POPULATION BIOLOGY OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM*, CAUSAL AGENT OF FUSARIUM WILT OF COTTON, IN THE SOUTHEASTERN UNITED STATES

by

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(Under the Direction of Marin Brewer)

ABSTRACT

Fusarium oxysporum f. sp. *vasinfectum* (FOV), has recently caused devastating Fusarium wilt outbreaks in cotton fields in the southeastern United States. FOV is often classified into races or genotypes, based on polymorphisms at the translation elongation factor (*EF-1*α) locus. The goal of this research was to use population biology to understand i) the genetic diversity and population structure, and ii) the aggressiveness of FOV causing Fusarium wilt in southeastern cotton fields. Sequence analysis of the *EF-1*α locus and SNPs identified through genotyping-by-sequencing revealed that populations of FOV in the southeastern U.S. are genetically diverse, but not genetically structured by *EF-1*α race or genotype. A greenhouse inoculation experiment identified variation in isolates' aggressiveness on cotton; these phenotypic differences were generally not associated with *EF-1*α sequence. These findings demonstrate that FOV is genetically and phenotypically diverse, and that *EF-1*α sequence polymorphisms are generally not biologically meaningful.

INDEX WORDS: *Fusarium oxysporum* f. sp. *vasinfectum*, Fusarium wilt, cotton, *Gossypium*

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DEDICATION

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LITERATURE REVIEW AND JUSTIFICATION

LITERATURE REVIEW

Cotton and Fusarium wilt of cotton

Cotton (*Gossypium* spp.) is a plant in the Malvaceae family that is well known for its soft bolls of fiber. The genus *Gossypium* is comprised of 39 wild and domesticated species which are globally distributed throughout the tropics and subtropics (Fryxell, 1979). The most economically important species of domesticated cotton are *Gossypium hirsutum*, which is known as Upland cotton, and *Gossypium barbadense*, known as Pima cotton. Cotton is typically grown in tropical and subtropical regions with an arid climate; however, cotton can also be grown in temperate areas. The United States is the 3rd largest cotton-producing country in the world, with national cotton production valued at approximately $7 billion in 2017 (USDA-ERS, 2018; USDA-NASS, 2019). Within the U.S., Georgia is the second largest cotton-producing state, behind Texas, with a production value of approximately $713 million in 2015 (Little, 2015; USDA-ERS, 2018).

Fusarium wilt of cotton is a re-emerging disease in cotton-producing regions of the United States, including Georgia. Plant diseases that were once mild or controllable can re-emerge due to environmental changes that are favorable for pathogens, or the evolution of new traits - such as increased virulence, chemical resistance, or the ability to overcome host resistance - in pathogen populations (Subbarao et al., 2015). Examples of re-emerging diseases include: Phomopsis stem canker of sunflower (*Helianthus annuus*), caused by two fungal species *Diaporthe gulyae* and *D. helianthi*, which re-emerged in the midwestern United States in 2010 and caused yield losses up to 40% after sunflower production practices shifted toward none or minimal tillage (Mathew et al., 2015); and wheat (*Triticum aestivum*) stem rust, which has become increasingly severe in several African and Middle-Eastern countries due to the emergence of a new, highly virulent lineage of *Puccinia graminis* f. sp. *tritici* known as Ug99 (Singh et al., 2015). Fusarium wilt of cotton is considered a re-emerging disease because it has historically only caused mild, late-season disease symptoms, and was relatively easy to manage; only in the past decade have Fusarium wilt outbreaks occurred earlier in the cotton-growing season and with more severe symptoms, despite formerly-effective management strategies being practiced (Collins et al., 2013; Whitaker et al., 2016). Fusarium wilt of cotton caused approximately $700,000 worth of damage in Georgia in 2015, demonstrating its economic importance and a need for management (Little, 2015). The most common symptoms of Fusarium wilt are discoloration of vascular tissues and wilting (Davis et al., 2006). Additional symptoms of Fusarium wilt depending on factors such as environmental conditions, cotton cultivar, and plant growth stage (Davis et al., 2006; Hao et al., 2009) include stunting, foliar chlorosis and necrosis, dieback, and even total plant death.

Fusarium wilt was first identified in 1892 in Auburn, Alabama by G.F. Atkinson (Atkinson, 1892). Since then, the causal organism - the fungus *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) - has been found in nearly every cotton-producing area in the world: FOV was reported in Egypt in 1902 (Fahmy, 1927), India in 1908 (Kulkarni, 1934), California in 1959 (Garber and Paxman, 1963), Sudan in 1960 (Ibrahim, 1966), Israel in 1970 (Dishon and Nevo, 1970), Brazil in 1978 (Armstrong and Armstrong, 1978), China in 1981 (Chen et al., 1985), and Australia in 1993 (Kochman, 1995). In addition to having a global distribution, FOV is able to infect every species of domesticated cotton (Armstrong and Armstrong, 1960; Fahmy, 1927).

Upland cotton is moderately susceptible to most genotypes of FOV, while Pima cotton has varying levels of tolerance or susceptibility depending on the cultivar (Doan and Davis, 2014; Holmes et al., 2009; Kim et al., 2006; Ulloa et al., 2006).

Fusarium oxysporum *species complex*

Fusarium oxysporum is a species complex comprised of over 100 distinct host-specific formae speciales (ff. spp.), with each forma specialis (f. sp.) defined by the host on which it causes disease (Armstrong and Armstrong, 1981; Michielse and Rep, 2009). Most formae speciales in the *Fusarium oxysporum* species complex (FOSC) are plant pathogens that cause vascular wilts or rots (Kang et al., 2014; O'Donnell et al., 2009). Host-specific formae speciales of *F. oxysporum* have been described on over 100 agriculturally and horticulturally important plant species in diverse environments including deserts, tropical forests, and even the Arctic Circle (Kommedahl et al., 1988; Mandeel et al., 2005; Michielse and Rep, 2009; Sangalang et al., 1995). There are also non-pathogenic isolates of *F. oxysporum* that can live endophytically or saprophytically in the environment (Kang et al., 2014; Kuldau and Yates, 2000). FOV, for example, has been reported to live asymptomatically on finger millet (*Eleusine coracana* L.), pearl millet (*Pennisetum glaucum* L.), snapdragon (*Antirrhinum majus* L.), sorghum (*Sorghum bicolor* L.), and sweet potato (*Ipomoea batatas* L.) (Armstrong and Armstrong, 1948; Davis et al., 2006).

Fusarium oxysporum is considered an asexual fungus, as sexual reproduction has never been observed in nature or artificially induced in a laboratory (Kang et al., 2014). *Fusarium oxysporum* produces three types of asexual spores: microconidia, macroconidia, and chlamydospores. Microconidia are oval-ellipsoid, one- or two-celled spores that are produced abundantly within the vascular system of the infected plant. Macroconidia are larger, three-to-five-celled canoe-shaped spores that are commonly found on the surface of infected plants. It is hypothesized that microconidia are involved in infection and colonization within the host, while macroconidia are produced for aerial dissemination from plant to plant (Katan et al., 1997). Chlamydospores are thick-walled survival spores that are produced on the tips or between septa of older hyphae (Kang et al., 2014). Unlike microconidia and macroconidia, chlamydospores are highly resistant to heat and chemicals and can survive in the soil for decades (Bennett, 2012; Freeman and Katan, 1988; Goyal et al., 1974). The genetic basis for the production of specific spore types is not well understood (Kang et al., 2014). Furthermore, spores cannot be used to distinguish formae speciales within the FOSC, as there are no consistent morphological distinctions among the spores of different formae speciales.

Many formae speciales in the FOSC, including FOV, are polyphyletic (Baayen et al., 2000; O'Donnell et al., 2009). O'Donnell et al. (2009) generated a phylogenetic tree for 256 *F. oxysporum* isolates among 48 different ff. spp. Isolates did not cluster according to forma specialis, suggesting that pathogenicity to the same host has independently arisen from different lineages. This most likely resulted from the horizontal transfer of lineage-specific pathogenicity chromosomes, also called accessory chromosomes (Ma et al., 2010). *Fusarium oxysporum* is characterized by the presence of lineage-specific (LS) chromosomes, which are enriched with transposable elements (TE's) and genes for small secreted proteins. The LS chromosomes are also highly divergent among different formae speciales, suggesting that they play a role in host-specialization (de Sain and Rep, 2015; Ma et al., 2010). In *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), a tomato wilt pathogen, the genes required for host infection are located on a single LS chromosome (chromosome 14). Horizontal transfer of this chromosome from a pathogenic to non-pathogenic *Fol* isolate confers pathogenicity to the formerly non-pathogenic isolate, demonstrating that host-specialization in *F. oxysporum* is defined by the presence of specific LS chromosomes containing virulence genes. The specific genes involved in virulence are currently being investigated in many *F. oxysporum* pathosystems, and there is evidence that the *Secreted-In-Xylem* (*SIX*) genes are important in pathogenicity and virulence across many formae speciales including *cubense*, *cucumerinum*, *lycopersici*, *melonis*, *niveum*, and *radicis-cucumerinum* (Czislowski et al., 2018; van Dam et al., 2016) The mechanism of horizontal chromosome transfer in *F. oxysporum* is currently unknown, but one hypothesis is that it occurs through anastomosis, i.e. hyphal fusion between compatible individuals (Mehrabi et al., 2011; Rep and Kistler, 2010).

Within a single forma specialis of *F. oxysporum*, there are usually several unique biotypes, such as vegetative compatibility groups or pathogenic races (Gordon and Martyn, 1997; Kang et al., 2014; Leslie, 1993). Vegetative compatibility is defined by the ability of two genetically distinct isolates to undergo anastomosis, or hyphal fusion (Leslie, 1993). Isolates in the same vegetative compatibility group (VCG) have identical alleles at specific loci that allow them to undergo anastomosis. The number of VCG's within a forma specialis is highly variable. FOV has over 20 known VCG's, whereas *Fusarium oxysporum* f. sp. *fragariae*, a strawberry wilt pathogen, only has three (Bell et al., 2017; Bell et al., 2019; Henry et al., 2017).

Some formae speciales also consist of different pathogenic races, as defined by virulence on a set of differentially resistant host cultivars (Kang et al., 2014). *Fol*, the tomato wilt pathogen, is divided into three pathogenic races - race 1, race 2, and race 3 - which are characterized by their ability to overcome specific plant immunity genes (Takken and Rep, 2010). Interestingly, multiple vegetative compatibility groups may exist within a single pathogenic race. In *F. oxysporum* f. sp. *cubense*, the causal agent of Panama disease of banana, for example, 25 distinct VCG's have been identified, but there are only 3 pathogenic races (Fourie et al., 2009; O'Donnell et al., 1998). In some formae specialies, including *cubense* and *vasinfectum*, associations have been made between several VCGs with pathogenic races, although the races corresponding with some VCGs are still undetermined (Bell et al., 2017; Czislowski et al., 2018).

Disease cycle and management of FOV

FOV is a soilborne pathogen that survives in the soil primarily as chlamydospores. In the presence of susceptible cotton roots, FOV spores germinate into infectious hyphae, then colonize and penetrate the root (Nelson, 1981). The hyphae usually penetrate the root through natural openings, including wounds, but may also penetrate the root directly using specialized infection structures (Nelson, 1981; Di Pietro et al., 2003). Once inside the host tissue, FOV grows intracellularly to reach the vascular tissue. In the vascular system, FOV produces microconidia which are transported through the plant xylem (Nelson, 1981). As the microconidia germinate and grow throughout the vascular system, the xylem becomes plugged and the plant's ability to transport water is impaired. This damage to the xylem is what causes the characteristic wilting of the cotton plant (Davis et al., 2006).

As FOV damages or kills its host, spores are produced on the surface of the plant and disseminated (Nelson, 1981). Macroconidia are aerially dispersed to neighboring plants, and chlamydospores drop to the soil where they may persist for decades (Katan et al., 1997; Smith et al., 2001). In addition to surviving as chlamydospores, FOV can persist between cotton-growing seasons as a saprophyte on dead or decaying plant material, or as an endophyte on a wide range of asymptomatic hosts (Armstrong and Armstrong, 1948; Davis et al., 2006). FOV can also survive in cottonseed, usually in plants that developed symptoms late in the growing season (Elliott, 1923; Hillocks, 1983).

The disease cycle of Fusarium wilt is often influenced by plant-pathogenic nematodes residing in soils. The most well-documented example of this is the disease complex of FOV with *Meloidogyne incognita*, the root knot nematode (RKN) (Atkinson, 1892; Garber et al., 1979; Jorgenson et al., 1978). In 1892, Atkinson observed a relationship between the incidence of wilt on cotton and population density of *M. incognita* (Atkinson, 1892). Nearly a century later, Jorgenson et al. (1978) confirmed this relationship by plotting the severity of cotton wilt symptoms against nematodes counted in soil samples from the field. Because of the clear relationship between *M. incognita* and Fusarium wilt, FOV has historically been managed by reducing population levels of *M. incognita* through fumigation, crop rotation, and the implementation of root-knot-nematode-resistant cotton cultivars (Chawla et al., 2012; Jorgensen et al., 1978; Kirkpatrick and Sasser, 1984; Ogallo et al., 1997). Recent surveys have shown that FOV may be associated with nematodes other than *M. incognita* (da Silva et al., in press). In these surveys, the presence of FOV was confirmed in soils where *M. incognita* was absent, but other plant pathogenic nematodes - including the reniform nematode (*Rotylenchulus reniformis*) and sting nematode (*Belonolaimus longicaudatus*) - were present.

Highly virulent isolates of FOV can cause severe disease on cotton even in the absence of plant pathogenic nematodes - FOV race 4 is especially well-known for this (Kim et al., 2005). Isolates that can directly penetrate the cotton root cannot be controlled simply by managing nematode populations. Instead, these aggressive genotypes of FOV must be managed directly via host resistance, chemical fumigation, soil solarization, or seed treatment.

Host resistance is considered the most economic and efficacious management strategy for FOV (Cianchetta and Davis, 2015; Ulloa et al., 2006). In 2006, 150 commercial and experimental cotton cultivars were evaluated for genetic resistance to FOV race 4, the highly virulent race that causes devastating crop loss even in the absence of plant pathogenic nematodes (Ulloa et al., 2006). Overall, Upland cotton (*Gossypium hirsutum* L.) is less sensitive to FOV race 4 than Pima cotton (*G. barbadense* L). When looking only among resistant cultivars, however, Pima cultivars appear to have more complete resistance than Upland, suggesting that resistance to FOV race 4 in Pima cultivars may be controlled by a single dominant gene. The Pima cultivar Phytogen 800 (Dow AgroSciences, Indianapolis, IN) is resistant to most races of FOV, including race 4 (Doan and Davis, 2014; Holmes et al., 2009). The Upland cultivars MD25-26ne and MD25-27 are also relatively resistant to aggressive genotypes of FOV (Doan and Davis, 2014; Meredith, 2013). Recently, multiple independent loci associated with resistance to FOV races 1 and 4 were identified in interspecific cotton (a cross between Pima and Upland varieties); these could provide useful sources of FOV resistance for breeding programs (Wang et al., 2018).

FOV can also be managed directly through chemical fumigation, soil solarization, or seed treatment. Ben-Yephet et al. (1988) found that combining soil solarization with an application of metam-sodium at 25 ml/m^2 significantly reduced the viability of FOV chlamydospores after 6 weeks. Bennett (2012) found that a soil solarization temperature of 39°C reduced *in vivo* populations of FOV chlamydospores from 16,000 CFU/g to less than 1 CFU/g within 18 days. Soil solarization at 41°C reduced populations to the same degree in just 6 days. Doan and Davis (2015) evaluated seed treatments as a means of eliminating FOV from infected cotton seeds. They found that immersing infected seeds in sterile water for 1 hour, then immersing the seeds in thiophanate-methyl (0.70 g a.i.) heated to 60°C for twenty minutes reduced FOV populations by 85% without reducing seedling germination or vigor. Because FOV produces chlamydospores which survive in the soil for decades, crop rotation is not a practical management option.

Genetic and pathogenic diversity of FOV

There are six nominal races of FOV which infect cotton worldwide; FOV races 1, 2, 3, 4, 6, and 8 (Cianchetta et al., 2015; Davis et al., 2006). Originally, eight pathogenic races were described, but multigene genealogies and pathogenicity tests demonstrated that races 3 and 5 are genetically indistinguishable, as are races 4 and 7 (Armstrong and Armstrong, 1960; Armstrong and Armstrong, 1978; Nirenberg et al., 1994; Skovgaard et al., 2001). It should be noted that when the nominal races of FOV were first described, the term "race" was not defined based on a set of differential reactions on a single host, but rather based on virulence on a set of cotton and non-cotton hosts. Sequence analyses in the past two decades have shown that the six nominal races can be distinguished by their sequences at the translation elongation factor 1α (*EF-1α*) locus and intergenic spacer region (IGS). (Kim et al., 2005; Skovgaard et al., 2001). These variants of FOV would be more accurately described by the term "genotype", but the term "race" has been maintained by the cotton industry (Davis et al., 2006). Of the six nominal races of FOV present worldwide, five - races 1, 2, 3, 4, and 8 - are found in major cotton-growing regions of the United States (Holmes et al., 2009; Kim et al., 2005). Races 1, 2, 3, and 8 are all widely distributed, having been detected both in California and throughout the Southeast. These genotypes of FOV are only mildly virulent on cotton, but cause more serious disease symptoms when *Meloidogyne incognita*, the root knot nematode, is also present (Garber and Paxman, 1973; Kim et al., 2005). Race 4, which is the only nominal race to cause severe disease in the absence of plant-pathogenic nematodes, appears to be limited within the U.S. to California and Texas (Cianchetta et al., 2015; Davis et al., 1996; Halpern et al., 2017; Kim et al., 2005).

In addition to the nominal races, there are five unique genotypes of FOV that are found throughout the southeastern U.S. - LA 108, LA 110, LA 112, LA 127/140, and MDS-12. The LA genotypes each have unique *EF-1α* sequences that differentiate them from the previously described nominal races (Holmes et al., 2009). MDS-12 has an *EF-1α* sequence identical to that of FOV race 4, but a unique IGS sequence (Bennett et al., 2013; Holmes et al., 2009). The LA genotypes and MDS-12 are often found in association with severe Fusarium wilt outbreaks in the southeastern U.S, but the virulence associated with these genotypes is not well-characterized (Holmes et al., 2009).

Our current understanding of the genetic diversity of FOV is primarily based on genetic polymorphism at the *EF-1α* locus and IGS region (Bennett et al., 2009; Cianchetta et al., 2015). *EF-1α* is a highly conserved gene among eukaryotes that is involved in the binding and delivery of transfer RNA (tRNA) during protein synthesis (Reed et al., 1994). It is considered to be an especially good marker for distinguishing formae speciales in the *Fusarium oxysporum* species complex (O'Donnell et al., 1998). IGS is a non-coding region of the nuclear ribosomal RNA (Appel and Gordon, 1996; White et al., 1990). Taken together, *EF-1α* and IGS sequences have been used to create a comprehensive database of two-locus sequence types (ST's) for 848 unique *Fusarium oxysporum* isolates, providing a reliable molecular method to identify pathogenic *Fusarium oxysporum* isolates (O'Donnell et al., 2009).

Although *EF-1α* and IGS are useful for identification purposes and for understanding the evolution of FOV, these markers do not seem to be associated with differences in pathogenicity or virulence among isolates, and they may not describe FOV's full genetic diversity. Ideally, molecular markers could be used to identify true pathogenic races or unique disease phenotypes of FOV. Molecular discrimination of unique FOV phenotypes is complicated because FOV possesses high levels of genetic diversity and current commercial cotton cultivars may not be able to differentiate diverse genotypes (Cianchetta et al., 2015; Holmes et al., 2009).

Genotyping-by-sequencing (GBS)

In population genetic studies, polymorphic molecular markers are used to analyze genetic variation and make inferences about the evolutionary and demographic processes which led to observed allele frequencies. In plant pathology, population genetic studies have been used to understand how pathogen populations are structured, identify pathogens' centers of origin, determine the genes and genotypes associated with particular disease phenotypes, and understand the evolution of traits such as virulence or fungicide resistance (Burchhardt and Cubeta, 2015; Goss et al, 2014; Talas et al., 2016). In the context of asexual fungi, like FOV, molecular markers are often used to determine the clonal lineages comprising population structure, or to identify novel genetic diversity (Hansen et al., 2016; Rafiei et al., 2018).

Examples of molecular markers used for population genetic studies include restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), microsatellite markers, also known as simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNP's) (Milgroom, 2015). Microsatellites and SNP's are two of the preferred molecular markers for population genetic analyses because they are repeatable, provide many loci for analysis, and also provide high resolution of genetic differences among individuals. Microsatellite markers are tandem repeats of 1-6 nucleotides which vary in length across populations or individuals, making them useful for identifying unique alleles within and among populations. SNPs are single nucleotide polymorphisms, or differences in bases at individual sites across the genome. SNPs are usually discovered by conducting whole or reduced-representation genome sequencing. Microsatellites are generally more polymorphic than SNPs, and more accessible and affordable than whole or reduced-representation genome sequencing; however, SNPs have the advantage of providing a higher number of markers for population genetic analyses, making them especially useful for making evolutionary inferences about highly diverse or fast-evolving species (Rafiei et al., 2018).

Genotyping-by-sequencing (GBS) is a type of reduced representation genome sequencing in which genomic DNA is digested using a common cutter enzyme, and short fragments are amplified and sequenced via next-generation sequencing (Andrews et al., 2016; Elshire et al., 2011). This approach allows for the identification of thousands of SNPs among a large sample of individuals; it provides high resolution of genetic differences among individuals and allows for robust evolutionary inferences, while being more affordable and practical than whole genome sequencing. GBS has been used in population genetic analyses of plant pathogens to determine population structure, identify cryptic sexual recombination in seemingly clonal populations, find associations between SNP markers and phenotypes of interest, and make inferences about the inheritance of virulence genes (Mhora et al., 2016; Milgroom et al., 2014; Rafiei et al., 2018; Yuan et al., 2018). Using GBS to discover SNPs for population genetic analyses is an ideal approach for FOV, because FOV is known to be genetically diverse, and because there are knowledge gaps in how the current classification (races and genotypes based on *EF-1α* sequence) relates to the organism's population structure, evolutionary history, and phenotypic diversity.

JUSTIFICATION AND OBJECTIVES:

Fusarium wilt of cotton is an economically important re-emerging disease in the southeastern United States, causing $700,000 worth of damage in Georgia alone in 2015 (Little, 2015). The causal agent of Fusarium wilt of cotton is the soilborne fungus *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). Pathogenic races of FOV were characterized in the late 1900's but are now considered invalid, leaving knowledge gaps in FOV's pathogenicity and virulence on modern cotton cultivars (Armstrong and Armstrong, 1960; Amrstrong and Armstrong, 1978; Holmes et al., 2009; Nirenberg et al., 1994; Skovgarrd et al., 2001). Additionally, the genetic diversity of FOV has only been characterized at a few housekeeping genes, so there are knowledge gaps in the pathogen's genetic diversity as well (Cianchetta et al., 2015; Kim et al., 2005; Skovgaard et al., 2001). Based on these knowledge gaps, the objectives of this study were to:

1) Characterize the genetic diversity and population structure of FOV using a genotyping-by-sequencing (GBS) approach and

2) Determine the *EF-1*α genotypes of FOV found in Georgia cotton fields, and determine if there is an association between *EF-1*α genotype and aggressiveness on cotton.

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CHAPTER 2

GENETIC DIVERSITY AND POPULATION STRUCTURE OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* (FOV), CAUSAL AGENT OF FUSARIUM WILT OF COTTON, USING GENOTYPING-BY-SEQUENCING (GBS)[1]

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[1] Halpern, H. C., Qi, P., Kemerait, R. C., and M. T. Brewer. To be submitted to *G3*.

ABSTRACT

Fusarium oxysporum f. sp. *vasinfectum* (FOV) is a fungal pathogen of cotton (*Gossypium* spp.) causing severe Fusarium wilt outbreaks in cotton-producing regions of the United States. Six nominal races and eleven genotypes of FOV have been characterized based on sequences at the partial translation elongation factor (*EF-1α*) locus and intergenic spacer region (IGS), yet genetic diversity beyond these housekeeping genes has not been reported, and it is unclear how race or genotype based on these loci relates to evolutionary relationships within this *forma specialis*. We used genotyping-by-sequencing to identify SNPs and determine genetic structure and levels of diversity among 86 genetically, geographically, and temporally diverse FOV isolates. Six isolates other *formae speciales* were genotyped and included in some analyses. Between 193 and 358 SNPs were identified and included in the analyses depending on the pipeline and filtering criteria used. Phylogenetic trees, minimum spanning networks (MSNs), principal components analysis (PCA), and discriminant analysis of principal components (DAPC) based on the SNPs demonstrated that races and genotypes of FOV are generally not structured by *EF-1α* genotype, nor are they monophyletic groups with the exception of race 4 isolates, which are distinct. Furthermore, DAPC identified an optimum of between 11 and 14 genetically distinct clusters of FOV, whereas only eight *EF-1α* genotypes were represented among isolates; suggesting that FOV, especially isolates within the widely distributed and common race 1 genotype, is more genetically diverse than currently recognized.

INTRODUCTION

Fusarium oxysporum f. sp. *vasinfectum* (FOV) is a soilborne fungus that causes Fusarium wilt of cotton (*Gossypium* spp.) in virtually every cotton-producing region of the world

(Atkinson, 1892; Armstrong and Armstrong, 1978; Chen et al., 1985; Dishon and Nevo, 1970; Fahmy, 1927; Garber and Paxman, 1963; Ibrahim, 1966; Kochman, 1995; Kulkarni, 1934). FOV is haploid, and it is considered asexual among plant pathologists because it reproduces clonally and does not undergo meiotic recombination. In the southeastern United States, Fusarium wilt of cotton was historically a relatively minor disease problem because effective disease management practices - especially the management of plant pathogenic nematodes which aggravate Fusarium wilt symptoms - were used (Garber et al., 1979; Jorgenson et al., 1978). In the past decade, despite the same disease management practices being followed, Fusarium wilt has become increasingly severe and widespread in the southeastern United States, potentially suggesting changes in local populations of FOV (Bell et al., 2017; Whitaker et al., 2016).

Although FOV has been studied for decades, there are still knowledge gaps in the pathogen's genetic diversity. Eight pathogenic races of FOV were reported between 1958 and 1985, and although these races are widely regarded as invalid (Armstrong and Armstrong, 1958; Armstrong and Armstrong, 1960; Armstrong and Armstrong, 1978; Atkinson, 1892; Bell et al., 2017; Chen et al., 1985; Davis et al., 2006; Ibrahim, 1966), they are widely used in the cotton industry for characterizing FOV. Typically, a race refers to a pathogen's ability to cause disease on its host and implies a gene-for-gene interaction between host and pathogen (Anderson et al., 2010). A set of differential host cultivars with unique combinations of resistance genes are frequently used to discriminate pathogenic races. The races of FOV, however, were characterized based on isolates' virulence not only on a differential set of cotton cultivars, but also on many non-host plants including tobacco, soybean, and okra (Armstrong and Armstrong, 1960; Armstrong and Armstrong, 1978; Chen et al., 1985). Additionally, many of the cotton cultivars that were originally used to differentiate races of FOV have not been maintained, and differential virulence reactions are not reproducible on modern cotton cultivars (Bell et al., 2017; Davis et al., 2006; Holmes et al., 2009). Furthermore, multigene genealogies, pathogenicity tests, and other bioassays demonstrated that races 3 and 5, as well as 4 and 7, are genetically and phenotypically indistinguishable and should therefore be recognized as single groups (Hering et al., 1999; Nirenberg et al., 1994; Skovgaard et al., 2001). As a result, only six races of FOV are recognized today.

Each race of FOV is associated with a unique sequence at the transcription elongation factor (*EF-1*α) locus, with the exception of races 1 and 6, but race 6 is apparently limited to South America; *EF-1*α sequence is the primary tool used to characterize races of FOV today (Cianchetta et al., 2015; Kim et al., 2005; Skovgaard et al., 2001). "Genotype" would be a more valid term than "race" to describe these polymorphisms, but the term "race" is still used nominally in the United States cotton industry (Davis et al., 2006). In addition to the six nominal races, there are four other genotypes of FOV characterized by unique *EF-1*α sequences, referred to as LA108, LA110, LA112, and LA127; and one genotype, MDS-12, that is identical to FOV race 4 in *EF-1*α sequence but unique in intergenic spacer region (IGS) sequence (Bennett et al., 2013; Holmes et al., 2009).

Although housekeeping gene sequences, especially *EF-1*α*,* have been used to characterize FOV isolates in the United States, there is some evidence that these polymorphisms do not fully explain the genetic diversity and evolutionary history of FOV. For example, a phylogeny generated from *EF-1*α, mitochondrial small subunit ribosomal DNA (mtSSU rDNA), nitrate reductase (*NIR*), and phosphate permease-like protein (*PHO*) sequences, showed race 2 to be a polyphyletic group (Skovgaard et al., 2001). A separate phylogeny generated from IGS, *PHO*, *EF-1*α, and beta-tubulin (*BT*), showed that MDS-12 is also polyphyletic (Cianchetta et al., 2015). Given that FOV is considered a highly diverse pathogen, there may be other polymorphisms being overlooked in characterizations based on housekeeping gene sequences.

Genotyping-by-sequencing (GBS) is a form of reduced-representation genome sequencing in which genomic DNA is digested with a restriction enzyme, and short fragments are amplified and sequenced via next-generation sequencing (Andrews et al., 2016; Elshire et al., 2012). Depending on coverage, this approach allows for the identification of hundreds to thousands of single nucleotide polymorphisms (SNPs) among a large sample of individuals, thus providing high resolution of genetic differences among individuals. GBS has been used in population analyses of plant pathogens to identify novel genetic diversity in high-diversity organisms, identify cryptic sexual recombination among clonal pathogens, and conduct genome-wide-association-studies (GWAS) linking quantitative trait nucleotides (QTNs) with pathogen virulence and mycotoxin production (Hansen et al., 2016; Milgroom et al., 2014; Talas et al., 2016).

The goal of this study was to characterize the genetic diversity, evolutionary history, and population structure of FOV using GBS to identify SNPs among diverse isolates of FOV. Additionally, we wanted to compare two data analysis pipelines using two different reference genomes - one publicly available annotated reference genome, and one reference assembled *de novo* from the raw GBS data - to assess the effect of the reference on population analyses.

MATERIALS AND METHODS

Isolate collection

One-hundred-and-fourteen single spore cultures of FOV were isolated from symptomatic plants throughout Georgia cotton fields, following the protocol described by da Silva et al.

(2019). Isolates were putatively genotyped based on their translation elongation factor (*EF-1α*) sequence, using the primers EF1 and EF2 (Cianchetta et al., 2015; O'Donnell et al., 1998). Each PCR reaction consisted of 1.25 µl mM dNTP's, 1.25 µl 10x ExTaq buffer (Takara Bio USA), 0.56 µl of each 10 µM primer, 0.3 µl ExTaq (Takara Bio USA), and 1 µl of genomic DNA (10 - 300 ng/µl). Amplification was conducted in a thermal cycler (PTC-100; MJ Research, Watertown, MA) under the following conditions: 95°C for 1 min; 40 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min; and a final extension of 72°C for 5 min (Cianchetta et al., 2015). Amplification of the *EF-1α* locus was confirmed by 1% agarose gel electrophoresis, and PCR products were purified with an ExoSAP-IT kit (Thermo Fisher Scientific, Waltham, MA) per manufacturer instructions. A 320 ng sample of DNA combined with 4 µl of 10 µM primers were sequenced by EuroFins (Louisville, KY). Sequences were aligned to the publicly available race and genotype references used by Cianchetta et al. (2015). Alignments were performed with Geneious R11 using a global alignment with free end gaps and a 70% BLOSUM cost matrix (Kearse et al., 2012). *EF-1α* sequence was insufficient to unambiguously to determine the race or genotype of FOV race 4 and MDS-12 isolates (Bennett et al., 2013); to differentiate between race 4 and MDS-12, the intergenic spacer region was sequenced following the protocol described by Cianchetta et al. (2015). Additionally, *EF-1α* sequence is insufficient to distinguish races 1 and 6 (Skovgaard et al., 2001). However, reports of race 6 are limited to South America so isolates with an *EF-1α* sequence indicative of race 1 or 6 were assumed to be race 1 (Amstrong and Armstrong, 1978; Cianchetta et al., 2015).

In addition to the 114 FOV isolates collected from symptomatic plants in Georgia cotton fields, 54 *Fusarium oxysporum* isolates were obtained from out-of-state collaborators Jeffrey Coleman (Auburn University), R. Michael Davis (University of California Davis), and Jinggao

Liu (USDA-ARS Southern Plains Agricultural Research Center), as well as the USDA-ARS Northern Regional Research Laboratory (NRRL) culture collection for the purpose of maximizing genetic, temporal, and geographic diversity. Six of these 54 isolates were other *formae speciales* of *Fusarium oxysporum* - *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), *Fusarium oxysporum* f. sp. *cubense*, and *Fusarium oxysporum* f. sp. *dianthi* - which can be closely related to FOV (O'Donnell et al., 2009).

Genomic DNA extraction and genotyping-by-sequencing (GBS)

Genomic DNA was extracted from a total of 168 single-spore *Fusarium oxysporum* isolates. Isolates were grown on potato dextrose agar (PDA) overlain with sterile cellophane for 6-7 days, after which time mycelia were harvested and lyophilized (Milgroom et al., 2014). Approximately 50 milligrams of lyophilized mycelia were frozen in liquid nitrogen and macerated in 2-ml tubes with glass beads in a Geno/Grinder® (SPEX SamplePrep, Metuchen, NJ). DNA was extracted using a DNeasy Plant Mini kit (QIAGEN, Valencia, CA) according to manufacturer protocols with the following modification: in the final step of the protocol, samples were eluted in 25 µl AE buffer to increase the final concentration of DNA. Concentration and quality were determined using a Nanodrop spectrophotometer ND-1000 (Nanodrop Technologies, Wilmington, DE) and by 0.7% agarose gel electrophoresis, respectively.

Genotyping-by-sequencing was performed following the protocol described by Elshire et al. (2011) at the Georgia Genomics and Bioinformatics Core (Athens, GA). Briefly, samples of ≥100 ng genomic DNA were digested with the restriction endonuclease *Ape*KI, ligated with combinatorial barcode adapters, pooled, PCR-amplified, and purified. Samples were then sequenced on an Illumina NextSeq PE150 high output flowcell using 150-bp paired end reads (Illumina, San Diego, CA).

SNP calling and data filtering

Single nucleotide polymorphisms (SNPs) were identified and called using two modified versions of the uGbS-Flex pipeline (Qi et al., 2018). In the first pipeline, SNP calling was performed using a reference-based alignment; in the second, a *de novo* reference was generated from GBS reads following the protocol described by Qi et al. (2018) and SNP calling was based on alignment to the *de novo* reference. The rationale for using two separate data analysis pipelines was that the reference genome used in data processing and SNP calling could potentially impact the results of population analyses.

For reference-based SNP calling, quality control was performed using FastQC and raw sequence data was sorted using the process_radtags command in Stacks version 2.0 (Catchen et al., 2013; Andrews, 2010). Processed reads were trimmed to 120 base pairs and aligned to an annotated reference genome of FOV race 4 (NRRL 25433, Broad Institute) using FASTX Trimmer and bowtie2, respectively (Hannon, 2010; Langmead and Salzberg, 2012). Aligned reads were processed, validated, and sorted using SAMtools and Picard (Broad Institute; Li et al., 2009). SNPs were called using GATK HaplotypeCaller and GenotypeGVCFs (Van der Auwera et al., 2013). SNPs were filtered to retain only biallelic sites, and the resulting VCF file was further filtered for read depth of at least 10 and a minimum of 20 percent missing information for any site or individual (Danecek et al., 2011).

The *de novo* GBS reference was assembled following the protocol described by Qi et al. (2018). Briefly, reads were made equal lengths using FASTX Trimmer, overlapping forward and reverse reads were merged in FLASH, non-overlapping forward and reverse reads were artificially joined and made equal lengths using the in-house python script EL.1.4.py, reads within each sample were clustered using the ustacks function in Stacks version 2.0, and a consensus set of tags from the population was generated with cstacks (Catchen et al., 2011; Hannon, 2010; Magoc and Salzberg, 2011; Qi et al., 2018). GBS reads were processed, aligned to the *de novo* reference, and filtered as described above.

Population genetic analyses

Filtered VCF files were analyzed in R version 3.5.1 with the packages vcfR ,poppr 2.0, and adegenet (Jombart and Ahmed, 2011; Kamvar et al., 2015; R Core Team, 2018; Knaus and Grunwald, 2017). A genotype accumulation curve was generated to demonstrate the number of unique SNP genotypes in the population, as well as the minimum number of SNP loci needed to distinguish unique genotypes (Grunwald et al., 2003; Kamvar et al., 2015). Bitwise genetic distance was calculated and used to generate a UPGMA tree with 500 bootstrap replicates. Bitwise genetic distance was chosen because it was the most appropriate distance model for SNP data of the models provided in poppr. Bitwise distance was also used to construct a minimum spanning network (MSN) with the poppr.msn function, to show the relationships among all individuals in the population (Kamvar et al., 2015). Isolates in the minimum spanning network were colored according to their race (as inferred from *EF-1α* genotype) to observe potential clustering patterns by race. Principal component analysis (PCA) was conducted to determine whether isolates of particular races were distinct. PCA was run using the glPca command in adegenet, and results were plotted using the R package ggplot2 (Jolliffe, 2002; Jombart and Ahmed, 2011; Wickham, 2016). Discriminant analysis of principal components (DAPC), or K-means hierarchical clustering, was conducted using the find.clusters command in adegenet in order to determine the optimal number of genetically differentiated clusters across all FOV isolates (Jombart et al., 2010).

All analyses were performed on the VCF files generated from the reference-based alignment, and replicated using the VCF file generated from the *de novo* based alignment to test for differences in results obtained using the two processing methods.

RESULTS

SNP calling, data filtering, and genetic diversity

The unfiltered VCF file based on alignment to the annotated reference genome of NRRL 25433 (Broad Institute) contained 229,338 SNPs. After filtering for read depth and missing information 193 biallelic SNPs remained, and 86 FOV and 6 other *Fusarium oxysporum* isolates were retained for population genetic analyses. Across the 86 FOV isolates that were retained, 76 multilocus genotypes (MLGs) were identified (Fig 2.1A). The genotype accumulation curve generated in poppr never fully leveled off, suggesting that greater than 193 SNPs are needed to distinguish the full genetic diversity of FOV (Fig 2.1A). With the *de novo* reference, 358 SNPs, 86 FOV isolates, and 6 other *Fusarium oxysporum* isolates were retained for analyses. Eighty-five of 86 FOV isolates were considered unique MLGs (Fig 2.1B). The genotype accumulation curve associated with this dataset leveled off at approximately 220 loci (Fig 2.1B).

Phylogenetic analyses and evolutionary relationships

In the UPGMA tree generated from the reference-based SNPs, all FOV race 4 isolates grouped together on a branch with bootstrap support of 100 (Fig 2A). Differentiation was observed within the clade of race 4 isolates; however isolates from China, California, and Texas were not separated by geographic location. The remaining isolates generally did not group by race or location, and most branches outside of the race 4 clade had bootstrap support below 50. Surprisingly, the other formae speciales (ff. spp.) of *Fusarium oxysporum* (*Fo*) did not form an outgroup nor did they all cluster together. The UPGMA tree based on alignment to the *de novo* reference was similar but not identical to the tree described above. Race 4 isolates, for example, still formed a monophyletic group, but were not separated from all other isolates as in the reference-based analysis, and furthermore no differentiation was observed within this clade (Fig 2B). There was also more clustering by race or *EF-1α* genotype in this tree, although several polyphyletic groups were still observed. Furthermore, most of the branches in this tree had bootstrap support below 50.

Population structure

In the minimum spanning network (MSN) constructed from the reference-based SNP dataset, FOV race 4 isolates were distant from all other isolates, and differentiation was observed within the group of race 4 isolates (Fig 2.3A). No other isolates clearly clustered by race or *EF-1α* genotype. In the MSN generated from the *de novo*-based SNP data, race 4 isolates still clustered together, but no differentiation was observed within that cluster (Fig 2.3B). Also, in the *de novo*-based MSN, more differentiation was observed among isolates of race 1, LA108, and LA110. Many reticulations - or the presentation of all equally probable connections - were observed among isolates of race 1, LA108, and LA110.

The PCA analysis of the reference-based SNP dataset demonstrated overlap among all races of FOV except for race 4, which formed a tight cluster with a narrow ellipse and was separated from other isolates by PC1 and PC2 (Fig 2.4A). LA127 isolates were also separated by both PC1 and PC2, but there were not enough samples (n=3) to construct an ellipse. On the *de novo*-based dataset, the PCA results were similar, though the race 4 cluster was more dispersed and overlapped with one race 1 isolate (Fig 2.4B).

For the reference-based SNPs, DAPC identified an optimum of k=14 genetically distinct clusters (Fig 2.5A). All individuals had a 100% membership probability to the group which they were assigned. All clusters contained between one and six individuals, except for cluster 5 which contained 42 individuals. Some clusters contained isolates of a single race or *EF-1α* genotype; for example groups 1, 2, and 3 all contained only race 1 isolates, groups 10 and 11 contained only race 4 isolates, and group 13 contained only race 2 isolates. However, some races spanned multiple groups (for example, race 1) and, conversely, some groups contained individuals of multiple races and genotypes - for example group 6 contained 44 isolates spanning race 1, LA108, LA110, and MDS-12.

When DAPC was run on the *de novo*-based SNPs, an optimum of k=11 clusters were identified (Fig 2.5B). As with the reference-based analysis, all individuals were assigned to groups with 100% membership probability. Some groups identified in this analysis were identical to those identified in the reference-based DAPC analysis. For example, group 5 in the *de novo*-based DAPC analysis was identical to group 10 in the reference based analysis. A major difference between the two analyses is that the *de novo*-based DAPC analysis showed less diversity among race 1 isolates: no groups contained only race 1 isolates; individuals of race 1 always grouped with other races and genotypes. Additionally, in the *de novo*-based analysis, more isolates fell into the largest genetic cluster as opposed to being placed in their own unique group; in this analysis, the largest cluster contained 50 individuals as opposed to 44 individuals in the reference-based analysis.

DISCUSSION

Using a genotyping-by-sequencing (GBS) approach, we identified novel genetic diversity in the cotton wilt pathogen *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). In our reference-based data processing pipeline, we identified 193 SNPs and 76 unique multilocus genotypes (MLGs) of FOV, and found that greater than 193 SNPs are most likely needed to fully distinguish unique genotypes of FOV in the population. In the *de novo*-based analysis, we identified 354 SNPs and 85 MLGs, and found that 220 SNP loci were needed to distinguish unique genotypes. The results of the reference-based and *de novo*-based analyses complement each other, and suggest that 220 is a realistic minimum number of SNPs needed to conduct high-resolution population genetic analyses of FOV. However, some of the SNPs identified in the *de novo*-based data analysis pipeline may be redundant, as the UGbS-Flex pipeline neither assessed redundancy nor removed redundant SNPs (Qi et al., 2018). Additionally, MLGs are not straightforward to interpret in the context of datasets with hundreds to thousands of SNPs - some of the unique MLGs identified in our study were only different by one SNP, which may not warrant classification as different genotypes (Grunwald et al., 2016). The concept that MLGs are problematic for SNP genotypes is further demonstrated by the fact that 85 of 86 individuals were considered unique MLGs in the *de novo*-based genotype accumulation curve, which was based on a larger number of SNPs than the reference-based genotype accumulation curve. Although the exact number of MLGs in the population is unclear, our genotype accumulation curves suggest that using one or a few housekeeping genes does not provide enough resolution for population analyses of FOV, and that such an approach is likely to overlook genetic diversity. Our DAPC results provide additional evidence that FOV is more genetically diverse than its races and *EF-1α* genotypes: only eight races and *EF-1α* genotypes were represented among the isolates analyzed by DAPC, but an optimum of k=14 and k=11 genetically distinct groups of FOV were identified using reference-based SNPs and *de novo*-based SNPs, respectively.

Although our population genetic analyses provide new evidence that FOV is more diverse than its races and *EF-1α* genotypes, we are not the first to suggest this. The U.S. cotton industry uses races and genotypes based on *EF-1α* sequence to characterize FOV isolates, but FOV can also be classified into vegetative compatibility groups (VCGs) (Bell et al., 2017). Vegetative compatibility is defined by the ability of isolates to undergo anastomosis, or hyphal fusion, and isolates are able to undergo anastomosis if they have identical alleles at several *vic* loci (Leslie, 1993). Because individuals in the same VCG must possess several identical alleles, vegetative compatibility is thought to be a good indication of isolates' relatedness (Puhalla, 1985). Additionally, Bell et al. (2017) reported that individuals in the same VCG typically possess the same disease phenotype on cotton, suggesting that VCG is an ideal way to characterize FOV isolates as it is indicative of both genetic relationship and disease phenotype. Twenty distinct VCG's have been identified in FOV (Bell et al., 2017; Davis et al., 1996; Fernandez et al., 1994; Katan and Di Primo, 1999), but our study identified a maximum of fourteen genetically distinct groups of FOV. One possible explanation for this is that some VCG's are rare - for example, between 1994 and 2017 only one isolate belonging to the VCG known as 0111 had been identified (Bell et al., 2017; Fernandez et al., 1994) - and therefore were not represented among the isolates analyzed in our study. Additionally, it is possible that some FOV isolates differ at *vic* alleles which govern vegetative compatibility but are otherwise genetically similar, resulting in individuals of different VCGs being placed in the same DAPC group in our study.

Our results also demonstrated that FOV race 4 isolates comprise a genetically distinct group, whereas the relationships among all other FOV isolates are not explained by their race or *EF-1α* genotype. This was demonstrated in maximum likelihood trees in which race 4 isolates formed a monophyletic group on a branch with high bootstrap support in both analyses, while the other races and genotypes of FOV were non-monophyletic and their depicted relationships generally had bootstrap support below 50. The fact that race or *EF-1α* genotype is not necessarily indicative of genetic relatedness was also demonstrated in the minimum spanning network (MSN) and principal components analysis (PCA), both of which showed a clear clustering of race 4 isolates, but no clustering of other races or genotypes. Additionally, the clusters identified by DAPC were also not structured by race or *EF-1α* genotype. Some clusters contained individuals of multiple races or genotypes, such as cluster six in the reference-based analysis, which contained 42 individuals spanning race 1, LA108, LA110, and MDS-12; this suggests that isolates with different *EF-1α* genotypes can possess a high degree of genetic similarity. Conversely, some races or genotypes based on *EF-1α* sequence were present in multiple genetic clusters: in the reference-based analysis, race 1 isolates were present in six of fourteen clusters, showing that individuals sharing the same *EF-1α* sequence are not necessarily closely related. Taken together, our population analyses provide compelling evidence that race or *EF-1α* genotype is not reflective of FOV isolates' genetic relatedness, with the exception of race 4.

The finding that race or *EF-1α* sequence is generally not indicative of isolates' genetic relatedness leads to the question of whether *EF-1α* genotypes are biologically meaningful. In previous studies, certain *EF-1α* genotypes have been associated with disease phenotypes. FOV race 4, for example, is considered the most virulent race and is associated with early-season damage and vascular discoloration of the taproot (Cianchetta et al., 2015; Kim et al., 2005). Races 1 and 2 have been described as generally mild, and characterizations of MDS-12 isolates range from non-virulent to highly aggressive (Bell et al., 2017; Cianchetta et al., 2015). Since *EF-1α* genotypes do not reflect the full genetic diversity or population structure of FOV, their reported disease phenotypes should be further investigated to determine if there is any biological meaning associated with *EF-1α* genotypes. The SNPs identified in this study should also be evaluated for association with disease phenotype, to determine if FOV would be better characterized by SNP genotype than *EF-1α*-based race or genotype.

Finally, this study is the first that we know of to assess the effect of the reference genome used in sequence alignment on the results of population analyses. Using the same SNP-calling and filtering parameters in both our reference-based and *de novo*-based data processing pipelines, more SNPs were retained for population genetic analyses using the *de novo*-based approach: 354 SNPs were retained using this approach, as compared to only 193 SNPs with the reference-based approach. This may suggest that using a consensus reference leads to more robust population analyses, especially for a high-diversity species like FOV, although it is currently unclear if any of the SNPs identified in our *de novo*-based reference are redundant. While we used the UGbs-Flex protocol to assemble a consensus reference *de novo* from the raw GBS reads, a consensus reference can also be constructed by mapping consensus reads to a reference genome (Li et al., 2009). Another important distinction between the two data-analysis pipelines was that the reference-based population genetic analyses, especially the minimum spanning network, showed increased differentiation of FOV race 4 isolates, which comprised only 8% of isolates analyzed, but less differentiation among the most prevalent races and genotypes in the population (race 1, LA108, and LA110); while the *de novo*-based population genetic analyses showed more differentiation among common races and less differentiation among race 4 isolates. Importantly, both the reference-based and *de novo*-based analyses showed similar results; their main distinction was the amount of differentiation observed among race 1, race 4, LA108, and LA110 isolates. These slight but important differences could mean that in population genomic analyses of high-diversity species, results are skewed toward increased differentiation of isolates that are genetically similar to the reference genome used for alignment and SNP calling.

CONCLUSIONS

In summary, we demonstrated through population genetic analyses that FOV is not structured by *EF-1α* genotype. We also found new evidence supporting the hypothesis that FOV is more genetically diverse than what is reported based on race or *EF-1α* genotype. Furthermore, we found that the reference genome used in sequence alignment and SNP calling influences the results of population genomic analyses: for a high-diversity organism like FOV, using a consensus reference seems to yield more high-quality SNPs with which to conduct population analyses, and results in increased differentiation of the most prevalent genotypes in the population. It is currently unclear how the genetic diversity and population structure of FOV relate to the pathogen's disease phenotypes; this should be investigated in order to characterize FOV in a biologically meaningful way and improve Fusarium wilt management.

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Table 2.1. Summary of *Fusarium oxysporum* f. sp. *vasinfectum* isolates analyzed, and the number of isolates that possessed the same unique combinations of EF genotypes and DAPC group assignments.

Race/genotype	Origin	# of isolates	Source	DAPC group (reference-based analysis)	DAPC group (*de novo*-based analysis)
Race 1	Alabama, USA	1	J.C.[b]	3	6
Race 1	Georgia, USA	1	R.K.[c]	3	6
Race 1	Georgia, USA	2	R.K.	1	3
Race 1	Georgia, USA	1	R.K.	5	1
Race 1	Georgia, USA	10	R.K.	5	6
Race 1	Georgia, USA	3	R.K.	3	2
Race 1	Georgia, USA	2	R.K.	2	6
Race 1	Georgia, USA	1	R.K.	4	2
Race 1	Louisiana, USA	1	NRRL[d]	5	6
Race 1	Louisiana, USA	1	NRRL	3	6
Race 2	Georgia, USA	2	R.K.	12	1
Race 2	Louisiana, USA	1	NRRL	12	1
Race 4	China	1	J.C.	13	4
Race 4	California, USA	1	R.M.D[e]	13	3
Race 4	California, USA	1	R.M.D	14	3
Race 4	Texas, USA	2	J.O.[f]	13	4
Race 4	Texas, USA	2	J.O.	14	4
Race 8	Alabama, USA	1	J.C.	11	6
Race 8	Georgia, USA	2	R.K.	10	5
Race 8	Georgia, USA	1	R.K.	10	5
LA108	Alabama, USA	2	J.C.	5	6
LA108	Georgia, USA	2	R.K.	6	7
LA108	Georgia, USA	1	R.K.	7	8
LA108	Georgia, USA	1	R.K.	4	6
LA108	Georgia, USA	1	R.K.	5	9
LA108	Georgia, USA	4	R.K.	5	6
LA110	Georgia, USA	2	R.K.	10	5
LA110	Georgia, USA	16	R.K.	5	6
LA110	Georgia, USA	2	R.K.	5	2
LA110	Georgia, USA	1	R.K.	3	6

LA127	Alabama, USA	1	J.C.	9	9
LA127	Arkansas, USA	1	NRRL	8	10
LA127	Georgia, USA	1	NRRL	9	9
MDS-12	Georgia, USA	1	R.K.	5	6
VCG0111[a]	unknown	1	J.L.[g]	1	1
VCG01114[a]	unknown	1	J.L.	4	6
VCG01115[a]	unknown	1	J.L.	2	11
VCG01116[a]	unknown	1	J.L.	2	11
VCG01117[a]	unknown	1	J.L.	5	6
VCG01118[a]	unknown	1	J.L.	2	1
VCG01119[a]	unknown	1	J.L	5	6
VCG01120[a]	unknown	1	J.L.	5	6
VCG01121[a]	unknown	1	J.L.	5	6
VCG01122[a]	unknown	1	J.L.	5	4

[a] FOV isolates sent by Jinggao Liu were characterized by vegetative compatibility group (VCG); their *EF-1α* race or genotype is currently unknown but will be provided prior to April 5th 2019.

[b] Isolate was provided by Dr. Jeffrey Coleman, Auburn University.

[c] Isolate was collected by authors of this paper.

[d] Isolate was provided by the USDA ARS Norther Regional Research Laboratory (NRRL) culture collection.

[e] Isolate was provided by Dr. R. Michael Davis, University of California Davis.

[f] Isolate was provided by Dr. James Olvey, O&A Enterprises.

[g] Isolate was provided by Dr. Jinggao Liu, Southern Plains Agricultural Research Center.

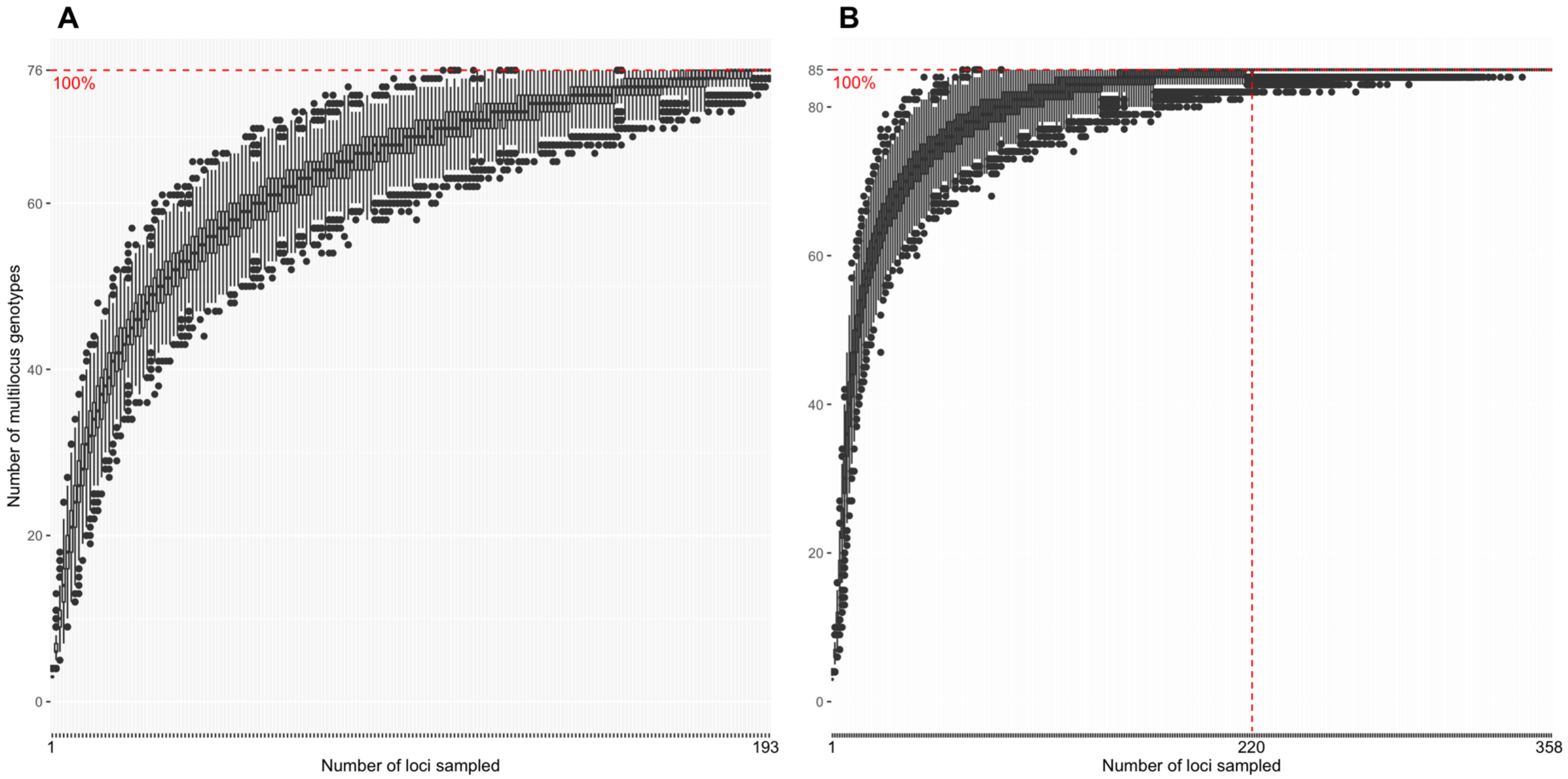


Figure 2.1. Genotype accumulation curves showing the number of multilocus genotypes (MLGs) identified in the population of FOV and the minimum number of SNPs needed to distinguish unique MLGs for **A.** the SNP genotypes based on alignment to a reference genome of FOV race 4 and **B.** the SNP genotypes based on alignment to the *de novo* assembled reference genome.

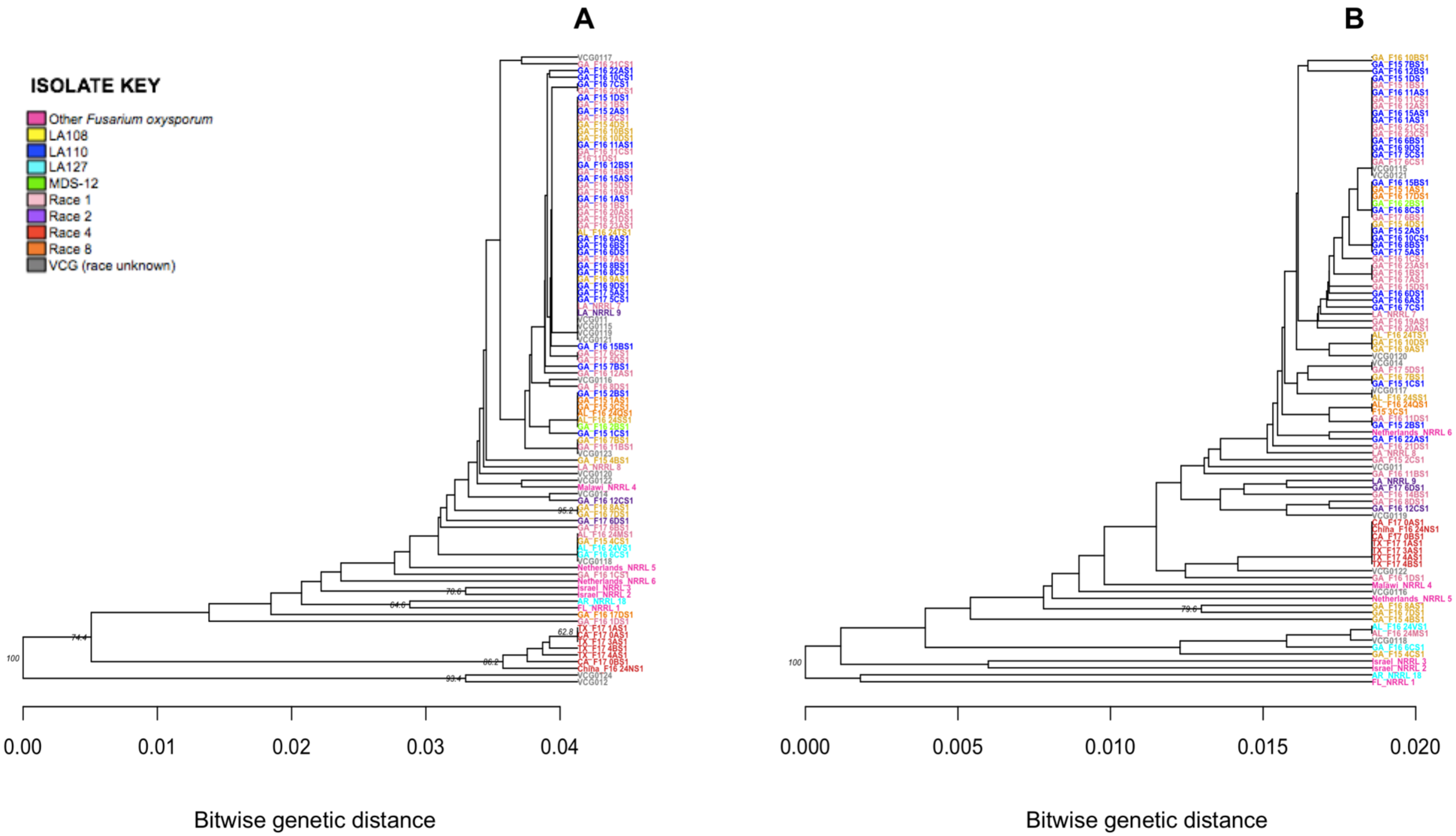


Fig 2.2. UPGMA trees based on bitwise genetic distance among individuals. **A.** The reference-based UPGMA tree shows little clustering by race or genotype, with the exception of race 4 isolates. **B.** The *de novo*-based UPGMA tree shows more clustering by race than Figure 2A, but polyphyly is still evident. Race 4 isolates cluster together in an undifferentiated clade.

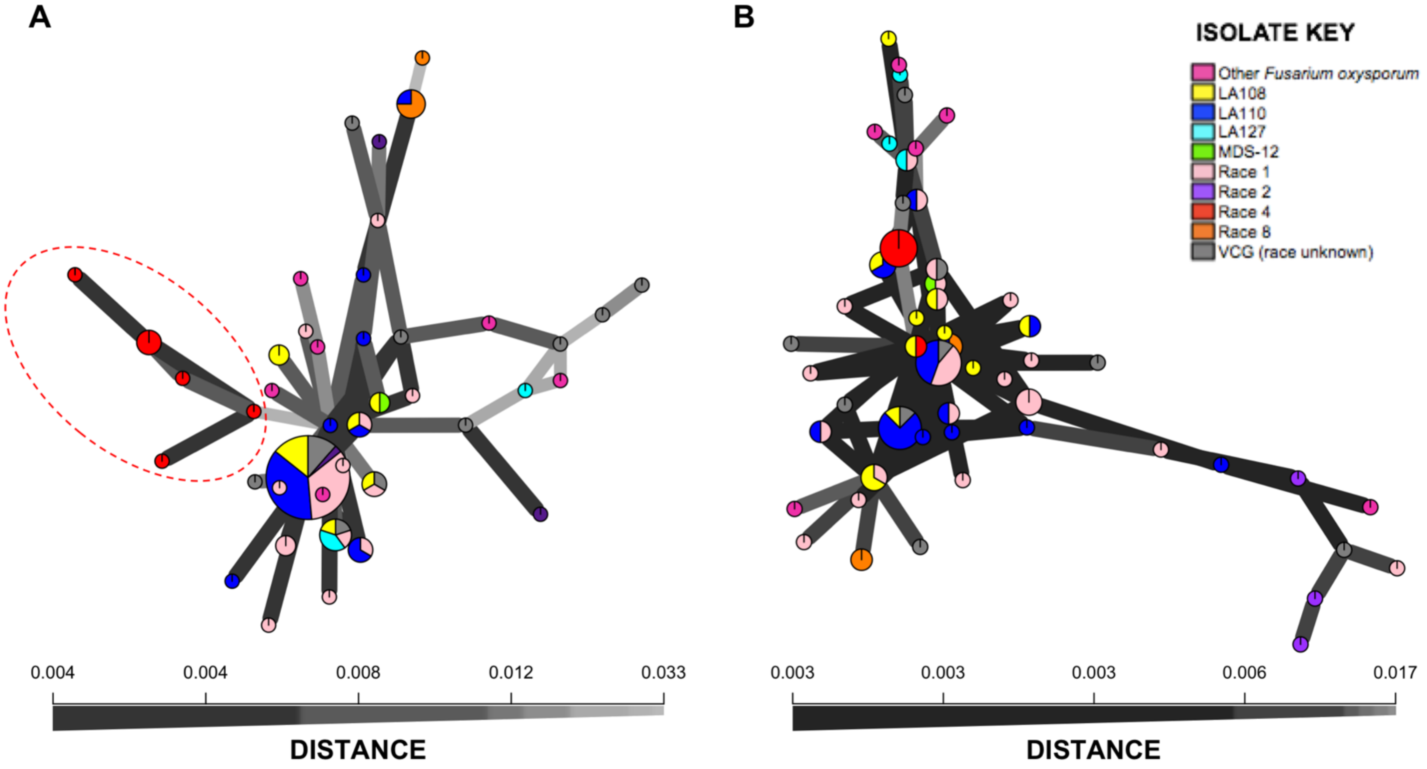


Fig 2.3. Minimum spanning networks (MSNs) constructed using the poppr.msn function in poppr. **A.** The MSN generated from reference-based SNPs shows race 4 isolates as genetically distinct, while LA108, LA110, and race 1 isolates are difficult to distinguish. **B.** The *de novo*-based MSN shows less differentiation among race 4 isolates (although they are still distinct), and more differentiation among LA 108, LA110, and race 1.

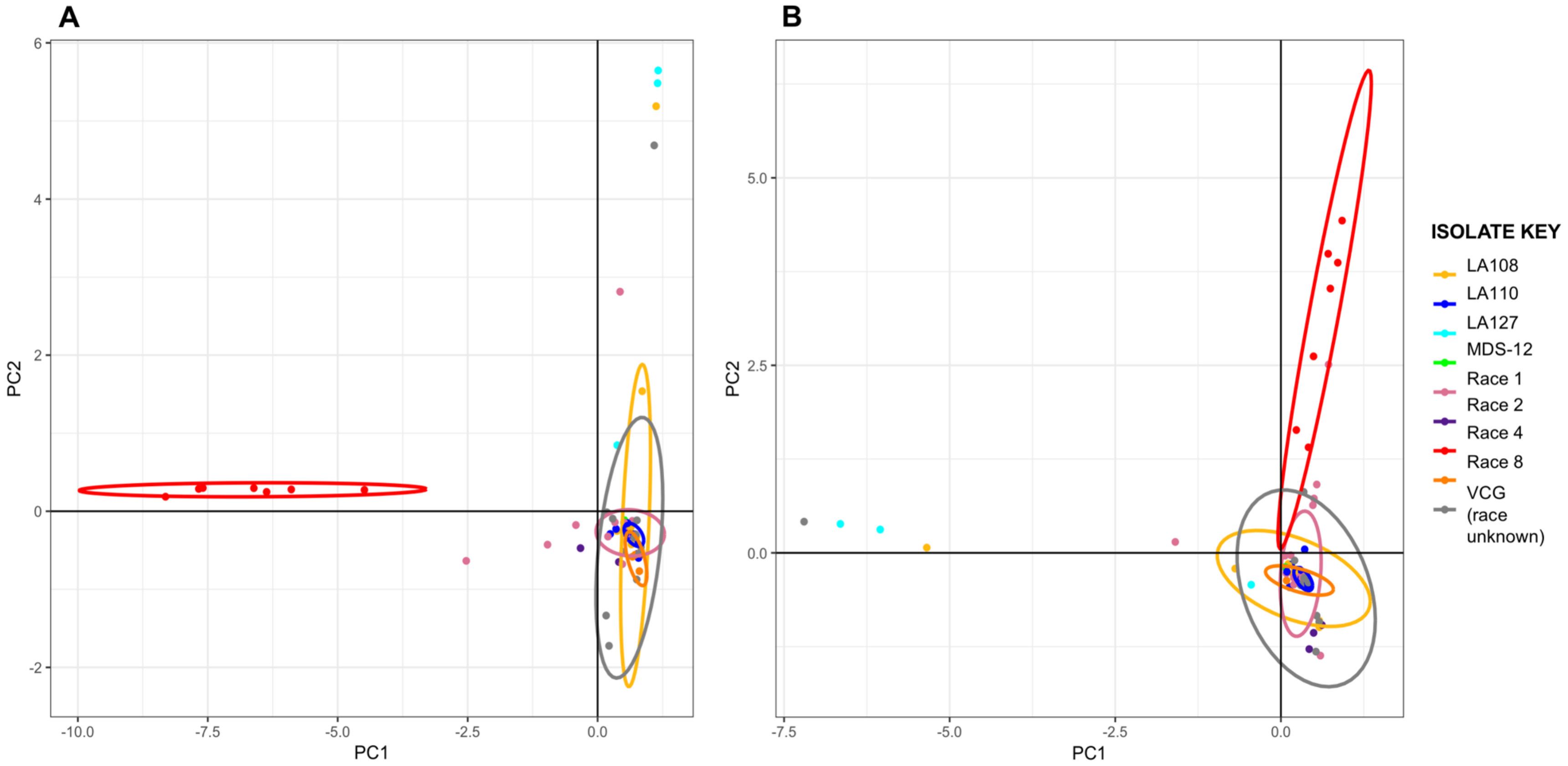
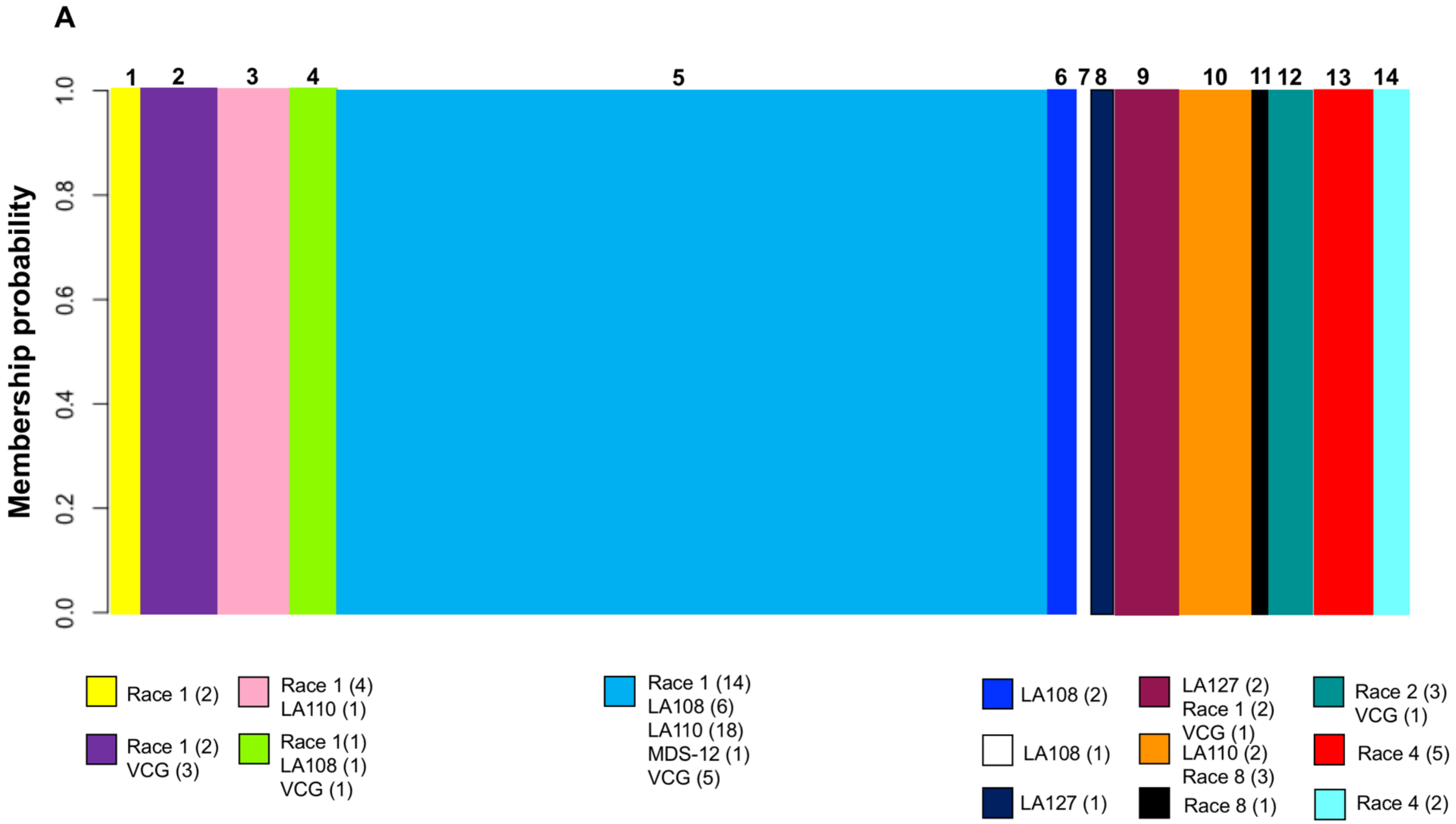


Fig 2.4. Principal component analysis (PCA) of FOV isolates. **A.** In the reference-based analysis, race 4 isolates clearly form a distinct cluster that is separated by two principal components. **B.** In the *de novo*-based analysis, race 4 isolates still cluster, but overlap slightly with race 1 and LA108 isolates.


A
Membership probability
0.0
0.2
0.4
0.6
0.8
1.0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Race 1 (2)
Race 1 (2)
VCG (3)
Race 1 (4)
LA110 (1)
Race 1(1)
LA108 (1)
VCG (1)
Race 1 (14)
LA108 (6)
LA110 (18)
MDS-12 (1)
VCG (5)
LA108 (2)
LA108 (1)
LA127 (1)
LA127 (2)
Race 1 (2)
VCG (1)
LA110 (2)
Race 8 (3)
Race 8 (1)
Race 2 (3)
VCG (1)
Race 4 (5)
Race 4 (2)

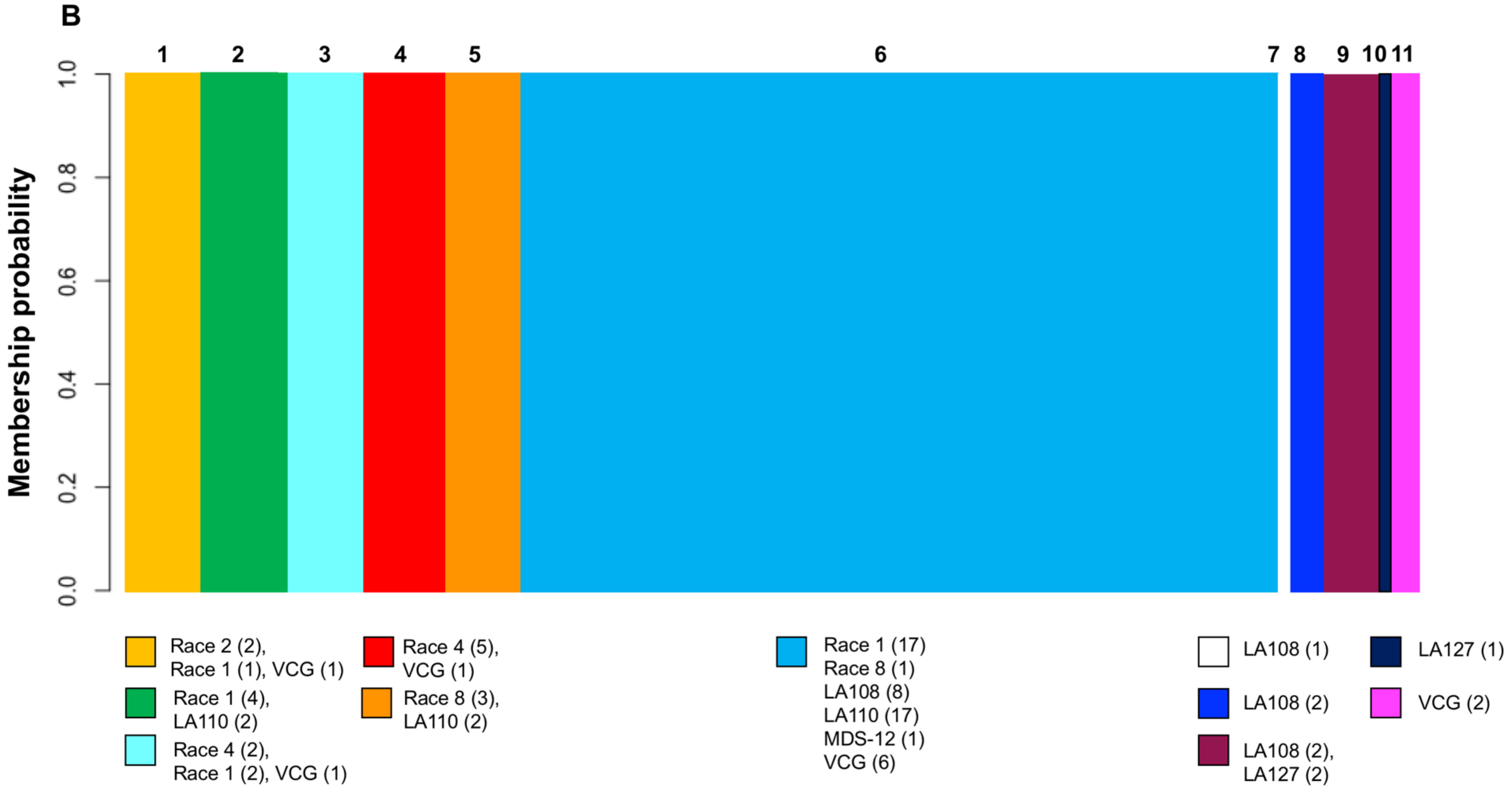


Fig 2.5. Genetically distinct groups of FOV identified through K-means hierarchical clustering. **A.** An optimum of k=14 groups were identified in the reference-based analysis. **B.** An optimum of k=11 groups were identified in the *de novo*-based analysis. Several groups are identical or nearly-identical across the two analyses.

CHAPTER 3

AGGRESSIVENESS AND DIVERSITY OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* IN THE SOUTHEASTERN UNITED STATES[2]

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[2] Halpern, H. C., Kemerait, R. C., and Brewer, M. T. To be submitted to *Plant Disease*.

ABSTRACT

Fusarium oxysporum f. sp. *vasinfectum* (FOV) has recently caused devastating outbreaks of Fusarium wilt of cotton (*Gossypium* spp.) in the southeastern United States. Additionally, some outbreaks were occurring earlier in the season during the seedling stage of growth. One-hundred-and-fourteen FOV isolates were collected from symptomatic cotton plants from disease foci in fields throughout Georgia to determine the races and genotypes associated with recent disease outbreaks and early-season outbreaks. In most cases, four single-spore isolates were obtained from each field, with each isolate coming from a different plant in the field. Analysis of the translation elongation factor (*EF-1α*) locus identified seven races or genotypes of FOV in Georgia cotton fields, with Race 1 and LA110 being the most common and widespread. Most fields contained more than one race or genotype of FOV, with an average of 2.2 races or genotypes per field, suggesting that outbreaks in fields were not caused by a single virulent strain. A subset of 39 genetically and geographically diverse isolates were inoculated onto three cotton cultivars to determine their pathogenicity and virulence. All isolates were pathogenic, and variation in virulence was observed within the previously reported races and genotypes of FOV. These results demonstrate that populations of FOV in Georgia are genetically and phenotypically diverse, and that *EF-1α* sequence is not a good predictor of disease phenotype.

INTRODUCTION

Fusarium wilt of cotton (*Gossypium* spp.), caused by the soilborne fungus *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), is an important disease in the southeastern United States. The disease was first identified in Alabama in 1892, and has since been reported in most cotton growing regions of the world (Atkinson, 1892; Armstrong and Armstrong, 1978; Chen et al., 1985; Dishon and Nevo, 1970; Fahmy, 1927; Garber and Paxman, 1963; Ibrahim, 1966; Kochman, 1995; Kulkarni, 1934). In the southeastern United States, Fusarium wilt was historically considered a minor disease problem that only became severe when plant-pathogenic nematodes, especially the root knot nematode (RKN) *Meloidogyne incognita*, were also present in soils (Davis et al., 2006). In the past decade, however, Fusarium wilt has become more prevalent in the southeast, and where it occurs the damage is devastating (Collins et al., 2013; Whitaker et al., 2016). Furthermore, some recent outbreaks occurred in fields with no history or symptoms of RKN infestation, which could suggest the introduction or evolution of highly virulent FOV isolates (Bell et al., 2017). Alternatively, the recent outbreaks in fields without RKN infestation could suggest that plant-pathogenic nematodes other than RKN are aggravating Fusarium wilt symptoms in southeastern cotton fields (da Silva et al., 2019).

Eight pathogenic races of FOV have been described, however they are considered invalid for several reasons. First, races were characterized based on host and non-host reactions; races 1, 2, and 6, for example, produced indistinguishable virulence reactions on cotton cultivars but produced different symptoms on soybean (Armstrong and Armstrong, 1960). Also, the set of differential cotton cultivars that were originally used to distinguish races are no longer available, and differential virulence reactions have not been reproducible on modern cotton cultivars (Holmes et al., 2009). Finally, sequence analyses and bioassays identified redundancy between races 3 and 5, and 4 and 7 (Hering et al., 1999; Nirenberg et al., 1994; Skovgaard et al., 2001). As such, only six of the races are recognized today, and they are often referred to as “nominal races.” The nominal races of FOV are each associated with a unique sequence at the translation elongation factor (*EF-1α*) locus, with the exception of races 1 and 6 which are differentiated by phenotypic differences and geographic distribution (Cianchetta et al., 2015).

Five of the nominal races of FOV - 1, 2, 3, 4, and 8 - are found in the United States; races 1, 2, 3, and 8 are widespread, whereas race 4 is limited to California and Texas (Halpern et al., 2018; Kim et al., 2005). In addition to the nominal races, there are five other genotypes of FOV that are found in the southeastern United States; named LA108, LA110, LA112, LA127, and MDS-12 (Bennett et al., 2013; Holmes et al., 2009). The LA genotypes possess unique *EF-1α* sequences, and therefore cannot be assigned to any of the nominal races. MDS-12 has an *EF-1α* sequence identical to that of FOV race 4, but has a unique sequence at the intergenic spacer region (IGS) and is therefore considered unique (Holmes et al., 2013). Of the nominal races and genotypes of FOV, race 4 is the most virulent. It is consistently associated with severe, early-season damage, and root-rotting symptoms; and while FOV is generally considered mild in the absence of nematodes, race 4 causes extreme damage regardless of nematode interactions (Kim et al., 2005).

Although FOV race 4 is associated with a specific disease phenotype, the phenotypic diversity among the remaining ten races and genotypes of FOV is not well characterized. A study in 2009 looked at the virulence of each race and genotype of FOV on a set of differential cotton cultivars; however, only one representative isolate of each race and genotype was assessed (Holmes et al., 2009). A recent survey of FOV in the United States assessed the pathogenicity of several representatives of each race and genotype, and reported variation in the disease symptoms caused by different MDS-12 isolates (Cianchetta et al., 2015). Additionally, a study of the genetic diversity and structure of FOV in the southeastern United States found that, with the exception of race 4, *EF-1α* races and genotypes do not reflect isolates' evolutionary relationships or genetic similarities: some isolates with the same *EF-1α* race or genotype were shown to be otherwise dissimilar from each other, while some isolates with different *EF-1α* races or genotypes were closely related (chapter 2). With variation in aggressiveness already reported in one *EF-1α* genotype, and with genetic diversity reported within several *EF-1α* races and genotypes, it is possible that FOV is more phenotypically diverse than currently reported in the literature.

An alternative to characterizing FOV by races and genotypes is classification into vegetative compatibility groups (VCGs). Vegetative compatibility is the ability of fungal isolates to undergo anastomosis, or hyphal fusion, a capability which is governed by isolates possessing identical alleles at several *vic* loci (Leslie et al., 1993). While the phenotypic diversity among races and genotypes of FOV is not well-understood, the phenotypic diversity associated with distinct VCGs is better characterized: isolates in the same VCG possess the same disease phenotype on cotton (Bell et al., 2017). Twelve VCGs are associated with a "vascular-competent" pathotype in which isolates are capable of causing vascular damage but not root rot symptoms to cotton plants, and three VCGs are associated with a "root rot" pathotype in which isolates cause extensive root damage but no vascular symptoms. In 2014, FOV was isolated from Georgia cotton fields and characterized by VCG and pathotype: the isolates spanned eight distinct VCGs, and all were associated with a vascular-competent pathotype (Bell et al., 2017). However, with FOV becoming increasingly severe and causing early-season Fusarium wilt outbreaks, there is a need to understand if isolates with the root-rot pathotype have evolved or been introduced in southeastern cotton fields.

The aim of our study was to infer the cause of recent Fusarium wilt outbreaks in the Georgia by understanding the populations of FOV responsible for those outbreaks. Our specific objectives were to i) determine the races and genotypes of FOV causing Fusarium wilt in Georgia, ii) determine the diversity of isolates in disease foci, iii) characterize the pathogenicity and virulence of these isolates, and iv) determine if there is an association of race or genotype with aggressiveness or particular disease phenotypes.

MATERIALS AND METHODS

Collection of fungal isolates. Cotton plants with symptoms of Fusarium wilt were collected from fields in Georgia between 2015 and 2017. In 2015, 8 fields were sampled across 4 counties (Tift, Grady, Valdosta, and Cook); in 2016, 19 fields were sampled across 7 counties (Colquitt, Tift, Cook, Coffee, Ware, Worth, and Tattnall); and in 2017, 2 fields were sampled across 2 counties (Tift and Madison). Two of the fields sampled in 2016 showed early-season symptoms of Fusarium wilt (both in Colquitt Co.), and both fields sampled in 2017 showed early-season damage. Ten cotton stems, each from a different infected plant, were taken from each field.

FOV was isolated from symptomatic cotton stems following the protocol described by da Silva et al (*in press*). Briefly, cotton stems were washed in soapy water and cut to a length of 3-5 cm. Stem segments were surface-sanitized for one minute in 95% ethanol, two minutes in 10% sodium hypochlorite solution, one minute in sterile double-deionized water, and then rinsed with Millipore water. Small slivers of each stem segment were cut with a utility knife, the outer layer of bark was removed, and the vascular tissue was plated onto acidified quarter-strength potato dextrose agar (AqPDA). Plates were incubated at 23°C with a 12-hour photoperiod for 6-7 days. Single spores of cultures resembling *Fusarium* were transferred to quarter-strength potato dextrose agar (qPDA) under a dissecting microscope. Four single-spore isolates were retained from each field and stored on sterile filter paper at 4°C.

DNA extraction, amplification, and sequencing. Genomic DNA was extracted from a total of 114 single-spore FOV isolates. Isolates were grown on potato dextrose agar (PDA) overlain with sterile cellophane for 6-7 days, after which time mycelia were harvested and lyophilized. Approximately 50 milligrams of lyophilized mycelia were placed in 2-ml microcentrifuge tubes with sterile glass beads, and macerated into a fine powder in a Geno/Grinder® (SPEX SamplePrep, Metuchen, NJ). DNA was extracted using a DNeasy Plant Mini kit (QIAGEN, Valencia, CA) following manufacturer protocols with the following modification: samples were eluted in 25 μl AE buffer (as opposed to 50 μl as stated in protocol), to increase the final concentration of DNA.

Isolates were assigned to a nominal race or genotype of FOV based on polymorphisms in the translation elongation factor (*EF-1α*) gene sequence. *EF-1α* was amplified using the primers EF1 and EF2 (Cianchetta et al., 2015; O'Donnell et al., 1998). Each PCR reaction consisted of 1.25 μl mM dNTP's, 1.25 μl 10x ExTaq buffer (Takara Bio USA), 0.56 μl of each 10 μM primer, 0.3 μl ExTaq (Takara Bio USA), and 1 μl of genomic DNA (10 - 300 ng/μl) . Amplification was conducted in a thermal cycler (PTC-100; MJ Research, Watertown, MA) under the following conditions: 95°C for 1 min; 40 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min; and a final extension of 72°C for 5 min. Amplification of the *EF-1α* locus was confirmed by 1% agarose gel electrophoresis, and PCR products were purified with an ExoSAP-IT kit (Thermo Fisher Scientific, Waltham, MA) per manufacturer instructions. A 320 ng sample of DNA combined with 4 μl of 10 μM primers were sequenced by EuroFins (Louisville, KY). PCR products were sequenced in both directions using the primers EF1 and EF2.

Race identification. *EF-1α* sequences were aligned, visually edited, and compared to publicly available reference sequences (from GenBank) of each race and genotype of FOV. In one instance, *EF-1α* sequence was insufficient to unambiguously determine genotype as race 4 or MDS-12 (also referred to as "race 4-like") (Bennett et al., 2013), so the intergenic spacer region (IGS) was also amplified and sequenced, following the protocol described by Cianchetta et al. (2015). All sequences were aligned in Geneious R11 using a global alignment with free end gaps and a 70% BLOSUM cost matrix (Kearse et al., 2012).

Assessment of pathogenicity and virulence. Thirty-nine genetically diverse isolates of FOV were inoculated onto the following set of cotton cultivars: Delta Pine 1454 (DP1454) (Monsanto Company, St. Louis, MO), Phytogen 805 (Ph805) (Dow AgroSciences, Indianapolis, IN), and Rowden. DP1454 is resistant to root-knot nematode but susceptible to FOV, Ph805 is the progeny of a cultivar which exhibited resistance to many races of FOV including race 4, and Rowden is susceptible to all races of FOV (Holmes et al., 2009). Seeds were planted in sandy soil (three parts sand, one part potting soil) and seedlings were inoculated at the two true leaf stage.

Isolates were selected to represent the genetic diversity of FOV, as determined by their *EF-1α* genotype. Five isolates each of races 1 and 8, four of race 2, two of race 3, six each of LA 108 and LA 110, five of LA 127 and one isolate of MDS-12 were evaluated (Table 3.1). Twelve isolates came from out of state (Alabama, Arkansas, and Louisiana), to distinguish potential disease phenotypes unique to Georgia FOV isolates. FOV race 4 was not included in this greenhouse study to avoid the risk of introducing this highly virulent pathogen into nearby Georgia cotton fields.

Inoculum was prepared from five-day-old single spore colonies of FOV grown on quarter-strength potato dextrose agar (qPDA) overlain with sterile cellophane. Conidia were harvested by flooding Petri plates with 10-15 ml sterile water, scraping the fungal tissue with a sterile disposable loop, homogenizing the solution using a Tissuemiser, and filtering the resulting suspension through three layers of cheesecloth. Suspensions were adjusted to a concentration of 10^5 conidia per ml using a hemacytometer or Cellometer X2 (Nexcelom Bioscience, Lawrence, MA).

Cotton seedlings were inoculated at two true-leaf stage using two separate inoculation methods per isolate. The rationale for using two inoculation methods was to account for previously observed differences in FOV, where some can directly penetrate the roots of cotton plants, whereas others can only enter the plant and cause disease through natural openings or wounds (Bell et al., 2017). The first inoculation method was a drench method designed to test the ability of isolates to directly enter the plant roots: cotton seedlings in seedling cells were immersed into a tray of 750 ml of inoculum for 15 minutes. The second method was injection with a sterile syringe and needle, which tested the ability of isolates to cause vascular damage once inside the xylem: cotton seedlings were pierced with a 25-gauge needle attached to a 1 ml syringe, and approximately 25 µl of inoculum was injected into the wound. Each isolate by cultivar by inoculation method treatment was replicated three or four times, depending on the amount of cotton seedlings available, and a water control was included in each treatment. Plants inoculated with different methods were kept on separate greenhouse benches. Within each bench, plants were randomized and maintained in the greenhouse at approximately 24°C for six weeks after which time plants were rated for symptoms of FOV. Due to technical issues, this experiment was conducted over the course of five greenhouse trials (ten isolates evaluated on

Ph805 and DP1454 in trial 1, ten isolates evaluated on Ph805 and Rowden in trial 2, 32 isolates evaluated on all cultivars in trial 3, 32 isolates evaluated on all cultivars in trial 4, and ten isolates evaluated on Rowden and DP1454 in trial 5; Table 3.1).

Foliar wilt symptoms, vascular discoloration, and root rot symptoms were evaluated on a qualitative scale of 0-5 with 0 indicating an asymptomatic plant and 5 indicating plant death. Additionally, shoot and root tissues were separated, placed into paper bags, and desiccated in a drying oven at 50°C for approximately 72 hours. Dry root mass and shoot mass (g) were recorded. Quantitative response variables (i.e. dry root mass and shoot mass) were evaluated using analysis of variance (ANOVA), while qualitative response variables (i.e. wilt, vascular discoloration, and root rot severity) were evaluated with the Kruskal-Wallis non-parametric test. In both analyses fixed effects included cultivar, fungal isolate, and any interaction between the two. When significant differences among isolates were detected, means separation was performed using Tukey's Honestly Significant Difference test or Wilcoxon test for quantitative and qualitative response variables, respectively. All statistical analyses were conducted using R version 3.5.1 (R Core Team, 2018).

Vascular damage, root rot, and wilt profiles were determined by adding all mean vascular discoloration, root rot, and foliar wilt ratings associated with each isolate. Total virulence was determined by adding the scores of three profiles described above. Isolates' virulence profiles were ranked from lowest and highest and fell into three discrete virulence categories: high virulence, moderate virulence, and low virulence. In the case of vascular discoloration, isolates fell into four discrete categories: high, moderate, low, and non-symptomatic.

Association between genotype and disease phenotype. To observe potential relationships between isolates' races and genotypes with their disease phenotype on cotton, cumulative virulence profiles were overlain onto a neighbor-joining tree based on isolates' *EF-1α* sequence. The *EF-1α* sequences of isolates evaluated in the greenhouse experiment were aligned in Geneious R11 using a global alignment with free end gaps and a 70% BLOSUM cost matrix, and the multi-sequence alignment was used to construct a neighbor-joining tree using the Tamura-Nei genetic distance model and 500 bootstrap replicates (Kearse et al., 2012). Isolates' virulence profiles were represented by unique symbols, which were overlain onto the neighbor-joining tree to determine if disease phenotype was associated with *EF-1α*-based races and genotypes (Fig. 3.1).

RESULTS

FOV race and genotype identification. One-hundred-and-fourteen isolates of *Fusarium oxysporum* f. sp. *vasinfectum* were collected from Georgia cotton fields between 2015 and 2017. Fourteen isolates were collected from the fields showing early-season symptoms of Fusarium wilt. All isolates possessed *EF-1α* sequences identical to previously reported races and genotypes of FOV. A total of seven genotypes were identified, with up to three genotypes being found in a single field and up to five genotypes found in a single county. Most fields sampled contained more than a single race or genotype of FOV, with a mean of 2.2 races or genotypes out of 4 isolates per field. The average number of races or genotypes per county was 2.6. The majority of isolates belonged to race 1 and LA110, with 48 and 35 isolates respectively (Table 3.2). The relative frequency of genotypes identified each year was consistent, with the exception of LA108 which accounted for 28% of isolates identified in 2015, but only comprised 12% of isolates in 2016 and was not detected at all in 2017 (Table 3.2). Among the early-season isolates, five genotypes of FOV were identified with the majority of isolates belonging to race 1 and

LA110, which comprised 50% and 29% of isolates, respectively (Table 3.3). The average number of races or genotypes of early-season isolates out of 4 sampled per field was 2.3, and the average number of races or genotypes of early-season isolates per county was 2.7.

Pathogenicity and virulence. Based on the *p*-values observed in ANOVA tests, fungal isolates did not significantly affect the dry root mass or dry shoot mass of cotton plants, with one exception: in trial 4, significant differences in dry root mass were observed among plants inoculated with different fungal isolates (Table 3.4). Cultivar generally had a significant effect on dry root mass and dry shoot mass, and no interaction between fungal isolate and cultivar was observed for dry root mass or dry shoot mass in any of the five trials. Based on the results of Kruskal-Wallis tests, fungal isolates always significantly affected wilt symptoms, vascular discoloration, and root rot severity. Cultivar also had a significant effect on qualitative disease severity, with only four exceptions: cultivar did not significantly affect root rot symptoms in trial 2, nor did it significantly affect any qualitative disease severity symptoms in trial 5. Additionally, an isolate by cultivar interaction was consistently observed for qualitative disease symptoms in all five trials. Accordingly, separation of means was performed separately for each cultivar within a given trial (Tables 3.5-3.10).

Based on cumulative virulence scores, all isolates evaluated were pathogenic (Tables 3.11 and 3.12). However, the three isolates that were included in all trials did not cause consistent disease symptoms among trials. As a result, the virulence profiles of isolates screened in trials 1, 2, and 5 were not directly compared to the virulence profiles of isolates screened in trials 3 and 4. In trials 1, 2, and 5, two isolates caused low wilting, five caused moderate wilting, and three isolates caused high wilt severity (Table 3.11). The isolates that caused low wilt severity belonged to races 1 and 8, and those that caused high wilt severity belonged to LA108, LA127, and MDS-12. Five isolates caused low root rotting, four caused moderate root rotting, and one - the MDS-12 isolate - caused high root rotting. Additionally, five isolates caused low vascular damage, four caused moderate vascular damage, and one - the MDS-12 isolate, again - caused high vascular damage. Some of the isolates that caused low root rotting caused moderate vascular damage, and vice versa. Based on total virulence scores, four isolates were weakly virulent, five were moderately virulent, and one was highly virulent. The weakly virulent isolates belonged to races 1, 2, and 8, and the highly virulent isolate had the MDS-12 genotype.

In trials 3 and 4, all isolates caused moderate wilt symptoms, with two exceptions: F16-2BS1, the MDS-12 isolate, was highly virulent with regard to wilt; while 34049, a race 2 isolate from the NRRL culture collection, caused low wilting (Table 3.12). Isolates exhibited more variation in root rot severity: three isolates caused low root rotting, thirteen caused moderate root rot severity, and sixteen caused high root rot severity. Isolates also showed variation in their ability to cause vascular damage: four isolates did not cause vascular symptoms, ten isolates caused low vascular damage, sixteen isolates caused moderate vascular damage, and two caused high vascular damage. The four isolates that were non-pathogenic in terms of vascular damage belonged to LA108, race 1, and race 3; the isolates that caused high vascular damage belonged to race 1 and MDS-12. When disease severity scores were added to analyze overall virulence, three isolates were considered weakly virulent, nineteen isolates were moderately virulent, and ten isolates were highly virulent. The isolates with low virulence belonged to races 2 and 3; the highly virulent isolates spanned races 1, 2, and 8, LA 108, LA110, and MDS-12.

When cumulative virulence profiles were overlain onto the neighbor-joining tree constructed from isolates' *EF-1α* genotypes, associations between race and virulence profile were observed among the isolates included in trials 1, 2, and 5, but were generally not observed among the isolates screened in trials 3 and 4. In trials 1, 2, and 5, the two LA110 isolates possessed identical disease phenotypes for every virulence profile evaluated. The two race 1 isolates possessed identical vascular, root rot, and total virulence profiles; as did the two LA108 isolates. In trials 3 and 4, race 3 isolates consistently possessed the same disease phenotype across all virulence profiles, and LA127 isolates shared a high-severity root rot phenotype (Figure 3.1). No other associations between race or genotype and disease phenotype were observed for isolates screened in trials 3 and 4. Because disease severity and *EF-1α* genotype were generally not associated, isolate virulence profiles were also overlain onto maximum likelihood trees based on SNPs that were recently identified in FOV (chapter 2). Seventeen isolates were analyzed for relationship between virulence and SNP genotype; fifteen isolates were excluded from this analysis because SNP genotype data was not collected for these isolates. No associations between SNP genotype and virulence profiles were observed (Figure 3.2).

DISCUSSION

This study identified seven different races and genotypes of *Fusarium oxysporum* f. sp. *vasinfectum* in Georgia cotton fields. No novel genotypes of FOV were identified, and importantly, neither was FOV race 4. Race 1 and LA110 were the most common and widespread genotypes in Georgia: race 1 accounted for 42% of isolates collected over three years and was found in 23 of 28 fields, and LA 110 accounted for 30% of isolates and was found in 21 of 28 fields. Despite being common in Georgia, neither race 1 nor LA110 were dominant in our study; they often occurred together, or in combination with isolates of other races and genotypes. In fact, we rarely found just one race or genotype of FOV in a field: the average number of races identified was 2.2. The actual number of races and genotypes present per cotton field is likely greater than 2.2, however, because we only genotyped 4 isolates per field. Since we usually found multiple races or genotypes of FOV per cotton field, it appears that there is not one single dominant race or genotype of FOV responsible for recent Fusarium wilt outbreaks in Georgia. Since pathogen populations do not appear to be evolving toward a particular race or genotype of FOV, recent Fusarium wilt outbreaks may be better explained by changes in cotton cultivars or in the environment. Additionally, the same trends described above were observed among the early-season FOV isolates: race 1 and LA110 were the most common races or genotypes found among early-season isolates, and on average 2.3 races or genotypes of FOV were identified in fields where early-season isolates were collected. Since there is not just one race or genotype of FOV causing early-season disease outbreaks, this further suggests environmental or host factors as the reason for recent changes in Fusarium wilt disease onset and severity.

Although the genetic diversity of FOV in Georgia cotton fields rules out the introduction or evolution of a single virulent race or genotype as the cause of recent Fusarium wilt outbreaks, it is still possible that changes in local populations of FOV are responsible, at least in part, for the outbreaks. Our greenhouse inoculation experiment demonstrated that some FOV isolates from Georgia cotton fields are capable of causing extensive damage to cotton plants: three isolates from Georgia caused severe wilting, eight caused severe root rotting, one caused severe vascular discoloration, and six were considered to be highly virulent overall. One isolate, F16-2BS1, an MDS-12 isolate that was associated with an early-season outbreak, was highly virulent across all cumulative virulence profiles. It is currently unclear what makes this isolate so virulent on cotton: MDS-12 is identical to race 4 in *EF-1α* sequence, but genetically dissimilar from race 4 at many other positions throughout the genome (chapter 2). Additionally, a previous survey of FOV in the United States reported that MDS-12 isolates are not identical to race 4 in their disease phenotype on cotton: in that study, MDS-12 isolates were generally less virulent than race 4, but on the cotton cultivar Phytogen 72 one MDS-12 isolate was more virulent than the race 4 control (Cianchetta et al., 2015). We hypothesize that MDS-12 can possess a wide range of disease phenotypes on cotton, and that the highly virulent phenotype of the MDS-12 isolate identified in this study is isolate-specific, not genotype-specific.

The disease phenotype of our MDS-12 isolate being isolate-specific, not genotype-specific, is supported by our observation of phenotypic diversity within most other races and genotypes of FOV. In trials 1, 2, and 5, races and genotypes of FOV frequently seemed to be associated with a disease phenotype. The two LA110 isolates examined possessed identical virulence profiles across all categories, while the two LA108 isolates and race 1 isolates both possessed identical virulence profiles in three of four categories. In trials 3 and 4, however, those patterns were not replicated: in these trials, the only races and genotypes associated with specific disease phenotypes were race 3, which was consistently weakly aggressive (or non-symptomatic in the case of vascular discoloration) on cotton, and LA127, which was associated with a root rot phenotype. Holmes et al. (2009) also reported that race 3 was weakly virulent on several American cotton cultivars. The remaining isolates screened in trials 3 and 4 showed a wide range of phenotypes within races and genotypes, suggesting that many of the disease phenotypes reported by Holmes et al. (2009) were not actually race specific, but unique to the individual isolates assessed. A potential explanation for associations between race or genotype with disease phenotype in trials 1, 2, and 5, but not in trials 3 and 4 could be the number of isolates screened in the respective trials. In trials 1, 2, and 5, the maximum number of isolates of the same race or genotype screened was two; whereas in trials 3 and 4, up to six isolates of a single race or genotype were assessed, allowing phenotypic diversity within races and genotypes to be detected. Not only were disease phenotypes generally unrelated to *EF-1α* sequence, but they also did not associate with the SNP genotypes identified in chapter 2.

Since we were unable to find genetic characterizations of FOV that were consistently associated with disease phenotype, a natural follow-up question is: what genes are correlated with disease phenotype and what genes are directly responsible for virulence? In other *formae speciales* (ff. spp.) of *Fusarium oxysporum*, the *secreted-in-xylem* (*Six*) genes have been identified as playing an important role in virulence (Czislowski et al., 2018; Lanubile et al., 2016; Van Dam et al., 2016). In the tomato wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*), *Six1*, *Six3*, *Six4*, *Six5*, and *Six6* have been confirmed as bona fide effectors that interact with host resistance genes to induce disease in tomato (Gawhens et al., 2014; Houterman et al., 2007; Ma et al., 2015; Rep, 2004; Takken and Rep, 2010). In the pathogen that causes Panama disease of banana, *Fusarium oxysporum* f. sp. *cubense* (*Foc*), isolate virulence is correlated with the specific combinations of *Six* homologs that an isolate possesses (Czislowski et al., 2018; Widinugraheni et al., 2018). Homologs of *Six* genes have been identified in Australian FOV isolates, but their exact contributions to virulence are undetermined (Chakrabarti et al., 2011). Further investigation of the presence of *Six* homologs in FOV may provide insights into the relationship between the pathogen's genetic diversity and disease phenotype.

An additional knowledge gap that should be addressed is the relationship of FOV with plant-pathogenic nematodes. Most studies of FOV as a disease complex with nematodes have focused on the southern root knot nematode (RKN), *Meloidogyne incognita*, which is commonly found in association with FOV in the southeastern United States (Atkinson, 1892; Chawla et al., 2012; Jorgenson et al., 1978; Lawrence et al., 2015). It was the observation of severe Fusarium wilt outbreaks in the absence of RKN that prompted questions about the introduction or evolution of highly virulent strains of FOV in the southeastern U.S. However, our survey demonstrated that many races of FOV contributed to recent Fusarium wilt outbreaks, and that these pathogen populations possess a wide range of disease phenotypes on cotton. Since there is not a dominant race or genotype of FOV causing these outbreaks, and not all of the isolates associated with recent outbreaks are highly virulent, the presence of plant-pathogenic nematodes other than RKN might explain the increase in Fusarium wilt prevalence and severity in the southeastern U.S. Between 2011 and 2018, the nematicide Aldicarb was phased out in the United States, potentially allowing previously uncommon plant pathogenic nematodes to reach higher population levels in Georgia cotton fields (EPA, 2016). The Aldicarb phase-out might have led to the amplification of nematodes other than RKN, because RKN is well-managed through the use of *M. incognita*-resistant cotton cultivars in addition to nematicides (Davis and Kemerait, 2009). Also, plant-pathogenic nematodes including the reniform nematode (*Rotylenchulus reniformis*), the lesion nematode (*Pratylenchulus penetrans*), the sting nematode (*Belonolaimus longicaudatus*), and the burrowing nematode (*Radopholus similis*) have all been reported to amplify Fusarium wilt severity on a range of crops, demonstrating that RKN is not the only plant-pathogenic nematode capable of interacting with *Fusarium oxysporum* in a disease complex (Almeida et al., 2018; Cooper and Brodie, 1963; Minton et al., 1985; Seinhorst and Kuniyasu, 1971; Vats, 1997). A recent survey of nematode diversity in Georgia found that the sting nematode was common in Georgia cotton fields with symptoms of Fusarium wilt; 45% of fields surveyed found *B. longicaudatus* but not RKN in cotton fields with Fusarium wilt, suggesting that *B. longicaudatus* may play an important role in recent Fusarium wilt outbreaks in the southeastern U.S. (da Silva et al., *in press*).

In summary, our study demonstrated that populations of FOV in Georgia are genetically diverse, and that recent Fusarium wilt outbreaks are not the result of FOV race 4 or any other single, dominant race or genotype of FOV. Our study also showed phenotypic variation among populations of FOV from the southeastern United States, and that isolates' differences in disease phenotype are not correlated with polymorphisms in *EF-1α* sequence. This study shows compelling evidence that *EF-1α* sequence alone is not useful for characterizing the virulence of FOV, and also demonstrates the need to identify the genes that are responsible for disease phenotype on cotton in order to improve management through resistance breeding.

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Table 3.1. Summary of *Fusarium oxysporum* f. sp. *vasinfectum* isolates used in greenhouse virulence experiments.

Isolate Name	**Early-Season?**	**Race/Genotype**	**Year Collected**	**Geographic Origin**	**Greenhouse Trials Screened**
F16-2BS1	X	MDS-12	2016	GA, Colquitt Co.	1, 2, 3, 4, 5
F16-6CS1	-	LA 127/140	2016	GA, Tift Co.	1, 2, 3, 4, 5
309N-14[a]	-	LA 127/140	2013	AL	3, 4
2165-145[a]	-	LA 127/140	2013	AL	3, 4
32900[b]	-	LA 127/140	2007	AR	3, 4
34051[b]	-	LA 127/140	2007	AR	3, 4
F15-1CS1	-	LA 110	2015	GA, Tift Co.	3, 4
F16-22AS1	-	LA 110	2016	GA, Tattnall Co.	1, 2, 5
F16-8BS1	-	LA 110	2016	GA, Tift Co.	1, 2, 5
F16-21BS1	-	LA 110	2016	GA, Tattnall Co.	3, 4
F16-1AS1	X	LA 110	2016	GA, Colquitt Co.	3, 4
F17-5CS1	X	LA 110	2017	GA, Tift Co.	3, 4
F15-4AS1	-	LA 108	2015	GA, Grady Co.	3, 4

F15-7AS1	-	LA 108	2015	GA, Cook Co.	3, 4
F16-8AS1	-	LA 108	2016	GA, Tift Co.	1, 2, 5
F16-10BS1	-	LA 108	2016	GA, Tift Co.	1, 2, 5
F16-10DS1	-	LA 108	2016	GA, Tift Co.	3, 4
F16-7BS1	-	LA 108	2016	GA, Tift Co.	3, 4
C15044[a]	-	LA 108	2013	AL	3, 4
401N-3A[a]	-	LA 108	2013	AL	3, 4
F15-6BS1	-	Race 1	2015	GA, Lowndes Co.	3, 4
F16-16CS1	-	Race 1	2016	GA, Ware Co.	1, 2, 5
F16-20CS1	-	Race 1	2016	GA, Tattnall Co.	1, 2, 5
F16-7AS1	-	Race 1	2016	GA, Tift Co.	3, 4
TF2[a]	-	Race 1	2013	AL	3, 4
34076[b]	-	Race 1	2007	LA	3, 4
F17-6BS1	X	Race 1	2017	GA, Madison Co.	3, 4
F15-5DS1	-	Race 2	2015	GA, Lowndes Co.	3, 4
F16-12CS1	-	Race 2	2016	GA, Coffee Co.	1, 2, 3, 4, 5

F16-12DS1	-	Race 2	2016	GA, Coffee Co.	3, 4
F17-6DS1	X	Race 2	2017	GA, Madison Co.	3, 4
34049[b]	-	Race 2	2007	LA	3, 4
34082[b]	-	Race 3	2007	LA	3, 4
34083[b]	-	Race 3	2007	LA	3, 4
F15-3CS1	-	Race 8	2015	GA, Tift Co.	3, 4
F16-9CS1	-	Race 8	2016	GA, Tift Co.	1, 2, 5
F17-6AS1	X	Race 8	2017	GA, Madison Co.	3, 4
207N-18[a]	-	Race 8	2013	AL	3, 4
32876[b]	-	Race 8	2007	AR	3, 4

[a] Isolate provided by Dr. Jeffrey Coleman, Auburn University

[b] Isolate provided by USDA-ARS NRRL Culture Collection

Table 3.2. Number of individuals of races or genotypes of *Fusarium oxysporum* f. sp. *vasinfectum* isolates in Georgia based on translation elongation factor (*EF-1α*) sequence; relative frequency of each race or genotype in parentheses.

No.[a]	**Year**	**Race 1**	**Race 2**	**Race 8**	**LA 108**	**LA 110**	**LA 127/140**	**MDS-12**
12	2013[b]	5 (0.42)	2 (0.17)	1 (0.08)	1 (0.08)	3 (0.25)	0 (0.00)	0 (0.00)
32	2015	8 (0.25)	1 (0.03)	4 (0.13)	9 (0.28)	10 (0.31)	0 (0.00)	0 (0.00)
74	2016	36 (0.49)	2 (0.03)	2 (0.03)	9 (0.12)	23 (0.31)	1 (0.01)	1 (0.01)
8	2017	4 (0.50)	1 (0.13)	1 (0.13)	0 (0.00)	2 (0.25)	0 (0.00)	0 (0.00)

[a] Total number of isolates collected in each year

[b] 2013 data reported by Cianchetta et al. (2015), included for comparison

Table 3.3. Number of individuals of races or genotypes of early-season *Fusarium oxysporum* f. sp. *vasinfectum* isolates in Georgia based on translation elongation factor (*EF-1α*) sequence; relative frequency of each race or genotype in parentheses.

No.[a]	**Year**	**Race 1**	**Race 2**	**Race 8**	**LA 108**	**LA 110**	**LA 127/140**	**MDS-12**
6	2016	3 (0.50)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.33)	0 (0.00)	1 (0.171)
8	2017	4 (0.50)	1 (0.13)	1 (0.13)	0 (0.00)	2 (0.25)	0 (0.00)	0 (0.00)

[a] Total number of early-season isolates collected in each year

Table 3.4 *p*-values showing the statistical significance of fungal isolate, cotton cultivar, and isolate by cultivar interaction on symptoms of Fusarium wilt in all greenhouse trials, with a score of 0 indicating $p < 0.01$.

Variable	**Dry Root Mass**[a]					**Dry Shoot Mass**[a]					**Wilt Severity**[b]					**Vascular Discoloration**[b]					**Root Rotting**[b]				
	T1	**T2**	**T3**	**T4**	**T5**	**T1**	**T2**	**T3**	**T4**	**T5**	**T1**	**T2**	**T3**	**T4**	**T5**	**T1**	**T2**	**T3**	**T4**	**T5**	**T1**	**T2**	**T3**	**T4**	**T5**
Fungal Isolate	**.07**	.12	.22	.05	.24	.33	.99	.99	.99	.22	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Cotton Cultivar	**.01**	.53	**0**	**0**	.69	**0**	.29	**0**	**0**	**0**	**0**	**0**	**0**	**0**	.46	**0**	**0**	**0**	**0**	.92	**0**	**0**	**0**	**0**	.92
Isolate*cultivar	.63	.22	.50	.45	.18	.99	.92	1.0	.99	.83	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

[a] Significance was determined using analysis of variance (ANOVA) for dry root mass and dry shoot mass.

[b] Significance was determined using Kruskal Wallis non-parametric test for wilt severity, vascular discoloration, and root rotting.

Table 3.5. Disease severity ratings from inoculation of FOV isolates onto the cotton cultivar Phytogen 805 using injection with a sterile syringe and needle.

ISOLATE	ORIGIN	GENOTYPE	DISCOLORATION	WILT	SHOOT MASS
			T1, T2	**T1, T2**	**T1, T2**
F16-2BS1[z]	GA	MDS-12	1.25^{A}, 1.50^{a}	0.75^{AB}, 1.50^{a}	5.37, 8.77
F16-6CS1	GA	LA127	0.75^{AB}, 0.50^{bc}	0.50^{B}, 1.75^{a}	5.48, 12.30
F16-22AS1	GA	LA110	0.50^{AB}, 0.25^{c}	1.00^{A}, 1.00^{b}	4.17, 7.90
F16-8BS1	GA	LA110	0.50^{AB}, 0.50^{bc}	0.25^{BC}, 1.50^{a}	5.25, 10.08
F16-8AS1	GA	LA108	0.50^{AB}, 0.75^{bc}	1.00^{A}, 0.50^{d}	4.91, 6.69
F16-10BS1	GA	LA108	1.00^{A}, 0.25^{c}	0.50^{B}, 0.75^{bc}	5.36, 7.93
F16-16CS1	GA	Race 1	0.00^{C}, 1.50^{a}	0.00^{C}, 0.25^{cd}	5.68, 9.53
F16-20CS1	GA	Race 1	0.50^{B}, 0.50^{bc}	0.50^{B}, 1.00^{b}	4.73, 7.41
F16-12CS1	GA	Race 2	0.00^{C}, 0.25^{c}	0.50^{B}, 1.25^{ab}	4.96, 9.60
F16-9CS1	GA	Race 8	0.25^{BC}, 1.00^{b}	0.00^{C}, 0.00^{d}	5.85, 6.05
			T3, T4	**T3, T4**	**T3, T4**
F16-2BS1[z]	GA	MDS-12	1.75^{A}, 1.25^{ab}	1.50^{A}, 1.25^{ab}	5.06, 6.67
F16-6CS1	GA	LA127	1.25^{AB}, 1.25^{ab}	0.25^{BC}, 1.00^{abc}	4.62, 6.80
309N-14[x]	AL	LA127	1.75^{A}, 0.75^{bc}	1.50^{A}, 1.00^{b}	5.15, 6.23
2165-145[x]	AL	LA127	1.00^{ABC}, 0.50^{bc}	0.25^{BC}, 1.50^{a}	5.33, 6.54
32900[y]	AR	LA127	0.50^{BC}, 0.50^{bc}	0.50^{B}, 0.50^{bcd}	5.53, 6.09
34051[y]	AR	LA127	0.75^{BC}, 0.75^{bc}	0.50^{B}, 0.50^{d}	5.56, 7.15
F15-1CS1	GA	LA110	1.00^{B}, 0.50^{bc}	0.25^{BC}, 1.00^{b}	4.60, 6.30
F16-21BS1	GA	LA110	1.75^{A}, 0.50^{bc}	0.50^{B}, 0.00^{d}	5.03, 7.91
F16-1AS1[z]	GA	LA110	1.00^{B}, 1.00^{b}	0.75^{B}, 0.50^{c}	4.56, 6.60
F17-5CS1[z]	GA	LA110	1.00^{B}, 0.50^{bc}	0.75^{B}, 0.50^{c}	5.58, 6.15
F15-4AS1	GA	LA108	1.00^{B}, 0.50^{bc}	0.75^{B}, 0.75^{bc}	5.76, 7.34

F15-7AS1	GA	LA108	0.75^{BC}, 0.75^{bc}	1.25^{AB}, 0.75^{abc}	6.78, 6.98
F16-10DS1	GA	LA108	0.50^{BC}, 0.50^{bc}	0.75^{B}, 1.00^{b}	6.05, 6.74
F16-7BS1	GA	LA108	0.25^{C}, 0.75^{bc}	0.50^{B}, 0.25^{cd}	4.90, 7.19
C15044[x]	AL	LA108	1.25^{AB}, 0.75^{bc}	0.75^{B}, 0.50^{d}	5.06, 7.60
401N-3A[x]	AL	LA108	1.25^{AB}, 1.25^{ab}	1.00^{AB}, 0.00^{d}	4.89, 6.37
F15-6BS1	GA	Race 1	0.50^{BC}, 0.50^{bc}	1.50^{A}, 0.50^{bcd}	6.93, 6.09
F16-7AS1	GA	Race 1	0.50^{BC}, 0.50^{bc}	1.00^{AB}, 0.25^{cd}	5.85, 7.15
TF2[x]	AL	Race 1	1.50^{AB}, 1.75^{a}	0.00^{C}, 0.00^{d}	5.04, 7.32
34076[y]	LA	Race 1	0.50^{BC}, 0.25^{cd}	1.00^{AB}, 0.75^{bc}	5.54, 7.81
F17-6BS1[z]	GA	Race 1	1.00^{B}, 0.75^{bc}	0.75^{B}, 0.75^{bc}	5.23, 5.99
F15-5DS1	GA	Race 2	1.00^{B}, 0.75^{bc}	1.25^{AB}, 0.00^{d}	6.42, 6.88
F16-12CS1	GA	Race 2	0.75^{BC}, 1.00^{b}	0.75^{B}, 0.25^{cd}	5.56, 6.80
F16-12DS1	GA	Race 2	0.25^{C}, 0.50^{bc}	0.50^{B}, 0.75^{bc}	5.27, 6.10
F17-6DS1[z]	GA	Race 2	0.75^{BC}, 1.00^{b}	0.25^{BC}, 0.75^{abc}	5.21, 7.65
34049[y]	LA	Race 2	1.00^{AB}, 0.75^{bc}	0.25^{BC}, 0.25^{cd}	5.77, 7.82
34082[y]	LA	Race 3	0.50^{BC}, 0.00^{d}	0.25^{BC}, 0.25^{cd}	5.60, 7.49
34083[y]	LA	Race 3	0.50^{BC}, 0.50^{bc}	0.50^{B}, 0.25^{cd}	6.00, 7.01
F15-3CS1	GA	Race 8	0.50^{BC}, 0.50^{bc}	0.50^{B}, 0.75^{abc}	5.58, 6.55
F17-6AS1[z]	GA	Race 8	0.00^{C}, 0.50^{bc}	0.25^{BC}, 0.75^{bc}	5.39, 6.36
207N-18[x]	AL	Race 8	0.50^{BC}, 1.25^{ab}	0.50^{B}, 0.50^{d}	5.18, 6.25
32876[y]	AR	Race 8	0.50^{BC}, 1.00^{b}	0.75^{B}, 0.50^{d}	6.43, 7.10

[x] Isolate provided by Dr. Jeffrey Coleman, Auburn University

[y] Isolate provided by USDA-ARS NRRL Culture Collection

[z] Early season isolate

Table 3.6. Disease severity ratings from inoculation of FOV isolates onto the cotton cultivar Phytogen 805 using a soil-drench assay.

ISOLATE	**ORIGIN**	**GENOTYPE**	**DISCOLORATION**	**WILT**	**ROOT ROT**	**SHOOT MASS**	**ROOT MASS**
			T1, T2	**T1, T2**	**T1, T2**	**T1, T2**	**T1, T2**
F16-2BS1[z]	GA	MDS-12	0.50^{A}, 1.00^{a}	0.50^{C}, 2.00^{a}	1.50^{AB}, 2.67^{a}	3.97, 6.76	2.45, 5.95
F16-6CS1	GA	LA127	0.00^{B}, 1.00^{a}	1.00^{B}, 2.00^{a}	0.50^{CD}, 2.33^{ab}	3.57, 2.12	2.74, 1.84
F16-22AS1	GA	LA110	0.50^{A}, 1.00^{a}	1.00^{B}, 1.33^{bc}	0.75^{CD}, 1.67^{bc}	4.09, 4.56	2.17, 3.20
F16-8BS1	GA	LA110	0.00^{B}, 0.67^{ab}	0.25^{CD}, 2.00^{a}	1.00^{C}, 1.67^{bc}	4.19, 3.14	2.54, 3.07
F16-8AS1	GA	LA108	0.00^{B}, 1.00^{a}	1.00^{B}, 1.00^{c}	0.75^{CD}, 2.33^{ab}	4.43, 3.82	2.03, 4.24
F16-10BS1	GA	LA108	0.50^{A}, 0.67^{ab}	0.50^{C}, 1.33^{bc}	2.00^{A}, 2.00^{b}	3.72, 5.20	2.50, 5.67
F16-16CS1	GA	Race 1	0.00^{B}, 0.33^{bc}	0.00^{D}, 1.33^{bc}	0.25^{D}, 2.00^{b}	5.16, 5.44	2.35, 4.61
F16-20CS1	GA	Race 1	0.00^{B}, 1.00^{a}	0.50^{C}, 1.67^{ab}	0.50^{CD}, 2.00^{ab}	4.18, 4.90	2.11, 5.04
F16-12CS1	GA	Race 2	0.00^{B}, 0.00^{c}	1.75^{A}, 1.00^{c}	0.50^{CD}, 2.00^{ab}	4.16, 3.60	1.88, 2.86
F16-9CS1	GA	Race 8	0.00^{B}, 0.33^{bc}	1.00^{B}, 1.33^{bc}	1.00^{C}, 2.33^{ab}	3.97, 4.48	2.75, 3.22
			T3, T4	**T3, T4**	**T3, T4**	**T3, T4**	**T3, T4**
F16-2BS1[z]	GA	MDS-12	1.33^{A}, 1.00^{a}	1.67^{AB}, 1.67^{a}	2.33, 2.33^{a}	1.92, 4.32	2.42, 2.17
F16-6CS1	GA	LA127	0.33^{BC}, 1.00^{abc}	1.00^{BC}, 1.33^{ab}	2.00, 2.00^{a}	1.79, 4.39	1.74, 2.76
309N-14[x]	AL	LA127	0.67^{AB}, 0.67^{ab}	1.67^{AB}, 1.33^{ab}	2.00^{AB}, 2.00^{a}	1.73, 4.17	1.73, 2.94
2165-145[x]	AL	LA127	1.00^{A}, 1.00^{a}	1.00^{BC}, 1.33^{ab}	2.00^{AB}, 2.00^{a}	1.46, 4.14	1.46, 2.16
32900[y]	AR	LA127	1.00^{A}, 0.67^{ab}	1.33^{B}, 1.00^{b}	1.33^{BC}, 1.67^{a}	1.41, 5.00	1.41, 2.34

34051[y]	AR	LA127	0.67^{AB}, 1.00^{abc}	1.00^{BC}, 1.33^{ab}	1.33^{BC}, 1.33^{b}	1.92, 5.16	1.92, 2.60
F15-1CS1	GA	LA110	0.33^{BC}, 0.67^{ab}	2.00^{A}, 2.00^{a}	2.00^{B}, 1.33^{b}	1.77, 3.91	1.77, 1.65
F16-21BS1	GA	LA110	0.67^{AB}, 0.33^{bc}	0.67^{BC}, 0.33^{c}	1.33^{BC}, 1.67^{ab}	2.30, 4.45	2.30, 2.50
F16-1AS1[z]	GA	LA110	0.33^{BC}, 1.33^{a}	1.33^{B}, 1.33^{ab}	1.33^{BC}, 2.00^{a}	2.22, 3.86	2.22, 1.98
F17-5CS1[z]	GA	LA110	0.00^{C}, 0.67^{ab}	0.67^{BC}, 0.33^{c}	2.00^{AB}, 1.33^{ab}	1.64, 3.75	1.64, 1.78
F15-4AS1	GA	LA108	0.33^{BC}, 1.00^{a}	1.33^{B}, 1.00^{b}	1.67^{B}, 1.33^{ab}	1.47, 4.88	1.47, 1.56
F15-7AS1	GA	LA108	0.00^{C}, 0.67^{ab}	0.33^{CD}, 1.33^{ab}	2.00^{B}, 2.00^{ab}	2.84, 4.38	2.84, 2.45
F16-10DS1	GA	LA108	0.67^{AB}, 0.67^{ab}	0.67^{BC}, 0.67^{bc}	1.33^{BC}, 2.00^{a}	1.70, 5.08	1.70, 2.50
F16-7BS1	GA	LA108	0.33^{BC}, 1.00^{ab}	0.67^{BC}, 0.67^{bc}	1.67^{B}, 1.33^{b}	1.40, 3.71	1.40, 2.34
C15044[x]	AL	LA108	0.33^{BC}, 0.33^{bc}	1.00^{BC}, 0.67^{bc}	1.33^{BC}, 1.00^{b}	1.89, 4.00	2.05, 1.99
401N-3A[x]	AL	LA108	0.67^{AB}, 1.00^{a}	1.00^{BC}, 1.00^{b}	1.33^{BC}, 1.33^{ab}	2.22, 3.71	2.22, 1.86
F15-6BS1	GA	Race 1	0.33^{BC}, 1.00^{a}	0.33^{CD}, 1.00^{b}	2.67^{A}, 1.67^{ab}	1.54, 4.10	1.54, 2.09
F16-7AS1	GA	Race 1	0.67^{AB}, 0.67^{ab}	0.00^{D}, 1.00^{b}	1.00^{C}, 1.33^{b}	1.67, 4.17	1.04, 1.76
TF2[x]	AL	Race 1	0.33^{BC}, 1.33^{a}	0.00^{D}, 0.33^{c}	2.33^{AB}, 1.67^{ab}	1.04, 3.34	1.46, 2.15
34076[y]	LA	Race 1	0.00^{C}, 0.33^{bc}	0.67^{BC}, 0.33^{d}	0.33^{C}, 1.00^{b}	1.44, 3.78	1.44, 1.94
F17-6BS1[z]	GA	Race 1	0.67^{AB}, 0.67^{ab}	0.67^{BC}, 0.67^{bc}	1.33^{BC}, 1.00^{b}	1.55, 4.03	1.89, 2.10
F15-5DS1	GA	Race 2	1.00^{A}, 1.33^{abc}	0.67^{BC}, 0.00^{c}	1.33^{BC}, 1.33^{b}	1.41, 3.99	1.41, 2.03
F16-12CS1	GA	Race 2	0.33^{BC}, 1.00^{a}	1.00^{BC}, 0.67^{bc}	1.33, 1.67^{ab}	1.82, 4.93	1.60, 1.75
F16-12DS1	GA	Race 2	0.67^{AB}, 0.67^{ab}	0.67^{BC}, 1.67^{ab}	1.00^{C}, 1.33^{b}	1.74, 4.45	1.74, 1.56
F17-6DS1[z]	GA	Race 2	0.33^{BC}, 1.33^{a}	0.67^{BC}, 1.00^{b}	0.67^{C}, 1.33^{b}	2.42, 4.32	2.42, 2.37

34049[y]	LA	Race 2	1.00^{AB}, 1.00^{a}	0.67^{BC}, 0.67^{bc}	1.00^{C}, 1.33^{b}	1.21, 4.67	1.21, 1.95
34082[y]	LA	Race 3	0.00^{C}, 0.33^{bc}	0.67^{BC}, 0.33^{c}	0.67^{C}, 1.00^{b}	2.81, 4.03	2.81, 2.60
34083[y]	LA	Race 3	0.33^{BC}, 0.00^{c}	0.67^{BC}, 1.00^{abc}	0.33^{C}, 1.00^{b}	2.24, 4.16	2.24, 2.34
F15-3CS1	GA	Race 8	1.00^{B}, 0.33^{bc}	0.67^{BC}, 1.00^{b}	1.67^{B}, 2.00^{a}	1.84, 4.37	1.84, 2.09
F17-6AS1[z]	GA	Race 8	0.33^{BC}, 0.67^{ab}	0.67^{BC}, 0.67^{bc}	2.00^{AB}, 2.33^{a}	1.58, 3.66	1.58, 1.65
207N-18[x]	AL	Race 8	0.67^{AB}, 0.67^{ab}	0.67^{BC}, 0.67^{bc}	0.67^{C}, 1.00^{b}	2.39, 5.03	2.55, 3.03
32876[y]	AR	Race 8	0.67^{AB}, 0.33^{bc}	1.00^{BC}, 1.33^{ab}	1.67^{AB}, 1.67^{a}	2.55, 4.79	2.39, 2.11

[x] Isolate provided by Dr. Jeffrey Coleman, Auburn University

[y] Isolate provided by USDA-ARS NRRL Culture Collection

[z] Early season isolate

Table 3.7. Disease severity ratings from inoculation of FOV isolates onto the cotton cultivar Delta Pine 1454 using injection with a sterile syringe and needle.

ISOLATE	ORIGIN	GENOTYPE	DISCOLORATION	WILT	SHOOT MASS
			T1, T5	**T1, T5**	**T1, T5**
F16-2BS1[z]	GA	MDS-12	2.50^{A}, 2.33^{a}	2.00^{A}, 1.33^{a}	2.94, 7.74
F16-6CS1	GA	LA127	2.00^{AB}, 1.67^{ab}	1.00^{BC}, 1.00^{ab}	4.18, 7.79
F16-22AS1	GA	LA110	1.50^{B}, 1.67^{ab}	1.25^{B}, 1.33^{a}	3.28, 8.43
F16-8BS1	GA	LA110	1.50^{B}, 1.00^{b}	1.25^{B}, 1.00^{ab}	3.38, 6.34
F16-8AS1	GA	LA108	1.50^{B}, 1.67^{ab}	1.25^{B}, 1.33^{a}	3.33, 7.50
F16-10BS1	GA	LA108	1.75^{AB}, 0.67^{bc}	1.25^{ABC}, 1.33^{a}	3.83, 7.25
F16-16CS1	GA	Race 1	1.50^{B}, 1.33^{b}	0.50^{C}, 0.67^{b}	3.41, 5.06
F16-20CS1	GA	Race 1	1.50^{B}, 0.33^{c}	1.25^{B}, 0.33^{b}	6.67, 8.45
F16-12CS1	GA	Race 2	1.00^{C}, 0.67^{bc}	1.00^{BC}, 0.67^{b}	3.10, 6.90
F16-9CS1	GA	Race 8	0.50^{C}, 1.00^{b}	0.75^{C}, 0.33^{b}	4.33, 8.77
			T3, T4	**T3, T4**	**T3, T4**
F16-2BS1[z]	GA	MDS-12	1.67^{AB}, 1.25^{ab}	1.67^{BC}, 1.00^{ab}	1.86, 5.82
F16-6CS1	GA	LA127	1.00^{BC}, 0.75^{b}	1.00^{CD}, 0.75^{bc}	1.58, 5.89
309N-14[x]	AL	LA127	2.33^{A}, 1.00^{ab}	0.67^{CD}, 0.50^{bc}	1.90, 4.77
2165-145[x]	AL	LA127	1.33^{B}, 1.00^{ab}	0.33^{DE}, 0.75^{b}	2.02, 5.45
32900[y]	AR	LA127	1.00^{BC}, 0.75^{bc}	1.67^{BC}, 1.00^{ab}	1.46, 5.71
34051[y]	AR	LA127	1.00^{BC}, 0.25^{bc}	2.33^{B}, 1.50^{a}	1.22, 5.19
F15-1CS1	GA	LA110	1.33^{B}, 0.50^{bc}	1.33^{C}, 1.00^{a}	1.42, 6.10
F16-21BS1	GA	LA110	0.67^{BC}, 0.75^{bc}	1.00^{C}, 0.50^{bc}	2.35, 4.78
F16-1AS1[z]	GA	LA110	0.33^{CD}, 0.75^{bc}	1.33^{C}, 1.25^{a}	1.19, 4.10
F17-5CS1[z]	GA	LA110	1.33^{AB}, 0.50^{bc}	2.33^{B}, 1.25^{ab}	1.11, 5.66
F15-4AS1	GA	LA108	1.33^{B}, 0.50^{bc}	1.33^{C}, 0.75^{bc}	1.31, 6.01

F15-7AS1	GA	LA108	0.67^{BC}, 0.75^{bc}	0.67^{CD}, 1.25^{a}	1.56, 5.34
F16-10DS1	GA	LA108	1.67^{AB}, 1.00^{b}	1.33^{C}, 0.25^{c}	1.47, 5.38
F16-7BS1	GA	LA108	0.00^{D}, 0.50^{bc}	3.00^{A}, 1.25^{a}	1.04, 4.78
C15044[x]	AL	LA108	1.33^{B}, 1.00^{ab}	1.33^{C}, 0.75^{bc}	1.31, 5.40
401N-3A[x]	AL	LA108	1.33^{B}, 0.75^{bc}	2.00^{BC}, 0.25^{c}	1.11, 4.39
F15-6BS1	GA	Race 1	0.33^{CD}, 0.50^{bc}	2.67^{AB}, 0.50^{bc}	1.04, 4.95
F16-7AS1	GA	Race 1	1.33^{AB}, 0.75^{bc}	1.33^{C}, 0.50^{bc}	1.23, 6.31
TF2[x]	AL	Race 1	1.00^{BC}, 1.25^{ab}	2.33^{ABC}, 1.00^{abc}	1.25, 6.68
34076[y]	LA	Race 1	0.67^{BC}, 0.25^{bc}	0.67^{CD}, 0.75^{abc}	1.90, 6.70
F17-6BS1[z]	GA	Race 1	1.33^{B}, 1.00^{b}	1.33^{C}, 1.00^{ab}	1.57, 5.39
F15-5DS1	GA	Race 2	1.00^{BC}, 1.25^{ab}	2.33^{B}, 1.00^{a}	1.77, 4.81
F16-12CS1	GA	Race 2	0.67^{BC}, 1.00^{a}	1.67^{C}, 1.00^{ab}	1.18, 5.78
F16-12DS1	GA	Race 2	0.67^{BC}, 0.75^{bc}	1.33^{CD}, 1.00^{a}	1.19, 6.08
F17-6DS1[z]	GA	Race 2	1.00^{BC}, 1.75^{a}	0.67^{CD}, 2.00^{a}	1.14, 4.35
34049[y]	LA	Race 2	1.00^{BC}, 0.75^{bc}	0.00^{E}, 0.00^{c}	1.83, 4.89
34082[y]	LA	Race 3	0.67^{BC}, 0.50^{bc}	2.33^{B}, 0.25^{c}	1.80, 5.98
34083[y]	LA	Race 3	1.67^{AB}, 0.25^{bc}	1.33^{ABC}, 0.00^{c}	1.84, 6.53
F15-3CS1	GA	Race 8	1.33^{ABC}, 1.25^{ab}	0.67^{CD}, 0.50^{bc}	1.66, 5.12
F17-6AS1[z]	GA	Race 8	0.67^{BC}, 0.75^{bc}	0.00^{E}, 0.50^{bc}	2.11, 4.74
207N-18[x]	AL	Race 8	0.33^{CD}, 1.00^{abc}	0.33^{DE}, 0.50^{bc}	1.88, 6.09
32876[y]	AR	Race 8	0.33^{CD}, 1.25^{ab}	0.33^{DE}, 0.50^{bc}	1.80, 6.37

[x] Isolate provided by Dr. Jeffrey Coleman, Auburn University

[y] Isolate provided by USDA-ARS NRRL Culture Collection

[z] Early season isolate

Table 3.8. Disease severity ratings from inoculation of FOV isolates onto the cotton cultivar Delta Pine 1454 using a soil-drench assay.

ISOLATE	**ORIGIN**	**GENOTYPE**	**DISCOLORATION**	**WILT**	**ROOT ROT**	**SHOOT MASS**	**ROOT MASS**
			T1, T5	**T1, T5**	**T1, T5**	**T1, T5**	**T1, T5**
F16-2BS1[z]	GA	MDS-12	2.00^{A}, 1.67^{a}	2.25^{A}, 1.67^{a}	2.75^{A}, 2.33^{a}	2.29, 2.31	1.75, 2.27
F16-6CS1	GA	LA127	1.50^{AB}, 1.67^{a}	2.00^{AB}, 1.67^{a}	1.50^{B}, 2.33^{a}	1.75, 2.39	2.71, 1.80
F16-22AS1	GA	LA110	1.00^{B}, 1.00^{b}	2.00^{AB}, 1.33^{ab}	2.25^{AB}, 1.67^{ab}	2.42, 3.01	1.74, 2.04
F16-8BS1	GA	LA110	1.25^{BC}, 1.00^{b}	2.00^{AB}, 1.33^{ab}	1.75^{AB}, 1.67^{ab}	2.95, 2.55	2.02, 1.76
F16-8AS1	GA	LA108	0.75^{C}, 1.00^{b}	2.00^{AB}, 1.33^{ab}	2.00^{AB}, 2.00^{a}	2.51, 3.11	1.98, 2.33
F16-10BS1	GA	LA108	1.25^{BC}, 1.33^{ab}	2.25^{AB}, 1.33^{ab}	1.75^{ABC}, 2.00^{a}	3.24, 2.52	1.91, 1.54
F16-16CS1	GA	Race 1	1.00^{C}, 0.67^{bc}	1.50^{B}, 1.00^{ab}	0.75^{C}, 1.33^{b}	2.67, 3.15	2.07, 2.37
F16-20CS1	GA	Race 1	1.00^{C}, 0.67^{bc}	2.00^{AB}, 1.33^{ab}	1.00^{C}, 1.33^{b}	3.25, 3.69	1.81, 2.46
F16-12CS1	GA	Race 2	0.50^{C}, 0.67^{bc}	1.75^{AB}, 0.67^{b}	0.50^{C}, 1.33^{b}	3.35, 3.12	1.88, 1.59
F16-9CS1	GA	Race 8	0.00^{D}, 0.33^{c}	1.50^{B}, 1.00^{ab}	2.25^{AB}, 1.67^{ab}	3.36, 3.75	2.00, 1.43
			T3, T4	**T3, T4**	**T3, T4**	**T3, T4**	**T3, T4**
F16-2BS1[z]	GA	MDS-12	1.67^{A}, 1.67^{a}	1.67^{B}, 1.00^{b}	2.33^{B}, 2.33^{a}	2.31, 2.79	2.27, 1.64
F16-6CS1	GA	LA127	1.67^{A}, 1.00^{ab}	1.67^{B}, 1.67^{ab}	2.33^{B}, 2.33^{a}	2.39, 3.02	1.80, 1.98
309N-14[x]	AL	LA127	1.00^{B}, 1.33^{ab}	1.33^{BC}, 1.33^{ab}	2.00^{B}, 1.67^{ab}	3.15, 3.31	2.13, 1.95

2165-145[x]	AL	LA127	1.33^{AB}, 1.00^{ab}	1.00^{C}, 0.67^{bc}	2.00^{B}, 1.00^{b}	3.31, 2.84	2.02, 1.65
32900[y]	AR	LA127	1.33^{AB}, 1.00^{ab}	1.00^{C}, 0.67^{bc}	2.00^{B}, 1.67^{ab}	2.89, 2.46	1.98, 2.22
34051[y]	AR	LA127	0.00^{D}, 1.00^{ab}	1.00^{CD}, 1.00^{b}	1.67^{B}, 2.33^{a}	2.67, 2.59	1.59, 1.45
F15-1CS1	GA	LA110	0.67^{BC}, 1.33^{ab}	1.33^{BC}, 0.67^{bc}	1.00^{C}, 2.67^{a}	2.87, 2.37	1.87, 1.59
F16-21BS1	GA	LA110	1.00^{B}, 1.00^{ab}	1.33^{BC}, 0.33^{c}	2.00^{B}, 1.33^{b}	3.16, 2.29	1.93, 1.53
F16-1AS1[z]	GA	LA110	0.33^{CD}, 0.67^{b}	0.33^{D}, 1.00^{b}	1.67^{BC}, 1.67^{ab}	2.98, 2.49	1.55, 1.98
F17-5CS1[z]	GA	LA110	0.67^{BC}, 1.33^{ab}	1.33^{BCD}, 1.00^{b}	3.00^{A}, 2.00^{ab}	2.05, 2.90	2.07, 2.63
F15-4AS1	GA	LA108	0.33^{CD}, 0.67^{b}	1.00^{C}, 0.33^{c}	1.33^{BC}, 1.67^{ab}	2.77, 2.69	1.62, 0.97
F15-7AS1	GA	LA108	1.00^{B}, 1.67^{a}	1.33^{BCD}, 0.33^{c}	2.00^{B}, 1.67^{ab}	2.60, 2.54	1.80, 1.97
F16-10DS1	GA	LA108	1.00^{B}, 0.67^{b}	1.00^{BCD}, 1.67^{a}	3.00^{A}, 2.67^{a}	3.78, 3.05	2.01, 1.57
F16-7BS1	GA	LA108	0.33^{CD}, 0.33^{bc}	1.33^{BC}, 0.33^{c}	1.00^{C}, 1.33^{b}	3.08, 2.28	2.10, 1.61
C15044[x]	AL	LA108	1.00^{B}, 0.67^{b}	1.00^{BCD}, 0.67^{bc}	1.00^{C}, 1.33^{b}	3.34, 2.75	1.88, 2.03
401N-3A[x]	AL	LA108	1.00^{B}, 1.00^{ab}	1.33^{BCD}, 0.67^{bc}	0.67^{C}, 2.00^{ab}	2.93, 2.57	1.45, 1.83
F15-6BS1	GA	Race 1	0.67^{BCD}, 0.33^{bc}	1.67^{B}, 0.67^{bc}	2.00^{B}, 1.67^{ab}	2.55, 2.60	1.76, 1.45
F16-7AS1	GA	Race 1	1.00^{ABC}, 1.00^{b}	1.00^{C}, 1.00^{b}	1.67^{BC}, 1.67^{ab}	2.97, 2.75	2.03, 1.38
TF2[x]	AL	Race 1	1.33^{AB}, 2.00^{a}	1.00^{C}, 1.33^{ab}	2.00^{B}, 2.67^{a}	2.34, 2.91	1.47, 1.66
34076[y]	LA	Race 1	1.00^{ABC}, 1.33^{ab}	0.67^{CD}, 0.67^{bc}	1.33^{BC}, 1.33^{b}	3.78, 3.05	1.59, 2.12
F17-6BS1[z]	GA	Race 1	1.00^{B}, 0.67^{b}	1.33^{BC}, 0.67^{bc}	1.67^{B}, 1.33^{b}	3.06, 2.98	1.97, 1.79
F15-5DS1	GA	Race 2	0.67^{BC}, 0.67^{b}	1.67^{B}, 1.67^{a}	1.67^{B}, 1.00^{bc}	3.41, 2.46	2.08, 1.00
F16-12CS1	GA	Race 2	0.67^{BC}, 0.67^{b}	0.67^{BCD}, 1.00^{b}	1.33^{BC}, 2.00^{ab}	3.12, 2.84	1.59, 1.27

F16-12DS1	GA	Race 2	1.33^{AB}, 1.67^{a}	1.33^{BC}, 0.67^{bc}	2.33^{B}, 1.33^{b}	2.76, 2.29	2.19, 1.37
F17-6DS1[z]	GA	Race 2	1.00^{B}, 1.67^{a}	1.67^{B}, 2.00^{a}	3.00^{A}, 2.67^{a}	2.21, 3.21	1.49, 1.55
34049[y]	LA	Race 2	0.33^{CD}, 0.67^{b}	1.00^{C}, 0.67^{bc}	1.67^{B}, 1.00^{bc}	3.09, 2.24	1.91, 1.64
34082[y]	LA	Race 3	0.33^{CD}, 0.00^{c}	1.00^{BCD}, 1.00^{b}	1.67^{B}, 0.33^{c}	2.40, 2.16	2.64, 1.96
34083[y]	LA	Race 3	1.00^{B}, 0.00^{c}	1.33^{BC}, 0.67^{bc}	1.33^{BC}, 0.33^{c}	2.60, 2.74	2.00, 1.82
F15-3CS1	GA	Race 8	0.33^{CD}, 1.00^{b}	1.67^{B}, 0.33^{c}	2.67^{AB}, 1.00^{b}	3.05, 2.41	2.17, 1.65
F17-6AS1[z]	GA	Race 8	1.33^{AB}, 1.67^{a}	1.33^{BC}, 1.00^{b}	2.67^{AB}, 1.67^{ab}	3.31, 2.51	1.35, 1.94
207N-18[x]	AL	Race 8	0.67^{BC}, 1.33^{ab}	2.67^{A}, 1.00^{b}	2.00^{B}, 2.33^{a}	2.97, 2.24	1.27, 1.55
32876[y]	AR	Race 8	1.00^{B}, 0.33^{bc}	1.00^{BCD}, 0.67^{bc}	1.00^{C}, 0.33^{c}	3.00, 3.77	1.96, 1.37

[x] Isolate provided by Dr. Jeffrey Coleman, Auburn University

[y] Isolate provided by USDA-ARS NRRL Culture Collection

[z] Early season isolate

Table 3.9. Disease severity ratings from inoculation of FOV isolates onto the cotton cultivar Rowden using injection with a sterile syringe and needle.

ISOLATE	ORIGIN	GENOTYPE	DISCOLORATION	WILT	SHOOT MASS
			T2, T5	**T2, T5**	**T2, T5**
F16-2BS1[z]	GA	MDS-12	1.75^{A}, 2.00^{a}	2.25^{AB}, 1.00^{ab}	6.59, 6.50
F16-6CS1	GA	LA127	1.00^{B}, 1.00^{b}	2.00^{ABC}, 1.67^{a}	7.53, 10.16
F16-22AS1	GA	LA110	1.25^{AB}, 2.33^{a}	1.50^{C}, 1.33^{ab}	7.30, 7.86
F16-8BS1	GA	LA110	1.00^{B}, 1.33^{ab}	2.50^{A}, 1.33^{ab}	8.54, 9.92
F16-8AS1	GA	LA108	1.25^{AB}, 1.67^{ab}	2.00^{B}, 0.00^{c}	8.10, 6.61
F16-10BS1	GA	LA108	1.50^{A}, 1.33^{ab}	1.75^{ABC}, 1.00^{ab}	9.50, 10.17
F16-16CS1	GA	Race 1	1.00^{B}, 0.67^{bc}	2.00^{B}, 1.00^{ab}	6.59, 10.85
F16-20CS1	GA	Race 1	1.50^{A}, 1.67^{ab}	1.50^{C}, 1.00^{ab}	5.33, 10.46
F16-12CS1	GA	Race 2	0.50^{C}, 1.00^{b}	1.75^{BC}, 0.67^{b}	8.81, 10.11
F16-9CS1	GA	Race 8	0.50^{C}, 2.00^{a}	0.50^{D}, 1.00^{ab}	9.24, 8.18
			T3, T4	**T3, T4**	**T3, T4**
F16-2BS1[z]	GA	MDS-12	2.00^{A}, 1.50^{ab}	2.00^{AB}, 2.00^{a}	2.13, 4.65
F16-6CS1	GA	LA127	1.50^{AB}, 1.75^{a}	1.50^{BC}, 1.75^{a}	2.80, 4.99
309N-14[x]	AL	LA127	1.75^{AB}, 1.00^{bc}	1.50^{BC}, 1.75^{a}	2.19, 5.67
2165-145[x]	AL	LA127	1.00^{BC}, 0.75^{bc}	2.00^{AB}, 1.50^{a}	2.57, 4.39
32900[y]	AR	LA127	1.25^{ABC}, 1.25^{b}	2.00^{AB}, 1.25^{b}	2.22, 4.97
34051[y]	AR	LA127	1.25^{B}, 0.75^{bc}	1.75^{AB}, 0.75^{bc}	2.76, 4.45
F15-1CS1	GA	LA110	1.25^{B}, 0.75^{bc}	1.00^{C}, 1.75^{a}	2.82, 5.88
F16-21BS1	GA	LA110	1.75^{AB}, 1.00^{b}	1.75^{B}, 0.75^{bc}	1.90, 5.90
F16-1AS1[z]	GA	LA110	1.25^{ABC}, 1.00^{bc}	1.00^{C}, 1.00^{b}	2.54, 4.37
F17-5CS1[z]	GA	LA110	1.75^{AB}, 1.50^{ab}	1.25^{BC}, 1.25^{ab}	2.20, 4.90
F15-4AS1	GA	LA108	0.75^{BC}, 1.25^{ab}	1.25^{BC}, 1.25^{ab}	2.40, 5.88

F15-7AS1	GA	LA108	1.50^{AB}, 1.00^{b}	2.00^{B}, 1.00^{b}	1.88, 5.91
F16-10DS1	GA	LA108	1.00^{BC}, 1.25^{ab}	1.50^{BC}, 1.25^{ab}	2.09, 4.15
F16-7BS1	GA	LA108	0.25^{CD}, 0.75^{bc}	2.25^{AB}, 1.25^{ab}	2.91, 5.65
C15044[x]	AL	LA108	1.25^{B}, 1.50^{ab}	1.75^{B}, 1.50^{a}	2.89, 5.30
401N-3A[x]	AL	LA108	1.50^{AB}, 1.25^{b}	1.25^{BC}, 0.50^{bc}	2.60, 5.11
F15-6BS1	GA	Race 1	0.50^{C}, 0.75^{c}	1.75^{B}, 0.75^{b}	2.70, 4.61
F16-7AS1	GA	Race 1	1.00^{BC}, 1.00^{b}	2.00^{B}, 1.00^{b}	2.07, 4.92
TF2[x]	AL	Race 1	1.50^{AB}, 1.50^{ab}	1.50^{AB}, 1.75^{ab}	2.69, 4.34
34076[y]	LA	Race 1	0.75^{BC}, 1.00^{bc}	1.75^{B}, 1.25^{ab}	2.70, 4.71
F17-6BS1[z]	GA	Race 1	1.25^{B}, 1.00^{bc}	1.75^{B}, 1.50^{ab}	2.67, 5.98
F15-5DS1	GA	Race 2	1.75^{AB}, 0.75^{bc}	2.00^{AB}, 1.25^{ab}	2.13, 5.60
F16-12CS1	GA	Race 2	0.50^{C}, 0.75^{bcd}	1.00^{BC}, 0.50^{bc}	2.27, 5.87
F16-12DS1	GA	Race 2	1.50^{AB}, 1.25^{b}	0.75^{C}, 0.75^{bc}	2.82, 5.39
F17-6DS1[z]	GA	Race 2	1.00^{BC}, 1.75^{ab}	1.00^{C}, 2.25^{a}	2.96, 4.68
34049[y]	LA	Race 2	1.25^{ABC}, 1.00^{bc}	1.75^{B}, 0.50^{bc}	2.54, 4.92
34082[y]	LA	Race 3	0.25^{CD}, 0.50^{c}	1.00^{C}, 0.50^{bc}	2.75, 5.19
34083[y]	LA	Race 3	0.75^{BC}, 0.25^{cd}	1.00^{C}, 0.75^{bc}	2.61, 5.47
F15-3CS1	GA	Race 8	0.75^{BC}, 1.25^{b}	1.00^{C}, 1.50^{ab}	2.92, 5.90
F17-6AS1[z]	GA	Race 8	1.50^{AB}, 0.50^{c}	2.50^{A}, 1.75^{ab}	2.29, 5.69
207N-18[x]	AL	Race 8	1.25^{B}, 1.25^{b}	1.00^{BC}, 1.00^{b}	2.27, 6.12
32876[y]	AR	Race 8	1.25^{B}, 0.75^{bc}	1.00^{C}, 1.50^{ab}	2.83, 4.57

[x] Isolate provided by Dr. Jeffrey Coleman, Auburn University

[y] Isolate provided by USDA-ARS NRRL Culture Collection

[z] Early season isolate

Table 3.10. Disease severity ratings from inoculation of FOV isolates onto the cotton cultivar Rowden using a soil-drench assay.

ISOLATE	**ORIGIN**	**GENOTYPE**	**DISCOLORATION**	**WILT**	**ROOT ROT**	**SHOOT MASS**	**ROOT MASS**
			T2, T5	**T2, T5**	**T2, T5**	**T2, T5**	**T2, T5**
F16-2BS1[z]	GA	MDS-12	1.33^{AB}, 1.67^{a}	2.33^{B}, 2.00^{a}	3.00^{A}, 2.00^{A}	3.68, 5.00	3.28, 1.98
F16-6CS1	GA	LA127	1.67^{A}, 1.33^{ab}	2.67^{AB}, 0.67^{bc}	2.33^{B}, 1.67^{AB}	3.99, 6.36	1.90, 1.19
F16-22AS1	GA	LA110	0.67^{BC}, 0.67^{b}	1.67^{CD}, 1.33^{ab}	1.67^{BC}, 1.33^{AB}	4.18, 5.23	5.60, 1.69
F16-8BS1	GA	LA110	1.67^{A}, 0.33^{b}	1.33^{D}, 0.33^{bc}	1.67^{BC}, 1.33^{AB}	1.93, 6.44	1.74, 1.53
F16-8AS1	GA	LA108	1.33^{AB}, 0.67^{b}	2.00^{C}, 0.67^{bc}	2.00^{BC}, 1.00^{B}	3.85, 6.71	4.47, 1.69
F16-10BS1	GA	LA108	1.00^{B}, 1.00^{ab}	2.33^{BC}, 2.00^{a}	1.67^{BC}, 1.67^{AB}	3.22, 5.56	6.30, 2.21
F16-16CS1	GA	Race 1	0.67^{BC}, 0.67^{b}	3.00^{A}, 0.33^{bc}	2.00^{B}, 2.33^{A}	2.60, 5.14	6.16, 1.33
F16-20CS1	GA	Race 1	0.33^{CD}, 0.67^{b}	1.67^{CD}, 0.33^{bc}	2.00^{BC}, 1.67^{AB}	1.77, 5.66	2.95, 2.00
F16-12CS1	GA	Race 2	0.00^{D}, 0.67^{b}	1.33^{D}, 1.00^{b}	2.33^{AB}, 1.67^{AB}	2.47, 5.34	2.94, 2.15
F16-9CS1	GA	Race 8	0.33^{CD}, 0.33^{b}	1.33^{D}, 1.33^{ab}	2.67^{AB}, 2.00^{A}	4.06, 8.07	10.30, 1.57
			T3, T4	**T3, T4**	**T3, T4**	**T3, T4**	**T3, T4**
F16-2BS1[z]	GA	MDS-12	1.67^{A}, 1.67^{ab}	2.33^{B}, 2.00^{a}	2.67^{A}, 3.00^{a}	0.82, 3.01	0.61, 1.49
F16-6CS1	GA	LA127	1.00^{AB}, 1.00^{b}	1.33^{C}, 1.67^{ab}	2.00^{B}, 1.67^{bc}	1.40, 3.45	1.12, 1.67
309N-14[x]	AL	LA127	1.00^{AB}, 0.67^{b}	2.00^{BC}, 2.00^{Aa}	1.33^{C}, 3.00^{a}	1.24, 3.47	1.22, 1.44
2165-145[x]	AL	LA127	1.00^{AB}, 1.00^{b}	2.67^{B}, 2.00^{a}	3.00^{A}, 3.00^{a}	1.26, 3.56	0.99, 1.57
32900[y]	AR	LA127	1.67^{A}, 1.00^{b}	3.00^{AB}, 2.00^{a}	2.67^{A}, 3.00^{a}	0.94, 3.09	1.05, 0.89

34051[y]	AR	LA127	1.33^{AB}, 0.67^{bc}	2.67^{B}, 2.33^{a}	1.67^{B}, 2.00^{b}	0.72, 3.18	0.92, 0.98
F15-1CS1	GA	LA110	0.33^{BC}, 0.33^{bcd}	3.00^{B}, 2.00^{a}	1.67^{BC}, 2.33^{b}	1.36, 2.51	0.80, 1.30
F16-21BS1	GA	LA110	0.67^{B}, 1.00^{abc}	2.00^{BC}, 2.33^{a}	1.67^{B}, 1.67^{bc}	1.10, 2.86	1.76, 1.44
F16-1AS1[z]	GA	LA110	0.67^{B}, 1.00^{bc}	1.33^{C}, 1.00^{b}	2.33^{AB}, 2.67^{ab}	1.41, 4.06	0.80, 1.52
F17-5CS1[z]	GA	LA110	1.33^{AB}, 1.33^{ab}	2.33^{B}, 2.00^{ab}	1.33^{BC}, 2.33^{abc}	1.13, 3.79	0.97, 0.79
F15-4AS1	GA	LA108	0.67^{B}, 1.33^{ab}	2.00^{BC}, 1.67^{ab}	1.67^{BC}, 2.00^{b}	1.43, 3.51	0.78, 1.39
F15-7AS1	GA	LA108	1.00^{AB}, 0.67^{bc}	2.00^{B}, 1.33^{Aab}	2.00^{ABC}, 2.00^{bc}	1.12, 4.00	0.95, 1.56
F16-10DS1	GA	LA108	1.33^{AB}, 2.00^{a}	2.00^{B}, 1.33^{ab}	2.33^{AB}, 2.67^{ab}	1.13, 3.65	1.20, 1.79
F16-7BS1	GA	LA108	1.00^{AB}, 0.33^{bcd}	2.33^{B}, 0.67^{b}	2.67^{A}, 1.67^{bc}	0.93, 2.97	0.78, 1.32
C15044[x]	AL	LA108	1.00^{AB}, 1.00^{b}	1.67^{BC}, 1.67^{ab}	2.00^{B}, 1.67^{bc}	1.75, 3.04	1.37, 1.40
401N-3A[x]	AL	LA108	1.33^{AB}, 1.67^{ab}	1.00^{CD}, 2.00^{ab}	2.00^{B}, 1.33^{c}	1.82, 3.11	1.44, 1.65
F15-6BS1	GA	Race 1	0.67^{B}, 0.00^{cd}	1.33^{C}, 2.00^{ab}	2.00^{B}, 2.33^{ab}	1.36, 3.65	1.00, 1.37
F16-7AS1	GA	Race 1	1.00^{AB}, 1.00^{b}	1.67^{BC}, 1.00^{b}	2.00^{ABC}, 1.67^{bc}	1.17, 3.23	0.86, 1.88
TF2[x]	AL	Race 1	0.33^{BC}, 1.67^{ab}	1.67^{BC}, 1.67^{ab}	2.33^{AB}, 1.67^{bc}	1.55, 3.30	2.10, 1.38
34076[y]	LA	Race 1	0.67^{B}, 1.00^{b}	1.00^{BCD}, 1.00^{ab}	1.67^{BC}, 1.67^{bc}	1.40, 2.84	1.13, 1.20
F17-6BS1[z]	GA	Race 1	1.00^{AB}, 1.33^{ab}	1.33^{C}, 1.67^{ab}	1.67^{BC}, 1.67^{bc}	1.56, 3.41	1.45, 1.35
F15-5DS1	GA	Race 2	1.33^{AB}, 1.00^{b}	2.00^{B}, 1.33^{ab}	1.33^{C}, 1.67^{bc}	1.60, 2.54	1.67, 1.54
F16-12CS1	GA	Race 2	0.67^{B}, 0.33^{bcd}	1.67^{BC}, 1.67^{ab}	1.67, 2.33^{ab}	1.32, 3.23	0.62, 1.29
F16-12DS1	GA	Race 2	1.00^{AB}, 1.33^{b}	2.67^{B}, 1.67^{ab}	2.00^{B}, 1.67^{bc}	0.97, 3.15	1.02, 0.98
F17-6DS1[z]	GA	Race 2	0.67^{B}, 1.67^{ab}	2.33^{BC}, 1.33^{ab}	2.67^{A}, 3.00^{a}	0.80, 2.50	0.75, 1.47

34049[y]	LA	Race 2	1.67^{A}, 1.00^{abc}	1.00^{CD}, 1.33^{ab}	2.33^{AB}, 2.00^{bc}	0.70, 2.64	0.90, 1.20
34082[y]	LA	Race 3	0.33^{BC}, 0.33^{bcd}	2.00^{BC}, 1.00^{ab}	1.67^{ABC}, 2.00^{b}	1.16, 3.79	1.54, 1.67
34083[y]	LA	Race 3	0.67^{B}, 0.00^{cd}	1.67^{BC}, 1.67^{ab}	1.33^{BC}, 1.67^{bc}	1.36, 3.54	1.72, 1.91
F15-3CS1	GA	Race 8	1.00^{AB}, 0.33^{c}	1.67^{BC}, 1.67^{ab}	2.00^{B}, 2.00^{b}	1.28, 2.99	0.63, 1.37
F17-6AS1[z]	GA	Race 8	2.00^{A}, 1.33^{ab}	4.33^{A}, 1.33^{ab}	3.00^{A}, 2.67^{a}	0.46, 2.68	0.60, 0.99
207N-18[x]	AL	Race 8	1.33^{AB}, 1.33^{ab}	3.00^{AB}, 2.00^{a}	1.33^{C}, 1.67^{bc}	1.21, 3.51	2.66, 1.73
32876[y]	AR	Race 8	1.33^{AB}, 1.00^{ab}	3.67^{AB}, 1.67^{ab}	2.33^{AB}, 2.67^{a}	0.97, 2.67	1.30, 1.21

[x] Isolate provided by Dr. Jeffrey Coleman, Auburn University

[y] Isolate provided by USDA-ARS NRRL Culture Collection

[z] Early season isolate

Table 3.11. Virulence profiles determined from isolates' cumulative wilt, root rot, and vascular discoloration ratings in trials 1, 2, and 5.

ISOLATE	**RACE/ GENOTYPE**	**WILT SCORE**	**WILT CATEGORY**	**ROOT ROT SCORE**	**ROOT ROT CATEGORY**	**VASCULAR SCORE**	**VASCULAR CATEGORY**	**TOTAL VIRULENCE**	**VIRULENCE CATEGORY**
F16-2BS1[z]	MDS-12	19.58	High	14.25	High	19.50	High	53.33	High
F16-6CS1	LA127/140	17.92	High	10.66	Moderate	6.58	Low	42.67	Moderate
F16-22AS1	LA110	15.07	Moderate	9.34	Low	12.09	Moderate	36.74	Moderate
F16-8BS1	LA110	15.08	Moderate	9.09	Low	14.09	Moderate	34.92	Moderate
F16-8AS1	LA108	14.08	Moderate	10.08	Moderate	12.33	Moderate	36.25	Moderate
F16-10BS1	LA108	16.32	High	11.08	Moderate	12.25	Moderate	39.65	Moderate
F16-16CS1	Race 1	11.58	Low	8.66	Low	8.67	Low	29.57	Low
F16-20CS1	Race 1	13.08	Moderate	8.50	Low	9.33	Low	30.25	Low
F16-12CS1	Race 2	13.34	Moderate	8.33	Low	10.75	Moderate	27.93	Low
F16-9CS1	Race 8	10.08	Low	11.92	Moderate	6.26	Low	28.58	Low

[z] Early-season isolate

Table 3.12. Virulence profiles determined from isolates' cumulative wilt, root rot, and vascular discoloration ratings in trials 3 and 4.

ISOLATE	RACE/ GENOTYPE	WILT SCORE	WILT CATEGORY	ROOT ROT SCORE	ROOT ROT CATEGORY	VASCULAR SCORE	VASCULAR CATEGORY	TOTAL VIRULENCE	VIRULENCE CATEGORY
F16-2BS1[z]	MDS-12	20.01	High	14.99	High	18.43	High	53.43	High
F16-6CS1	LA 127/140	14.92	Moderate	12.67	High	13.50	Moderate	41.09	High
309N-14[x]	LA 127/140	16.58	Moderate	12.00	High	13.92	Moderate	42.50	High
2165-145[x]	LA 127/140	15.00	Moderate	13.00	High	11.91	Moderate	39.91	High
32900[y]	LA 127/140	15.92	Moderate	12.34	High	11.92	Moderate	40.18	High
34051[y]	LA 127/140	16.66	Moderate	10.33	High	9.42	Low	36.41	Moderate
F15-1CS1	LA 110	17.00	Moderate	11.00	High	8.99	Low	36.99	Moderate
F16-21BS1	LA 110	11.49	Moderate	9.67	Moderate	11.09	Moderate	32.25	Moderate
F16-1AS1[z]	LA 110	11.82	Moderate	11.67	High	9.66	Low	33.15	Moderate
F17-5CS1[z]	LA 110	14.99	Moderate	11.99	High	11.91	Moderate	38.89	High
F15-4AS1	LA 108	13.41	Moderate	9.67	Low	9.66	Moderate	32.74	Low
F15-7AS1	LA 108	13.57	Moderate	11.67	Moderate	10.43	Low	35.67	Moderate
F16-10DS1	LA 108	13.42	Moderate	14.00	High	12.76	Moderate	40.18	High
F16-7BS1	LA 108	14.50	Moderate	9.67	Moderate	5.82	Non-pathogenic	29.99	Moderate
C15044[x]	LA 108	13.26	Moderate	8.33	Moderate	11.41	Moderate	33.00	Moderate

401N-3A[x]	LA 108	12.00	Moderate	8.66	Moderate	14.00	Moderate	34.66	Moderate
F15-6BS1	Race 1	14.67	Moderate	12.34	High	6.08	Non-pathogenic	33.09	Moderate
F16-7AS1	Race 1	11.75	Moderate	9.34	Moderate	10.42	Low	31.51	Moderate
TF2[x]	Race 1	12.58	Moderate	12.67	High	15.49	High	40.74	High
34076[y]	Race 1	10.51	Moderate	7.33	Low	7.75	Low	25.59	Low
F17-6BS1[z]	Race 1	13.42	Moderate	8.67	Moderate	11.67	Moderate	33.76	Moderate
F15-5DS1	Race 2	15.17	Moderate	9.33	Moderate	12.50	Moderate	37.00	Moderate
F16-12CS1	Race 2	11.85	Moderate	9.33	Moderate	8.34	Low	29.52	Moderate
F16-12DS1	Race 2	13.76	Moderate	9.66	Moderate	11.34	Moderate	34.76	Moderate
F17-6DS1[z]	Race 2	15.92	Moderate	13.34	High	13.92	Moderate	43.18	High
34049[y]	Race 2	8.09	Low	9.33	Moderate	11.42	Moderate	28.84	Moderate
34082[y]	Race 3	10.58	Moderate	7.34	Low	3.74	Non-pathogenic	21.66	Low
34083[y]	Race 3	10.84	Moderate	5.99	Low	5.92	Non-pathogenic	22.75	Low
F15-3CS1	Race 8	11.93	Moderate	11.34	High	9.57	Low	32.84	Moderate
F17-6AS1[z]	Race 8	15.08	Moderate	14.34	High	11.25	Moderate	40.67	High
207N-18[x]	Race 8	13.84	Moderate	9.00	Moderate	11.58	Moderate	34.42	Moderate
32876[y]	Race 8	13.92	Moderate	9.67	Moderate	9.74	Low	33.33	Moderate

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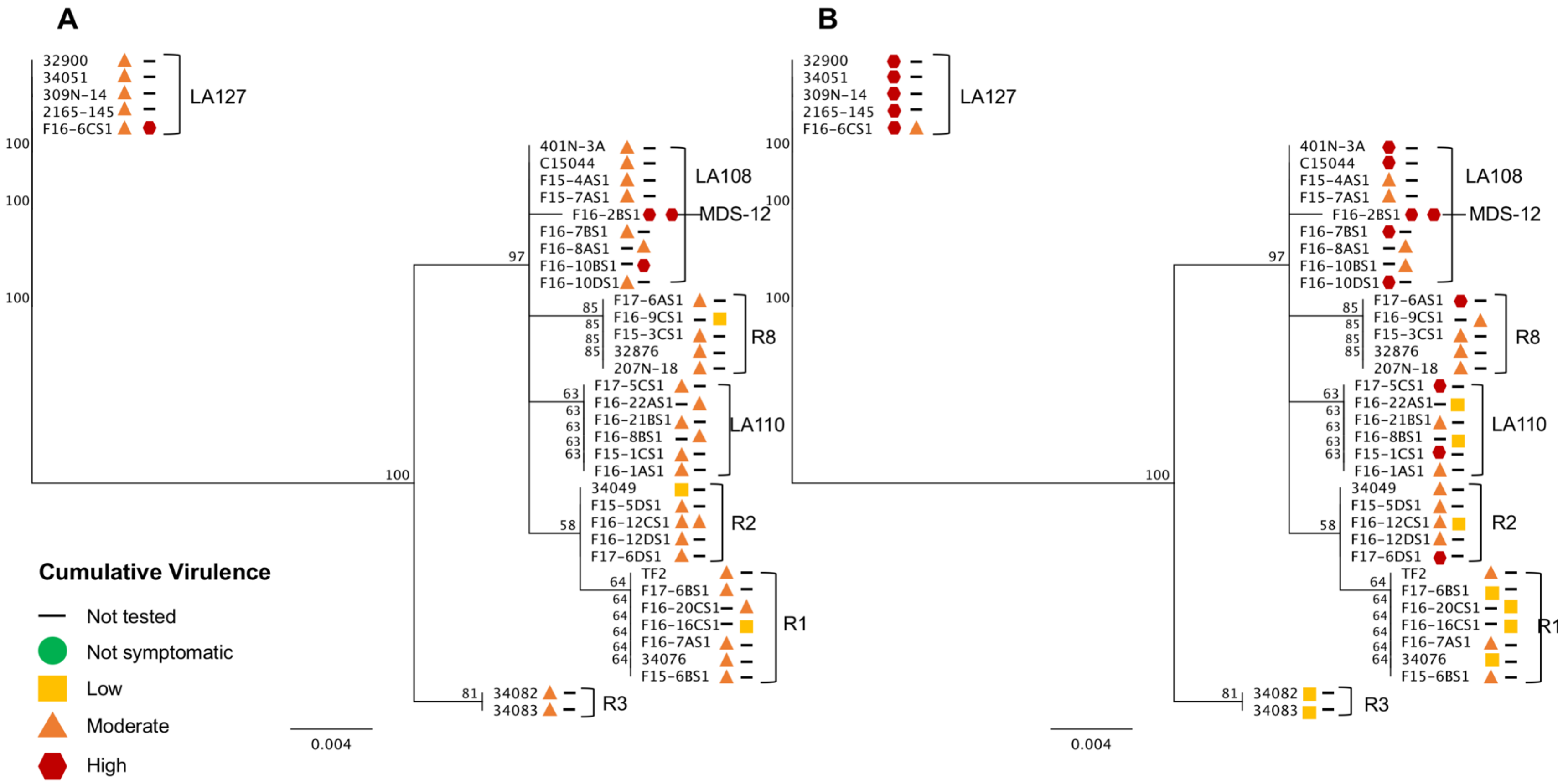
[x] Isolate provided by Dr. Jeffrey Coleman, Auburn University

[y] Isolate provided by USDA-ARS NRRL Culture Collection

[z] Early season isolate


A
32900
34051
309N-14
2165-145
F16-6CS1
LA127
401N-3A
C15044
F15-4AS1
F15-7AS1
F16-2BS1
MDS-12
F16-7BS1
F16-8AS1
F16-10BS1
F16-10DS1
LA108
F17-6AS1
F16-9CS1
F15-3CS1
32876
207N-18
R8
F17-5CS1
F16-22AS1
F16-21BS1
F16-8BS1
F15-1CS1
F16-1AS1
LA110
34049
F15-5DS1
F16-12CS1
F16-12DS1
F17-6DS1
R2
TF2
F17-6BS1
F16-20CS1
F16-16CS1
F16-7AS1
34076
F15-6BS1
R1
34082
34083
R3
100
100
100
97
85
63
58
64
100
81
0.004
B
32900
34051
309N-14
2165-145
F16-6CS1
LA127
401N-3A
C15044
F15-4AS1
F15-7AS1
F16-2BS1
MDS-12
F16-7BS1
F16-8AS1
F16-10BS1
F16-10DS1
LA108
F17-6AS1
F16-9CS1
F15-3CS1
32876
207N-18
R8
F17-5CS1
F16-22AS1
F16-21BS1
F16-8BS1
F15-1CS1
F16-1AS1
LA110
34049
F15-5DS1
F16-12CS1
F16-12DS1
F17-6DS1
R2
TF2
F17-6BS1
F16-20CS1
F16-16CS1
F16-7AS1
34076
F15-6BS1
R1
34082
34083
R3
100
100
100
97
85
63
58
64
100
81
0.004
Cumulative Virulence
Not tested
Not symptomatic
Low
Moderate
High

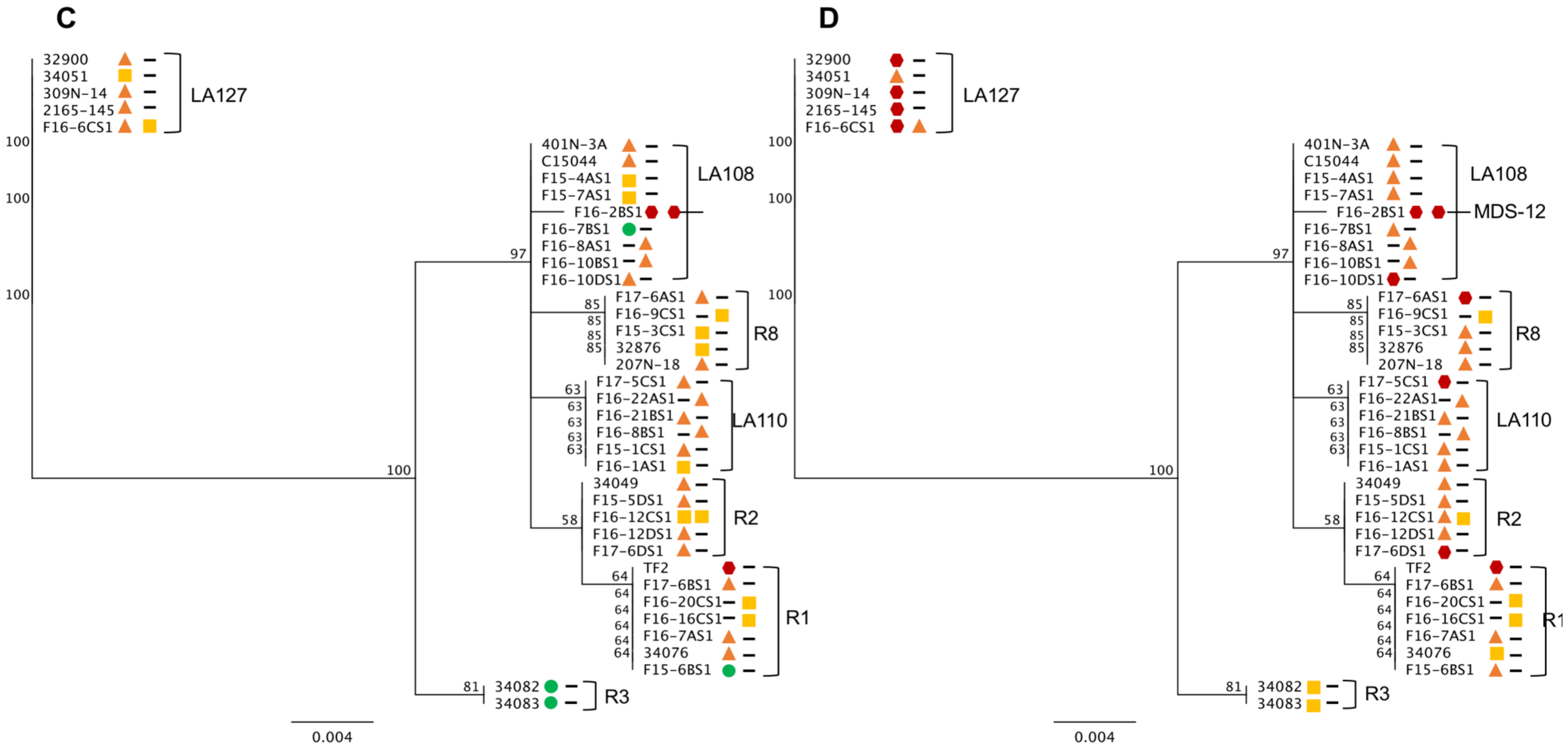
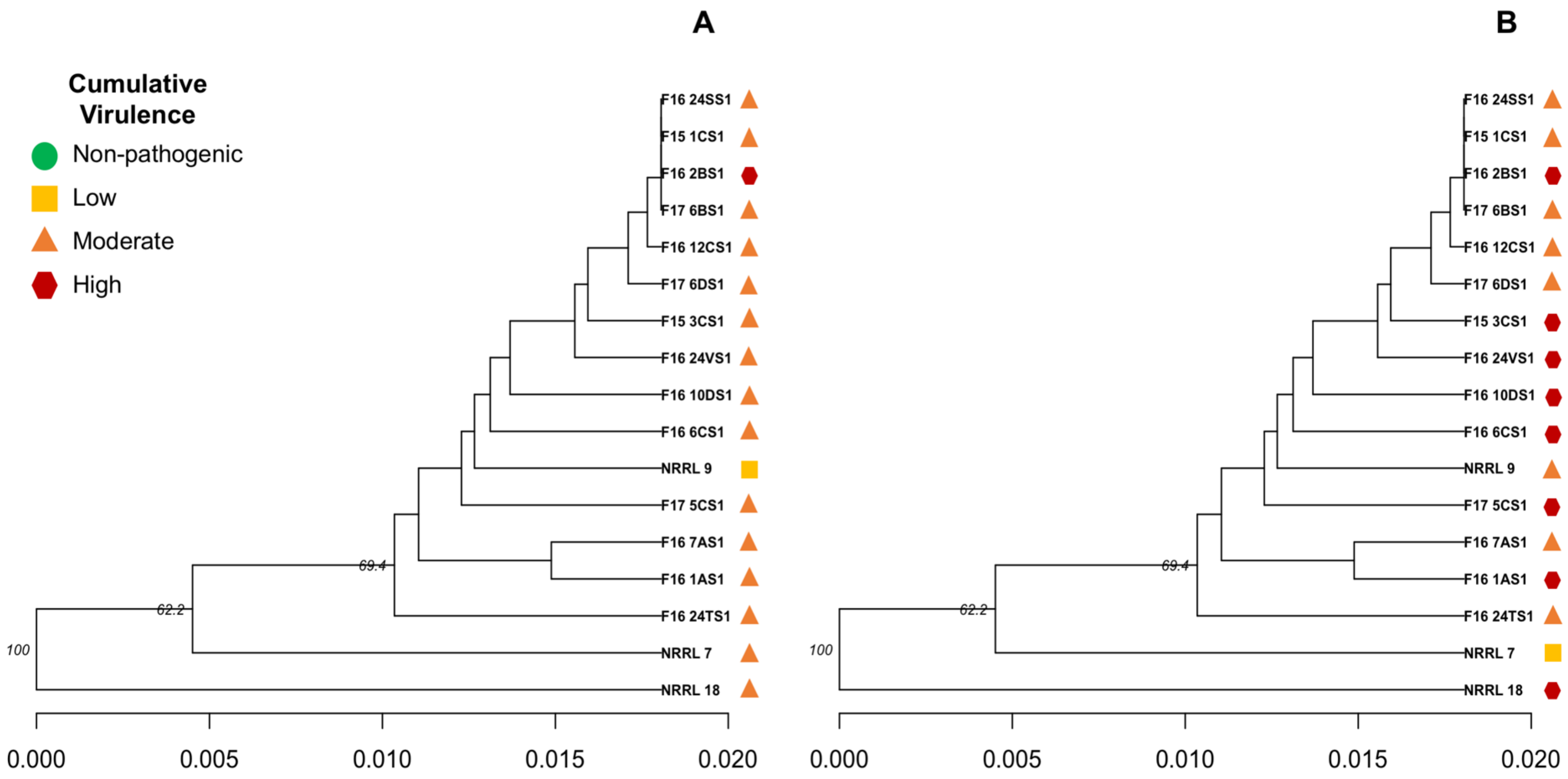


Fig 3.1. Virulence profile overlain onto neighbor-joining trees based on polymorphisms at the *EF-1α* locus, with trials 3 and 4 shown on the left and trials 1, 2, and 5 shown on the right, for the following profiles: **A)** wilt severity, **B)** root rot, **C)** vascular damage, and **D)** total virulence.


A
B
Cumulative Virulence
Non-pathogenic
Low
Moderate
High
F16 24SS1
F15 1CS1
F16 2BS1
F17 6BS1
F16 12CS1
F17 6DS1
F15 3CS1
F16 24VS1
F16 10DS1
F16 6CS1
NRRL 9
F17 5CS1
F16 7AS1
F16 1AS1
F16 24TS1
NRRL 7
NRRL 18
69.4
62.2
100
0.000
0.005
0.010
0.015
0.020

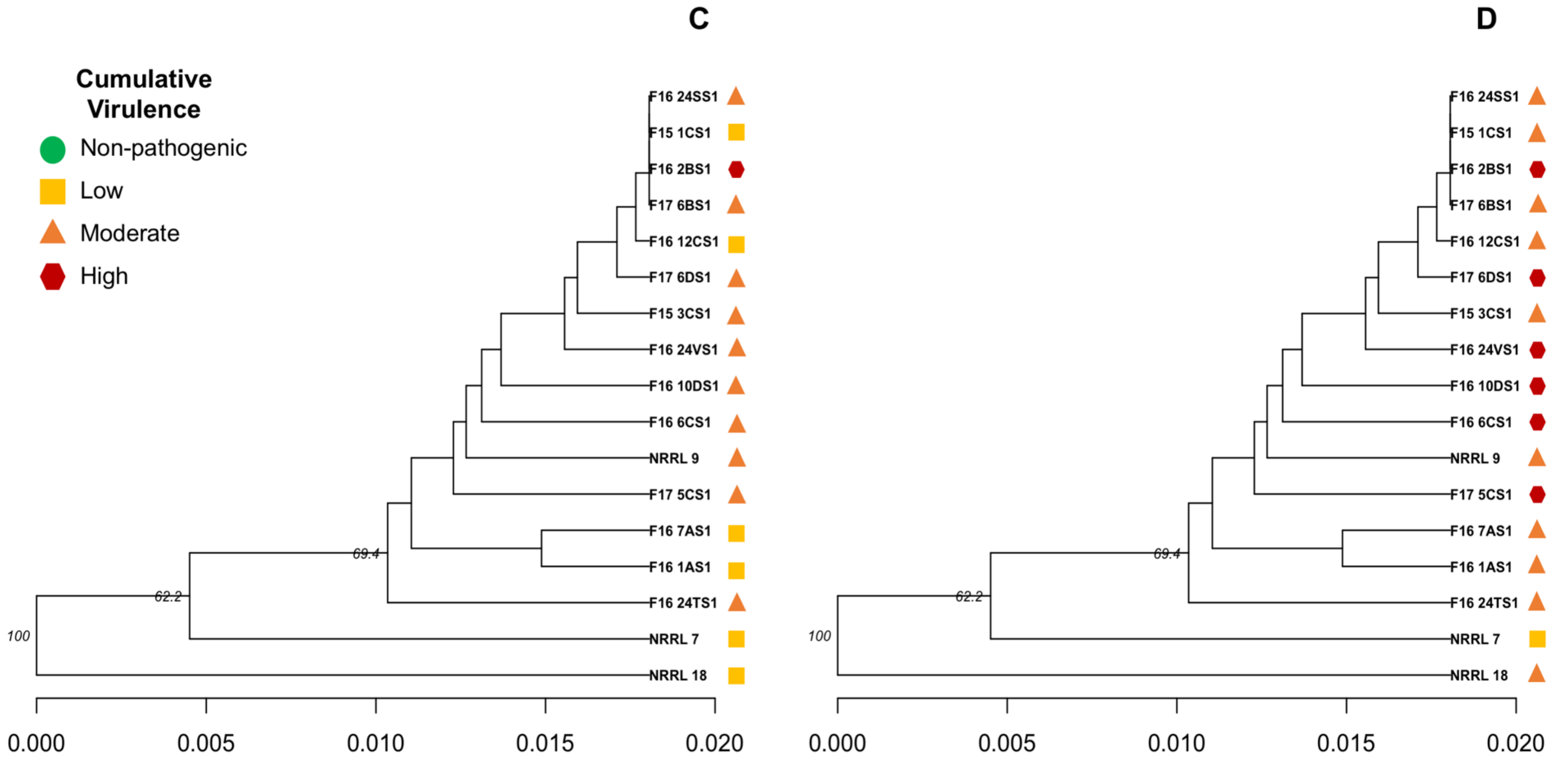


Fig 3.2. Virulence profiles overlain onto UPGMA trees based on bitwise genetic distance calculated from SNP genotypes, for the following profiles: **A)** wilt severity, **B)** root rot, **C)** vascular damage, and **D)** total virulence.

CHAPTER 4

SUMMARY

Populations of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) in the southeastern United States are genetically and pathogenically diverse, but that variation is generally not associated with *EF-1α* race or genotype. Population genetic analyses based on SNPs identified through genotyping-by-sequencing (GBS) showed that southeastern populations of FOV are not genetically structured by *EF-1α* race or genotype, with the exception of race 4 isolates, and that FOV possesses higher levels of genetic diversity throughout the genome than at the *EF-1α* locus. Greenhouse inoculation experiments demonstrated that FOV possesses a wide range of disease phenotypes, and that variation in disease phenotype is generally not associated with *EF-1α* sequence, with the exception of race 3 isolates consistently being weakly aggressive. Disease phenotypes were also not associated with the SNP genotypes identified in objective 2, leaving a knowledge gap in how the genetic diversity of FOV relates to disease phenotype on cotton. This knowledge gap should be further investigated; specifically, identifying the genes directly responsible for virulence on cotton may help to improve disease management through resistance breeding.

APPENDIX A

FIRST REPORT OF FUSARIUM WILT OF COTTON CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* RACE 4 IN TEXAS, U. S. A.[3]

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[3] Halpern, H. C., Bell, A. A., Wagner, T. A., Liu, J., Nichols, R. L., Olvey, J., Woodward, J. E., Sanogo, S., Jones, C.A., Chan, C. T., and Brewer, M. T. 2018. *Plant Dis.* 102(2):446.

Fusarium wilt of cotton (*Gossypium* spp.), caused by the soilborne fungus *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), is a widespread and economically important disease. FOV is genetically diverse with numerous described races and genotypes (Cianchetta et al. 2015), most of which cause disease only in the presence of plant-pathogenic nematodes; however, FOV race 4 is extremely virulent and can cause severe, early-season damage in the absence of nematodes. Race 4 was first described in India (Armstrong and Armstrong, 1960) and has likely spread to other cotton-producing regions through cotton seed. FOV race 4 was first detected in California in 2001 (Kim at el. 2005), and had not been confirmed elsewhere in the U.S.A. (Cianchetta et al. 2015) until recently. In June of 2016 and 2017, severe Fusarium wilt symptoms, including wilting, root rot and stem discoloration, that were consistent with FOV race 4 were observed on seedlings of Pima cotton (*Gossypium barbadense*) in the Upper Rio Grande Valley of Texas in El Paso and Hudspeth Counties. To test for FOV race 4, lower stem segments from symptomatic seedlings collected from three fields (two in El Paso County and one in Hudspeth County) in June 2017 were surface-disinfested, placed onto acidified quarter-strength potato dextrose agar, and incubated at 23°C for 4 days. Galls indicative of the cotton root-knot nematode (*Meloidogyne incognita*) were not observed on seedling roots; however, soil samples were not collected and assayed for nematodes at this time. The colony morphology of fungal isolates that emerged and grew from stem segments was characteristic of *Fusarium oxysporum.* Isolates were single-spored and DNA was extracted in 5% Chelex 100 (Bio-Rad, Hercules, CA). PCR was performed on eight isolates from the three fields ($n = 3, 3, 2$) using primers for the *translation elongation factor* (*EF-1*α) and intergenic spacer (IGS) regions, which together can be used to identify and distinguish described races and genotypes of FOV (Cianchetta et al. 2015). PCR products were sequenced (EuroFins, Louisville, KY) and aligned in Geneious v6.1.3

(Biomatters, Ltd., Newark, NJ) with reference sequences of all known FOV races and genotypes (Cianchetta et al. 2015). All eight isolates from the three fields were identical in nucleotide sequence to FOV race 4 reference isolates ATCC 16613 (Cianchetta et al. 2015) and NRRL 25434 for *EF-1α* (Genbank accession number FJ985279) and IGS (Genbank accession number FJ985475). Additionally, a representative isolate from each of the three fields in Texas contained the *Tfo1* insertion in the *PHO* gene that is considered diagnostic for virulent FOV race 4 isolates found in California (Ortiz et al. 2017). Forty-four isolates from the three fields were tested for virulence on *G. barbadense* 'Pima S-7' using a soil infestation assay (Bell et al. 2017) and found to be highly virulent with 33 of the isolates killing the seedlings and the other 11 causing severe stunting greater than 80%. These results confirm that FOV race 4 is causing wilt in cotton fields in western Texas and is now present in the U.S.A. outside of California.

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2. Bell, A. A., Kemerait, R. C., Ortiz, C. S., Prom, S., Quintana, J., Nichols, R. L., and Liu, J. 2017. Genetic diversity, virulence, and Meloidogyne incognita interactions of Fusarium oxysporum isolates causing cotton wilt in Georgia. Plant Dis. 101:948-956.
3. Cianchetta, A. N., Allen, T. W., Hutmacher, R. B., Kemerait, R. C., Kirkpatrick, T. L., Lawrence, G. W., Lawrence, K. S., Mueller, J. D., Nichols, R. L., Olsen, M. W., Overstreet, C., Woodward, J. E., and Davis, R. M. 2015. Survey of Fusarium oxysporum f. sp. vasinfectum in the United States. J. Cotton Sci. 19:328-336.

4. Kim, Y., Hutmacher, R. B., and Davis, R. M. 2005. Characterization of California isolates of Fusarium oxysporum f. sp. vasinfectum. Plant Dis. 89:366-372.
5. Ortiz, C. S., Bell, A. A., Magill, C. W., and Liu, J. 2017. Specific PCR detection of Fusarium oxysporum f. sp. vasinfectum California Race 4 based on a unique Tfo1 insertion event in the PHO gene. Plant Dis. 101:34-44.