |
| **β-tubulin (BT)** | **550 bp** | | |
| BT3 | CGT CTA GAG GTA CCC ATA CCG GCA | Tooley et al., 2001 | 95°C for 5 min followed by 35 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 1.5 min and a final extension at 72°C for 10 min |
| BT5 | GCT CTA GAC TGC TTT CTG GCA GAC C | | |
| **Intergenic spacer (IGS) region 1.5kb** | | | |
| CNS1R | GAG ACA AGC ATA TGA CTA C | Appel and Gordon, 1996; Harrington and Wingfield, 1995; White et al., 1990 | 40 cycles of 94°C for 2 min, 55°C for 2 min, and 72°C for 2.5 min followed by a final extension at 72°C for 10 min |
| 28F | CTG AAC GCC TCT AAG TCA GAA | | |

**Table 3. Genbank accessions of *Fusarium oxysporum* f. sp. *vasinfectum* EF-1α, BT, PHO and IGS sequences used in this study.**

| FOV Race/Genotype | Isolate | EF-1α[z] Genbank No. | BT[y] Genbank No. | IGS[x] Genbank No. | PHO[w] Genbank No. |
|---|---|---|---|---|---|
| Race 1 | CA 10 | AY714099 | AY714084.1 | FJ985472 | AY714114.1 |
| Race 2 | ATCC 16611 | AY714105 | AY714091.1 | FJ985473 | AY714119.1 |
| Race 3 | CA 3 | FJ466734 | AY714088.1 | FJ985539 | AY714113.1 |
| Race 3 | CA 11 | AY714100. | AY714085.1 | FJ985539 | AY714115.1 |
| Race 4 | CA 14 | AY714101 | AY714086.1 | DQ 831885 | AY714116.1 |
| Race 4 | ATCC 16613 | FJ985279 | - | FJ985475 | - |
| Race 6 | ATCC 36198 | AY714103 | AY714089.1 | FJ985476 | AY714117.1 |
| Race 8 | CA 1 | AY714098 | AY714087.1 | - | AY714112.1 |
| 108 | LA 108 | FJ466731 | FJ466736.1 | FJ985544 | FJ466741.1 |
| 110 | LA 110 | FJ466732 | FJ466737.1 | FJ985546 | FJ466742.1 |
| 112 | LA 112 | FJ466733 | FJ466738.1 | FJ985547 | FJ466743.1 |
| 127 | LA 127 | FJ466734 | FJ466739.1 | FJ985551 | FJ466744.1 |
| 140 | LA 140 | FJ466735 | FJ466740.1 | FJ985550 | FJ466745.1 |
| *F. redolens* | Fo1502 | AY714109.1 | AY714094.1 | AY714122.1 | HM057287[v] |

[z] Partial translation elongation factor.

[y] Beta-tubulin.

[x] Intergenic spacer.

[w] Phosphate permease-like protein.

[v] From isolate NRRL 31075.

**Greenhouse pathogenicity tests.** Seed of Phytogen 830 (Ph830) (Dow AgroSciences, Indianapolis, IN), a commercial Pima cultivar susceptible to race 4, Phytogen 800 (Ph800), a Pima cultivar resistant to race 4, and Phytogen 72 (Ph72), an Upland cultivar with intermediate resistance to race 4, were planted in 128 cell-styrofoam trays (each cell 3.5 cm x 3.5 cm x 6.5 cm deep), filled with UC potting mix (one part coarse sand, one part compost (redwood shavings and turkey manure) and one part peat moss and 1.36 kg Dolomite/m). One fungal culture submitted from Arizona was directly evaluated on cotton cultivars Delta Pine 744 (Pima) (Monsanto, St. Louis, MO), Phytogen 89 (Upland), and Phytogen 725 (Upland) in addition to Phytogen 830, Phytogen 72, and Phytogen 800. Representative isolates of each genotype collected from Alabama, Arkansas, Georgia, Louisiana, Mississippi, and Texas in 2013 were selected for pathogenicity testing. A total of four isolates of race 1, 2, and 8; two of race 3, LA110 and LA108; one of LA127/140 and LA112; and eleven isolates of MDS-12 were evaluated. DNA was extracted from each isolate originally grown from a single conidium. Partial EF sequences confirmed the identity of each culture. Isolates AL1A, AL28A, GA4E, LA1O, and LA9A were cultured from hyphal tips rather than a single conidium. Colonized APDA plates were wrapped in Parafilm and kept in a clear plastic box on the lab bench for one week. Conidia of each isolate were then harvested by adding approximately 10 ml of deionized water to the plate, scraping the agar surface with a clean microscope slide and filtering the suspension through four layers of cheesecloth. Each conidial suspension was quantified using a hemocytometer and adjusted to $10^6$ conidia/ml. Control inocula included deionized water and isolates of FOV race 1 (CA8), race 3 (CA11), race 4 (RBH1), and race 8 (CA7).

Approximately three-wk-old cotton plants at the one to two true-leaf stage were inoculated. Plant roots were rinsed of potting mix in water and then immersed in the conidial suspension for two to three min. Four plants of each cultivar were inoculated with each isolate and the culture received from Arizona. The inoculated seedlings were individually transplanted into new 8.5 cm-deep pots filled with sterilized UC potting mix. Plants were watered as needed in a greenhouse maintained at 18 to 24°C. After two weeks, pots were randomized in the greenhouse and kept at 18 to 24°C with at least 12 hours light for a total of four weeks.

Plant symptoms were then evaluated on two qualitative scales. The extent of foliar symptoms were assessed using a 1 to 4 scale, where: 1= no

foliar symptoms; 2= chlorosis or wilt restricted to cotyledons or the lowest true leaf; 3= mild chlorosis or wilt extending beyond the lowest true leaf; and 4= severe symptoms affecting the whole plant or plant death. The vascular discoloration rating scale was: 1= no vascular discoloration; 2= light brown streaks or isolated areas of discoloration; 3= light brown discoloration throughout the vasculature; and 4= dark brown discoloration or plant death. Re-isolation of FOV was attempted from two plants of each fungus-cultivar combination. Stem tissue immersed in a 0.5% hypochlorite solution for two min was plated on APDA. Partial EF sequences were determined from each resulting culture and aligned to reference strains to confirm the identity of each recovered genotype. An isolate was considered pathogenic if vascular discoloration was present and the pathogen was recovered from the inoculated plants. This experiment was repeated once.

**Phylogenetic analysis.** EF-1α sequences of representative isolates of each race or genotype of FOV from each state as well as the FOV reference strains were aligned in MEGA. The file was imported into MacClade (MacClade Phylogenetic Software 3.0, Sinauer Associates Inc., Sunderland, MA) and manually adjusted as needed. A phylogenetic tree was generated in PAUP (version 4.0b10, Sinauer Associates) with a heuristic search using maximum parsimony analysis with *F. redolens* as the outgroup (Kim et al., 2005). All characters were run unordered with equal weight and gaps were treated as missing data. Statistical support for the tree was derived by running 5,000 bootstrap replicates. Unique genotypes were further analyzed by performing the same search with the EF-1α sequence concatenated with sequences of the β-tubulin gene, phosphate permease-like protein, and intergenic spacer region. All sequences for the reference FOV isolates were obtained from GenBank (Table 3).

## RESULTS & DISCUSSION

Over the course of two years, this survey assayed 261 plant samples from California and 249 from other states in the Cotton Belt, including Alabama, Arkansas, Georgia, Mississippi, Louisiana, South Carolina, and Texas (Table 1). Fungal cultures were also submitted from Arizona in 2013. Among all samples, FOV race 4 was only detected in samples from California (Table 4).

Identification of samples received from states other than California found FOV race 1 in each participating state (Table 4). This race was first documented in the United States in 1958 in Alabama and has a wide distribution in areas within the U.S. where cotton is grown (Armstrong and Armstrong, 1958). The EF-1α sequence of FOV race 1 cannot be distinguished from that of race 6. Because reports of race 6 are limited to South America, these isolates collected from Alabama, Georgia, Louisiana, and Texas were identified as race 1. Race 2 had a more uneven distribution than did race 1 and was identified in samples from Texas and South Carolina in 2012 and Alabama, Georgia, Louisiana, Mississippi, and Texas in 2013. Initially reported to have a more limited distribution than race 1 in the Cotton Belt, FOV race 2 may have a wider range of pathogenicity on cotton cultivars than race 1 (Armstrong and Armstrong, 1960; Kappelman, 1983). FOV race 3 was identified in samples collected from Arkansas, Mississippi, and Texas in 2012 and Alabama and Mississippi in 2013 (Table 4). Previously, race 3 was documented in California and Louisiana (Holmes et al., 2009; Kim et al., 2005). Samples identified as FOV race 8 were collected from Alabama, Arkansas, and Louisiana in 2012 and Alabama, Georgia, and Louisiana in 2013. Holmes et al. (2009) identified isolates of FOV race 8 in Arkansas, Louisiana, Georgia, and Missouri in 2009. Samples collected from fields in California were only tested for FOV race 4. Races 1, 2, 3, 4, and 8 have all been previously identified in California (Kappelman, 1983; Kim et al., 2005). All isolates were pathogenic on at least one of the three cotton cultivars.

This survey also documented the distribution of four genotypes, LA108, LA112, LA110, and LA127/140, that caused relatively severe symptoms on cotton cultivars in pathogenicity tests (Holmes et al., 2009). In 2012, LA108 was detected in one sample each from Alabama and Louisiana (Table 4). In 2013, LA108 was identified in individual samples from Alabama, Arkansas, Georgia, and Mississippi. LA110 was identified in three samples from Georgia and LA112 in seven samples from Alabama. The genotype LA127/140 was only identified in one sample from Mississippi. Holmes et al. (2009) identified these genotypes primarily in Arkansas and at a relatively high frequency, indicating they were not rare. This survey reports a wider distribution of these genotypes.

**Table 4. Distribution of genotypes and races of *Fusarium oxysporum* f. sp. *vasinfectum* isolates[z] from cotton plant samples[y] collected in the United States in 2012 and 2013.**

| | | 2012 | | | | | | | 2013 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CA | AL | AR | LA | MS | TX | SC | CA | AL | AR | AZ | GA | LA | MS | TX |
| **Races of FOV and Reference Isolates** | | | | | | | | | | | | | | | | |
| **Race 1** | **CA 10** | | 4 | 1 | 5 | | 9 | 1 | | 9 | | | 5 | 7 | 2 | 9 |
| **Race 2** | **ATCC 16611** | | | | | | 2 | 1 | | 2 | | | 2 | 4 | 1 | 3 |
| **Race 3** | **CA 11** | | | 4 | | 1 | 1 | | | 4 | | | | | 1 | |
| **Race 4** | **CA 14** | 55 | | | | | | | 33 | | | | | | | |
| **Race 8** | **CA 1** | | 1 | 1 | 8 | | | | | 6 | | | 1 | 6 | | |
| **Other Genotypes** | | | | | | | | | | | | | | | | |
| **MDS-12** | | | | | | 2 | | | | 5 | 2 | | | 2 | 2 | |
| **LA108** | | | 1 | | 1 | | | | | 2 | 1 | | 1 | 1 | 1 | |
| **LA110** | | | | | | | | | | | | | 3 | | | |
| **LA112** | | | | | | | | | | 7 | | | | | | |
| **LA127/140** | | | | | | | | | | | | | | | 1 | |

[z] **Isolates were identified using partial translation elongation factor sequences (EF-1α) and pathogenicity tests.**

[y] **More than one cotton plant was submitted in each sample.**

The phylogenetic tree generated with partial EF-1α sequences of DNA from the races and genotypes collected in this survey had the same topology as previously published phylogenies describing the five nominal races of FOV in the U.S. (Fig. 1) (Holmes et al., 2009). Only one representative sequence from the race 3 lineage was used to construct the tree. Single nucleotide polymorphisms in the EF-1α sequences of samples MS5B and AL27 placed them in the same clades as FOV race 2 and LA112, respectively. The unique genotype AR2 shared a clade with LA112.

The presence of the genotype MDS-12, referred to as 'race 4-like' in a previous publication (Bennett et al., 2013), was observed in Mississippi in 2012 and in a total of four states, Alabama, Arkansas, Louisiana, and Mississippi, in 2013. The designation 'MDS-12' is in reference to the identification of these isolates in the mid-southern United States in 2012 and 2013. Although EF, BT, and PHO sequences for these samples were identical to FOV race 4, they did not amplify with race 4 specific primers and had distinct IGS sequences. Phylogenetic analysis of isolates identified as MDS-12, with concatenated EF, BT, PHO, and IGS sequences, resolved three groups (Fig. 2). One group, comprised of isolates LA1O and AL28A, was in the same clade as FOV race 4; the second group containing two isolates, ALKL25 and MSGL10, identified in a previous study (Bennett et al., 2013), was in a separate clade; and the third group, AR10A, MS3, LA4B, MS11, and two isolates from a previous study, MSGL18 and ALKL11 (Bennett et

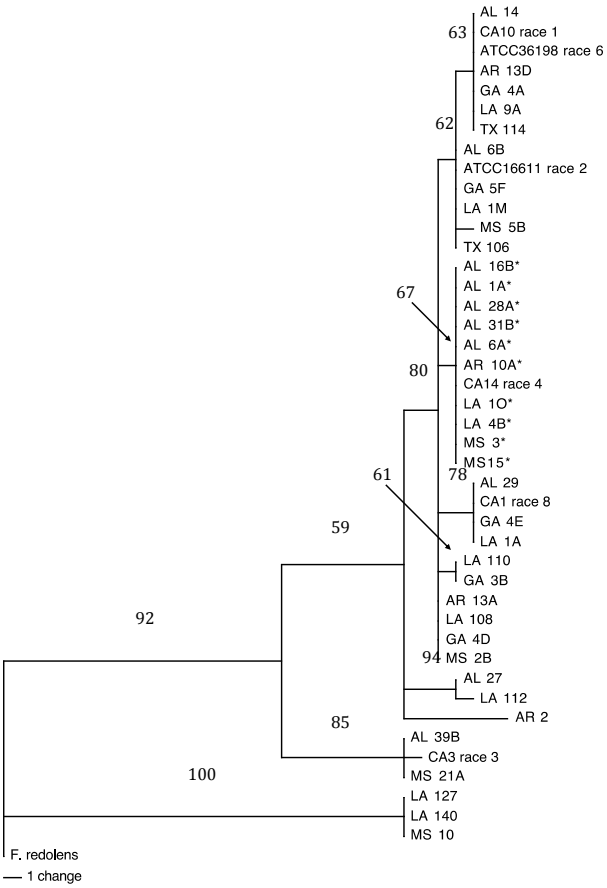


**Figure 1. Single most parsimonious tree generated from partial translation elongation factor (EF 1-α) sequences of selected isolates of *Fusarium oxysporum* f. sp. *vasinfectum*. The outgroup was *Fusarium redolens*. Bootstrap values from 5,000 replications are noted on the branches.**

al., 2013), was in the same clade as FOV race 6 (Fig. 2). Multiple IGS genotypes of MDS-12 isolates were identified across Alabama, Arkansas, Louisiana, and Mississippi. Isolates from each group were identified in Alabama, isolates from the second and third groups were identified in Louisiana and Mississippi and MDS-12 isolates from Arkansas were all from the third group. The isolates varied from each other and from FOV race 4 with respect to pathogenicity and virulence on Ph72, Ph800, and Ph830. For example, on Ph830 the positive race 4 control caused extensive vascular discoloration and AL28A and LA1O caused only light brown vascular discoloration, but on Ph72 the positive race 4 control and AL28A caused light brown vascular staining and LA1O killed the plant. Because the sequences of EF, PHO, and BT do not discriminate between isolates of MDS-12 and FOV race 4, any sample with EF identity to FOV race 4 must undergo further testing by PCR with race 4 specific primers or by sequencing of the IGS region.

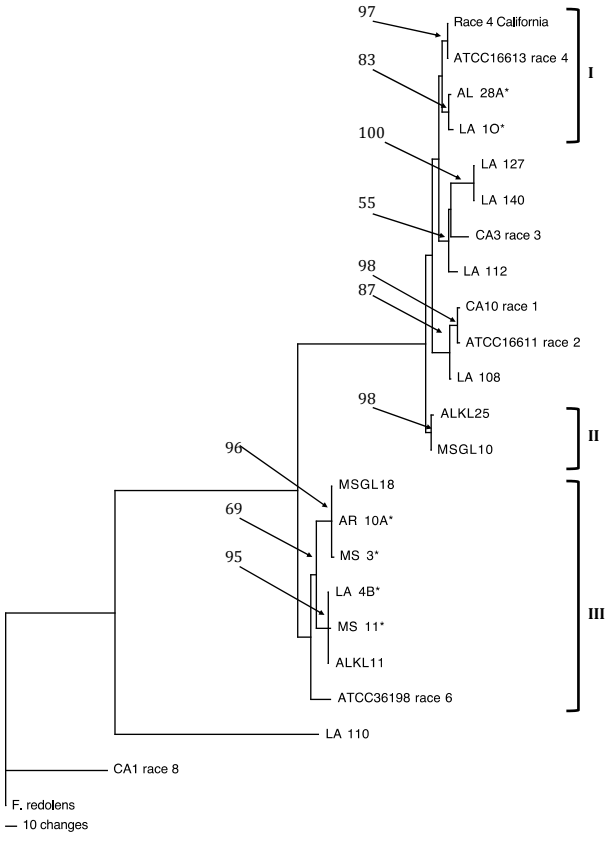


**Figure 2. One of 126 most parsimonious trees generated from concatenated partial translation elongation factor, phosphate permease-like protein, β-tubulin, and intergenic spacer sequences of selected isolates of *Fusarium oxysporum* f. sp. *vasinfectum* including those identified as MDS-12 (noted with an asterisk), with EF identity to FOV race 4, collected from cotton grown in the United States in 2013. *Fusarium redolens* served as the outgroup. Bootstrap values from 5,000 replications are noted on the branches.**

The completion of this two-year survey provides the cotton industry and research communities with an up-to-date assessment of the distribution of FOV genotypes in the U.S. A previously published analysis of FOV demographics was last completed in 2005. The current study uniquely accounts for both the distribution of the five nominal races of FOV as well as the occurrence of several recently identified genotypes from the southeastern United States, including LA108, LA110, LA112, and LA127/140. These genotypes are of interest as they demonstrated pathogenicity on commercial cultivars grown both in the southeastern U.S. as well as in California at degrees of virulence that exceeded that of California FOV race 4 on particular cultivars (Holmes et al., 2009). This survey also identified a unique genotype of FOV, AR2, which caused mild symptoms on Ph72, Ph800, and Ph830 and had a unique EF sequence, placing it in the same clade as LA112.

Six fungal cultures were submitted from Arizona, but none were identified as FOV. Arizona has a long history of Pima cotton breeding and production and due to its proximity to California is vulnerable to the spread of FOV race 4. Subsequent surveys must include more in-depth sampling from Arizona to appropriately assess the movement of FOV race 4 outside of California. In this survey, FOV race 4 was not detected outside of California.

The genotype distribution reported in this survey was not uniform. FOV race 4 was limited to California and genotypes LA108, LA110, LA112, and LA127/140 were localized to the southeast. Races 1 and 2 were the most widespread, as documented in previously published surveys (Kappelman, 1983). The diversity of genotypes of FOV that cause disease in cotton is unusual among pathogenic *Fusarium oxysporum* as confirmed by O'Donnell et al. (2009). This study identified at least 10 unique genotypes of FOV that are pathogenic on cotton. Fusarium wilt of asparagus is similar to cotton in that many pathogenic genotypes have been identified (LaMonda and Elmer, 1988). The reason for the extent of this diversity in both cotton and asparagus is unknown as effective pathogenicity and virulence is achieved in other crops by a limited number of *Fusarium* genotypes. A differential set of cotton cultivars capable of resolving these races and genotypes is needed to classify the range of genotypes of FOV infesting cotton.

## ACKNOWLEDGMENT

This research was partially supported by Cotton, Incorporated. California cotton samples were submitted by Pablo Guzman and Nicole Hostert.

## REFERENCES

Abd-Elsalam, K.A., M.R. Omar, Q. Migheli, and H.I. Nirenberg. 2004. Genetic characterization of *Fusarium oxysporum* f. sp. *vasinfectum* isolates by random amplification of polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP). J. Plant Dis. Prot. 111:534-544.

Abo, K., K.K. Klein, V. Edel-Hermann, N. Gautheron, D. Traore, and C. Steinberg. 2005. High genetic diversity among strains of *Fusarium oxysporum* f. sp. *vasinfectum* from cotton in Ivory Coast. Phytopathology 95:1391-1396.

Anonymous, USDA-NASS. 2015. Quick Stats Lite: Acreage, Yield, Production and Price for

Cotton. [Online]. Available at http://www.nass.usda.gov/Quick_Stats/Lite/ result.php?314A404E-861B-3787-81BA-DEA75B6FEC5E (verified 3 Feb 2015).

Appel, D.J., and T.R. Gordon. 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.

Armstrong, G.M., and J.K. Armstrong. 1960. American, Egyptian, and Indian cotton-wilt Fusaria: Their pathogenicity and relationship to other wilt Fusaria. Tech. Bull. S.C. Agric. Exp. Stn. 1219.

Armstrong, G.M., and J.K. Armstrong. 1978. A new race (race 6) of the cotton-wilt Fusarium from Brazil. Plant Dis. Rep. 62(5):421-423.

Armstrong, J.K., and G.M. Armstrong. 1958. A race of the cotton wilt *Fusarium* causing wilt of the Yelredo soybean and flue-cured tobacco. Plant Dis. Rep. 42:147-51.

Atkinson, G.F. 1892. Some diseases of cotton. III. Frenching. Bull. - Ala. Agric. Exp. Stn., Auburn Univ. 41:19-29.

Bennett, R.S., T.Z. Scott, K.S. Lawrence, and G.W. Lawrence. 2013. Sequence characterization of race-4 isolates of *Fusarium oxysporum* from Alabama and Mississippi. J. Cotton Sci. 17:125-130.

Chen, Q., X. Ji, and W. Sun. 1985. Identification of races of cotton wilt *Fusarium* in China. Agric. Sci. China 6:1-6.

Davis, R.M., P.D. Colyer, C.S. Rothrock, and J.K. Kochman. 2006. Fusarium Wilt of Cotton: Population Diversity and Implications for Management. Plant Dis. 90(6): 692-702.

Doan, H.K., S. Zheng, and R.M. Davis. 2014. Development and evaluation of AmplifyRP Acceler8 diagnostic assay for the detection of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 in cotton. Plant Health Prog. doi: 10.1094/PHP-RS-13-0115.

Fahmy, T. 1927. The *Fusarium* wilt disease of cotton and its control. Phytopathology 17:749-767.

Hao, J.J., M.E. Yang, and R.M. Davis. 2009. Effect of soil inoculum density of *Fusarium oxysporum* f. sp. *vasinfectum* race 4 on disease development in cotton. Plant Dis. 93:1324-13.

Harrington, T.C., and B.D. Wingfield. 1995. A PCR-based identification method for species of *Armillaria*. Mycologia 87:280-288.

Hering, O., H.I. Nirenberg, S. Köhn, and G. Deml. 1999. Characterization of isolates of *Fusarium oxysporum* Schlecht. f. sp. *vasinfectum* (Atk.) Snyd. & Hans., races 1-6, by cellular fatty acid analysis. J. Phytopathol. 147:509-514.

Holmes, E.A., R.S. Bennett, D.W. Spurgeon, P.D. Colyer, and R.M. Davis. 2009. New genotypes of *Fusarium oxysporum* f. sp. *vasinfectum* from the southeastern United States. Plant Dis. 93:1298-1304.

Hutmacher, R.B., M. Ulloa, S.D. Wright, B.T. Campbell, R. Percy, T. Wallace, G. Myers, F. Bourland, D. Weaver, P. Chee, P. Thaxton, J. Zhang, W. Smith, J. Dever, V. Kuraparthy, D. Bowman, D. Jones, and J. Burke. 2013. Elite upland cotton germplasm-pool assessment of Fusarium wilt resistance in California. Agron. J. 105:1635-1644.

Ibrahim, F.M. 1966. A new race of the cotton-wilt *Fusarium* in the Sudan Gezira. Emp. Cotton Grow. Rev. 43:296-299.

Kappelman, A.J., Jr. 1983. Distribution of races of *Fusarium oxysporum* f. sp. *vasinfectum* within the United States. Plant Dis. 67:1229-1231.

Kim, Y., R.B. Hutmacher, and R.M. Davis. 2005. Characterization of California isolates of *Fusarium oxysporum* f. sp. *vasinfectum*. Plant Dis. 89:366-372.

LaMonda, J.A., and W.H. Elmer. 1988. Pathogenicity and vegetative compatibility among isolates of *Fusarium oxysporum* and *F. moniliforme* colonizing asparagus tissues. Can. J. Bot. 67:2420-2424.

Lawrence, K.S., M.W. Olsen, T.R. Faske, R.B. Hutmacher, J.D. Muller, J. Mario, B. Kemerait, C. Overstreet, G.B. Sciumbato, G.W. Lawrence, S. Atwell, S. Thomas, S. Koenning, R.K. Boman, H.K. Young, J.E. Woodward and H.L. Mehl. 2014. Cotton disease loss estimate committee report – 2014. Poster session presented at: Beltwide Cotton Conferences; 2015 Jan 6-7; San Antonio, TX.

Nirenberg, H.I., G. Ibrahim, and S.H. Michail. 1994. Race identity of three isolates of *Fusarium oxysporum* Schlect. f. sp. *vasinfectum* (Atk.) Snyd. & Hans. from Egypt and the Sudan. Z. Pflanzenkr. Pflanzenschutz 101: 594-597.

O'Donnell, K., C. Gueidan, S. Sink, P.R. Johnston, P.W. Crous, A. Glenn, R. Riley, N.C. Zitomer, P. Colyer, C. Waalwijk, T. van der Lee, A. Moretti, S. Kang, H. Kim, D.M. Geiser, J.H. Juba, R.P. Baayen, M.G. Cromey, S. Bithell, D.A. Sutton, K. Skovgaard, R. Ploetz, H.C. Kistler, M. Elliott, M. Davis, and B.A.J. Sarver. 2009. A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. Fungal Genet. Biol. 46:936-948.

O'Donnell, K., H.C. Kistler, E. Cigelnik, and R.C. Ploetz. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. U.S.A. 95:2044-2049.

O'Donnell, K., H.C. Kistler, B.K. Tacke, and H.H. Casper. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc. Natl. Acad. Sci. U.S.A. 97:7905-7910.

Skovgaard, K., H.I. Nirenberg, K. O'Donnell, and S. Rosendahl. 2001. Evolution of *Fusarium oxysporum* f. sp. *vasinfectum* races inferred from multigene genealogies. Phytopathology 91:1231-1237.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30:2725-2729.

Tooley, P.W., E.D. Goley, M.M. Carras, R.D. Frederick, E.L. Weber, and G.A. Kuldau. 2001. Characterization of *Claviceps* species pathogenic on sorghum by sequence analysis of the β-tubulin gene intron 3 region and EF- 1α gene intron 4. Mycologia 93:541-551.

Ulloa, M., R.B. Hutmacher, R.M. Davis, S.D. Wright, R. Percy, and B. Marsh. 2006. Breeding and Genetics: Breeding for fusarium wilt race 4 resistance in cotton under field and greenhouse conditions. J. Cotton Sci. 10:114-127.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p 315-322. *In* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and J.W. White (eds.) PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, NY.

Yang, M.E., R.M. Davis, and R.B. Hutmacher. 2006. Fusarium wilt of cotton in California: characterization and PCR- based detection of race 4. p. 93-96. *In* Proc. Beltwide Cotton Conf., San Antonio, TX. 3-6 Jan. 2006. Natl. Cotton Counc. Am. Memphis, TN.