Cultivar Susceptibility to the Fusarium Wilt Complex and Race Characterization of *Fusarium oxysporum* f. sp. *vasinfectum*

by

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Abstract

Fusarium wilt of cotton is a serious fungal disease caused by *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) and highly impacted by root-knot nematode (*Meloidogyne incognita*). Cotton cultivars were tested for resistance to these pathogens and races of FOV were determined. Under high disease pressure in 2010, Phytogen 367 WRF appeared resistant to RKN and FOV. Comparatively, Phytogen 565 WRF was also resistant to FOV but tolerant to RKN supporting high populations of the nematode. In 2011, the lack of rainfall early in the season eliminated disease pressure by RKN and FOV; all varieties tested produced approximately twice as much cotton yield in 2011 than 2010 with timely rainfalls during bloom.

In addition, this study identified that FOV from lineage II, III, and IV exist within Alabama fields based on the partial sequence differences in EF-1α, BT, and PHO genes. These lineages correspond to molecularly recognized races 1, 4, and 8. However, pathogenically the Alabama race 4 isolate did not perform as the hyper-virulent race 4 CA strain that produces a Fusarium wilt which can kill cotton without the presence of nematodes.

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Introduction

Fusarium wilt of cotton (*Gossypium spp.*), caused by *F. oxysporum* Schlechtend.:Fr f. sp. *vasinfectum* (Atk.), is an extensive disease occurring in most cotton growing areas in the world. It was first identified in United States cotton fields in Alabama in 1892 by Atkinson (Armstrong, G. M. and Armstrong, J. K. 1981) and has been found to occur in all four domesticated cotton (Armstrong, G. M. and Armstrong, J. K. 1960; Davis, R. M. *et al*., 2006). Disease losses in cotton due to *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) are highly variable within a region and depend on a number of factors such as environmental temperatures, soils, and added pathogen presence. Over 100 years from its first citing, this disease is still causing important yield losses and remains a threat to future cotton production.

Symptoms

Symptoms of Fusarium wilt can appear at any stage of crop development depending on inoculum density, temperature, and host susceptibility. At high inoculum density or when infection initiates from the seed, plants may be killed at the seedling stage (Hillocks, R. J. 1992). In mildly-affected or older plants, the lower leaves will show symptoms but the plant will survive, although with reduced vigor. From visibility of the disease, the most diagnostic symptoms of Fusarium wilt is the loss of turgor resulting in wilt and a brown discoloration of the vascular tissue. This discoloration is localized in the cortical tissue of the vascular system of the upper tap root and lower stem when cut at a diagonal or in cross-section (Davis, R.M. *et al*., 2006).

Most typically, first symptoms become apparent in the field after one to two months after planting, when onset of flowering begins. In seedlings and young plants, symptoms begin to

appear on the cotyledons and leaves. Vein darkening, chlorosis, and necrosis progressively worsen on the leaves before eventual shed. In pinhead squared (6-8 leaf stage), symptoms are first seen on the lower leaves. Leaf chlorosis begins at the margin and spreads between the main veins to form a yellow interval pattern (Figure 1.1). More leaves become chlorotic as the disease spreads upwards in the plant. Infected plants appear stunted with flaccid leaves giving the plant a wilted appearance, easily visible during mid-day (Davis, R.M. *et al*., 2006). As the infection becomes fully systemic, all the leaves throughout the plant become necrotic as the plant dies from moisture stress. Severely diseased chlorotic plants may be defoliated quickly, leaving green petioles attached to the plant. In older plants, symptoms of Fusarium wilt begin as yellowing at the margins of one or more of the lower leaves (Hillock, R.J. 1992), followed by gradual progression of chlorotic and necrotic leaves that become easily detachable. Provided conditions are conducive to Fusarium wilt, plants which show symptoms within the first two months after planting usually die before setting bolls, while plants that develop symptoms after the onset of flowering often survive to produce bolls (Hillock, R.J. 1992).

The Pathogen

The causal organism of Fusarium wilt is recognized as *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). The species *F. oxysporum* is very variable, containing a large number of saprophytic and pathogenic forms which have morphological features in common. FOV is a soil-invading, weak saprophyte that survives in soil for long durations in the form of three types of spores, microconidia, macroconidia, and chlamydospores (DeVay, J. E. *et al*., 1997). Sporulation of the fungi starts quickly in the aerial mycelium; microconidia are borne on simple phialides, arising from a short conidiophore, and are produced the most within the infected plants. The microconidia are one or two celled, may be cylindrical to ellipsoid, oval, straight to slightly

curved, and 5-20 x 2.2-3.5 µm in size. Macroconidia are borne on conidiphores or in sporodociums, and are falcate in shape, predominately three to five celled, and 27-48 x 2.5-4.5 µm in size. They are usually moderately curved, sub-cylindrical, equally and gradually tapering toward both ends with a pointed apical cell, have a distinct pedicellate basal cell, (Nelson, P. E. e*t al*., 1981), and are commonly found on the surface of plants that have been infected by Fusarium wilt. Chlamydospores are the third type of spore and are readily formed in old cultures and in senescent host tissues. They are generally abundant in hyphae and conidia, terminal, smooth or rough walled, globose to sub-globose, one celled (7-11 µm), in pairs, short chains, or possibly found in clusters. They can remain dormant in the soil but germinate more frequently and grow vigorously in soils known to be wilt conducive compared to soils suppressive to Fusarium wilt (Smith, S. N. and Snyder, W. C. 1972). The main morphological features which distinguish *F. oxysporum* from most other *Fusaria* are the short micro conidiophore, together with the formation of chlamydospores (Hillock, R.J. 1992).

Infection Cycle

The pathogen is introduced into previously disease-free soil through the planting of infected seed or is carried in from adjoining infested areas on farm implements. Infection of the plant then arises directly from the seed or from chlamydospores in the soil. The spores germinate when nutrients from a nearby root exudes, promoting growth of the fungus. The germ tube grows towards the cotton root until it makes contact with the plant root system, where it enters through a wound in the epidermis or by direct penetration.

The fungus grows through the cortex to the vascular tissue and sporulates only when it has invaded the xylem. Conidia are then carried upwards in the transpiration stream and are

carried passively as far as the first vessel wall where they become trapped and must then germinate and grow through into the next vessel (Hillocks, R.J. 1992). Systemic spread within the plant occurs by spore transport; mycelia growth occurs in the vessels and later in the surrounding cortex. As the plant becomes fully infected, wilting and aging occur as a result of water stress induced by the combined effect of mycelia growth in the xylem, fungal metabolites, and vascular obstruction by the host, in an attempt to prevent total spread of the fungus (Skovgaard, K. *et al*., 2001). As the plant dies, the pathogen produces chlamydospores which return to the soil in falling leaf litter and crop residues, and the disease cycle is completed. The succeeding plant can be affected as early as the seedling, but mostly occurs during the reproductive stage.

Environmental Factors

Fusarium wilt is known for being a warm temperature disease. Optimum temperature for spore germination and growth through soil is 25°C (Nelson, P. E.*et al*., 1981; Nelson, P. *et al*., 1990), with *F. oxysporum* being able to grow from 10 to 32°C, with severe inhibition above or below these temperatures (Nelson, P.E. *et a*l., 1981; Larkin, R.P. and Fravel, D.R. 2002). Cotton plants may recover from the disease and re-grow if the temperature falls below the optimum for disease development after initial infection. Spore production and germination for FOV are greatest at 85% saturation according to Tharp and Young (1939), while according to El-Abyad and Saleh (1971), mycelia growth in soil is greatest when soils are at 40% moisture-holding capacity but retarded at increased moisture levels.

Individual species of *Fusarium* are limited to specific soil, climate, and biotic conditions. Typically, Fusarium wilt of cotton is most damaging in acid, sandy soils (Bell, A.A. *et al*., 2003)

with a pH of 5.0-6.5, and generally less of a problem in heavier clay soils (Larkin, R. P. *et al*., 1993). Natural suppression of Fusarium wilt disease has been known to occur in many soils (Larkin, R. P. *et al*., 1993). This suppression is generally biological in nature, but is also commonly associated with certain physical and/or chemical characteristics such as relatively high pH, clay, and organic matter content (Larkin, R.P. and Fravel, D.R. 2002). Smith and Snyder (1972) stated that red lateritic clay soils are usually suppressive to Fusarium wilt. Though, once a field is infested with FOV the fungus usually sustains itself continuously within the field (Smith, S. N. and Snyder, W. C. 1975).

Ecology

The *Fusarium* species was placed phylogenetically in the section *Elegans* along with nine other species in three subsections because of the variation in their asexual reproductive structures recognized by Wollenweber and Reinking (Wang, B. *et al*., 2007). The section *Elegan*, according to Snyder and Hansen, is highly variable and subject to environmental influence, and therefore was collapsed into the single species, *Fusarium oxysporum*. However, these authors also recognized true variants within this species. Although many or most isolates of *F. oxysporum* may be nonpathogenic soil inhabitants (Alves-Santos, F. M. *et al*., 1999), the concept of form or specialized form (forma specialis) arose to distinguish the ability of their members to cause a wilt disease on different plants.

Fusarium oxysporum has the ability to cause two types of plant disease, cortical rots and vascular wilts. In wilts, once infection has occurred spread of the fungus is systemic in the vascular tract and is faster in relation to plant growth than in cortical lesions. In cortical rots the extent of infection is proportional to the numbers of individual lesions pressed by the numerous

propagules as well as subsequent lesion growth (Toussoun, T.A. and Nelson, P.E. 1968). Despite the wide host range of the species as a whole, individual isolates usually cause disease only on a slim range of plant species. Pathogenic strains within the species have a limited host range. Strains with similar or identical hosts are assigned to intraspecific groups or forma specialis (Armstrong, G. M. and Armstrong, J. K. 1981); more than 75 forma specialis have been described (Smith, S. N. and Snyder, W.C. 1975; Zhou, X.G. and Everts, K.L. 2006). Some of the forma specialis are further divided into subgroups, named races, based on pathogenicity to a set of differential cultivars within the same plant species (Armstrong, G. M. and Armstrong, J. K. 1981; Namiki, F. *et al*., 1994).The cotton vascular wilt pathogen is referred to as forma specialis *vasinfectum*.

Race Characterization

Fusarium oxysporum races have been identified inconsistently (Kistler, H.C. 1997). Some occurrences define races as being strains compatible to cultivar-level specificity of the host genotype (Kistler, H.C. 1997). This definition of race is frequent in plant pathology and is consistent with cultivar selectivity, which is often determined by single genes in the host. However, in other instances race has been defined by the selectivity of isolates to distinct plant species (Armstrong, G. M. and Armstrong, J. K. 1981). As a result race designation was reserved for isolates with specificity to particular genotypes within a plant species. Eight races have been identified within *F. oxysporum* f. sp. *vasinfectum* based on particular genotypes pathogenicity on a number of *Gossypium spp.* (O'Donnell, K. *et al*., 1998; Hillocks, R.J. 1992), okra, alfalfa, and two cultivars of tobacco.

The first races of *F. oxysporum* f. sp. *vasinfectum* were described in the United States and Tanzania in 1958 (Assigbetse, K.B. *et al*., 1994). Distinctions were made between isolates capable of causing wilt symptoms in soybean and tobacco, race 2, and isolates that could not, race 1 (Armstrong, J. K. and Armstrong, G. M. 1958; Hyer, A.H. *et a*l., 1979). Race 6 from Brazil, caused wilt of *G. hirsutum* and okra, like races 1 and 2, but did not wilt alfalfa, soybean, tobacco, or lupine (*Lupinus luteus* L.), which are susceptible to race 1 and/or 2. Race 6 could not be distinguished from races 1 or 2 in pathogenicity tests on cotton cultivars and was therefore clustered into a single race containing races 1, 2, and 6 and was called the American race (Armstrong, G. M. and Armstrong, J. K. 1978). Race 3 was described in Egypt, Sudan, and Israel. Race 5 was described in Sudan but was eventually renounced according to Snyder and Hansen, because of cultural variability. Pathogenicity tests found that race 3 and 5 were identical (O'Donnell, K. *et al*., 1998). Race 4 is thought to be the most virulent strain of FOV and was described in India. Races 7 and 8 were confirmed in China based on the reactions of cotton, tobacco, alfalfa, okra, and soybean (Davis, R. D. *et al*., 1996). Isolates of race 7 were genetically indistinguishable from the race 4 isolates from India. However, isolates of races 4 and 7 can be separated by their pathogenicity toward cotton and non-cotton hosts (Smith, S. N. and Snyder, W.C. 1975).The latest described populations of FOV are two closely related biotypes discovered in Australian cotton crops in 1993 (Davis, R. M. *et al*., 2006). In Kim *et al*. (2005) isolates of *F. oxysporum* f. sp. *vasinfectum* were analyzed as polyphyletic with at least two independent evolutionary origins, race 3 and the Australian biotypes. All the rest were grouped to come from the same beginning; lineage I contained race 3, an independent clade, lineage II contained races 1, 2, and 6, lineage III contained race 8, lineage IV contained race 4, and lineage V included the Australian biotypes representing an independent origin (Table 1.1). The term race as used for *F.*

oxysporum f. sp. *vasinfectum* had been deemed invalid at this point, and no race description was made for these strains (Davis, R. D. *et al*., 1996; 2006). Knowledge about these population genetics of FOV in Australia is limited. Previous studies showed that the Australian isolates behaved similarly to race 6 on differential hosts (Davis, R.D. *et al*. 1996). More recent work demonstrated that FOV in Australia has arisen indigenously, as it is genetically related to a lineage of indigenous *F. oxysporum* found in both the rhizosphere soil of wild native cottons (*Gossypium* spp.) and uncultivated soil from cotton growing regions (Wang, B. *et al*., 2006). However, little is known about the degree and distribution of genetic variation within and among populations of FOV in Australia.

By the 1990s, DNA-based techniques were employed in conjunction with pathogenicity tests to distinguish the various races of *F. oxysporum* f. sp. *vasinfectum*. In 1993, vegetative compatibility groups (VCG- hyphae of two stains that fuse with each other) among races 1, 2, 3, and 4 were assessed (Bridge *et al*., 1993). Races 1 and 2 belonged to the same VCG and race 3 belonged to another. Within group A (races 1, 2, and 6 collectively), there were eight VCGs (0111, 0112, and 0115-01110). Races 3 and 4 were placed into VCGs 0113 and 0114, respectively. In this designation, the first three digits identify the forma specialis (011 for *F. oxysporum* f. sp. *vasinfectum*) and the last digit or digits identify the subgroup. VCGs of Australian strains were regarded unique in this process. They belong to two VCGs (01111 and 01112) that are both vegetatively incompatible with other forms of FOV found elsewhere in the world. In initial studies, representatives of the two VCGs were shown to be genetically distinct using DNA amplification fingerprints, but no further variation was detected within each VCG (Bentley *et al.*, 2000; Wang, C. *et al*., 2006).

Interactions with Root-knot Nematode

In the first report of Fusarium wilt of cotton, Atkinson (1892) observed a connection between Fusarium wilt and nematodes. These two soil borne pathogens usually infect cotton roots simultaneously, forming the Fusarium wilt complex that increases disease incidence (Gilbert, C. A. *et al*., 2008) and severity (Nelson, P. E. *et al*., 1981). The root-knot nematode (RKN), *Meloidogyne incognita* (Kofoid & White) Chitwood, is the most common of the nematodes that affect cotton roots. It is a sedentary endoparasite that retards growth and development of cotton, *Gossypium sp*., by attacking the root system, causing galling, stunting, and other adverse effects that occurs on cotton roots in all lighter soils of the Cotton Belt. Root infections by *M. incognita* increase the colonization of cotton plants by *F. oxysporum* f. sp. *vasinfectum* and their susceptibility to Fusarium wilt (Garber, R. H. *et al*., 1979). Though wilt is increased by the occurrence of root-knot, either disorder could occur in the absence of the other (Nelson, P. E. *et al*., 1981). Infections of other nematodes implicated in increasing the severity of wilt of *G. hirsutum* are *Rotylenchulus reniformis* (Neal, D.C. 1954), *Pratylenchus brachyurus* (Garber, R.H. *et al*., 1979) *Belonolaimus gracilis, B. longicaudatus* (Minton, N. A. and Minton, E. B. 1966), and *Hoplolaimus seinhorsti* (Rajaram, B. 1979).

The immature second stage juvenile (J2) of RKN infest cotton plants by invasion through the root tips. After entering the tip, the J2 push their way between the cells and feed by puncturing all the cell walls within reach with their spear-like stylet. The infected cells grow large, usually 6-10µm, forming a nutrient sink of knots or galls that are likely to decay and leave the ends of vascular bundles exposed to the wilt fungus (Figure 1.2). While the most diagnostic root-knot nematode damage occurs below ground, numerous symptoms can also be observed above ground. These above ground symptoms usually appear in patches or clusters of plants. Severely RKN affected plants will often wilt readily. Since galled roots have only limited ability

to absorb and transport water and nutrients to the rest of the plant, infected plants may wilt even in the presence of sufficient soil moisture, especially during the afternoon. Nematodes move through the soil and facilitate infestations that will gradually radiate outward from an initial point of infection. It is not yet clear in what manner nematodes increase the severity of Fusarium wilt, moreover this complex requires further investigation for clarification.

Disease Management

Once established in the soil, the fungus is almost impossible to eradicate (Hillocks, R.J. and Kiban, T.H. 2002). The chlamydospores remain viable in the soil for several years and the pathogen is able to multiply on the roots of many weed and crop species (Smith, S. N. and Snyder, W.C. 1975). Therefore, management of Fusarium wilt in cotton is difficult. An IPM approach specifically for the control of Fusarium wilt on cotton has not been described. Some advances have been made in the managing of Fusarium wilt with host resistance (Davis, R. M. *et al*., 2006); however, there are no immune cultivars.

Because of the close association with the root-knot nematode, some success in management of wilt has been obtained by focusing management strategies on the nematode. Since the root-knot nematode can increase the incidence of Fusarium wilt in both resistant and susceptible cultivars, planting cultivars with resistance to the nematode can reduce disease severity (Egel, D.S. and Martyn, R.D. 2007). Unfortunately, the development of cultivars with high levels of resistance and agronomically favorable traits has been poor (Starr, J. L. *et al*., 1989; Smith, S. N. and Snyder, W.C. 1972; Davis, R. D. *et al*., 1996). However variability for wilt resistance occurs in both Upland and *G. barbadense* cottons, with resistance more complete

in *G. barbadense* cultivars; only moderate resistance to the root-knot nematode is available in a few commercial cultivars (Koenning, S. R. 2001).

Crop rotation is often recommended as a management strategy (Smith, S.N. *et al.*, 2001). However, rotation to any crop other than cotton prevents an increase in the soil population of *Fusarium*, but may not reduce the number of spores in the soil. *Graminaceous* species have been considered non-susceptible to Fusarium wilt, and yet several of them are able to sustain high populations of the fungus on their roots (Hillocks, R.J. 1992). In California, populations of the pathogen in field soils did not decline during the five years when the field was planted to barley and wheat, while populations appeared greater after a crop of barley than in fields cropped continuously with cotton (Smith, S. N. and Snyder, W.C. 1975). Since root-knot nematodes increase the incidence of wilt, crop rotations to reduce nematode populations may prove to be helpful in reducing wilt. Conversely, since the fungus is likely to sustain itself on the roots of most plants, including weeds (without causing any symptoms), it cannot be eliminated by crop rotation alone.

Successful chemical management strategies have not been proven. *Fusarium* soil populations have been shown to be decreased by soil fumigation, using a mixture of chloropicrin and methyl bromide (Jorgenson, E.C. *et al*., 1978). However this type of treatment is usually confined to high-value crops grown on relatively small areas. In fields where Fusarium wilt is associated with root-knot nematodes, fumigants that reduce root-knot nematode populations like nematicides have been shown to reduce the incidence and severity of Fusarium wilt (Hyer *et al*., 1979; Jorgenson, *et al*., 1978). If nematode populations are sufficiently high and the crop potential is high-yielding, nematicide application may give an economic return (Jorgenson, *et al*., 1978). Some reports of systemic fungicides for seed treatments have been reported to eliminate

the pathogen from seed (Allen, S.J. and Kochman, J.K. 2001; Hillocks, R.J. 1992) and might be effective in limiting the spread of the pathogen on planting seed; however control of Fusarium wilt in cotton fields already infested with FOV still become susceptible to infection.

Increases in soil temperature which occur under polyethylene sheeting have been shown to decrease the population of *Fusarium* spp. (Katan *et al*., 1983). Soil solarization under clear plastic for a minimum of 5-6 weeks decreased the incidence of wilt and increased cotton yields in Israel. The beneficial effect was apparent over three seasons (Hillocks, R.J.1992). Ben-Yephet *et al*., (1987) also observed soil solarization reducing the survival of the pathogen in the soil and reducing the incidence of wilt. Although solarization appears to be effective, it is not economically feasible for large-scale cotton production. In addition to the costs associated with implementing solarization, treated fields are out of production for 1 year during the solarization.

Other containment options include tillage, restricting traffic in affected patches, especially when the soil is wet, destroying affected plants and surrounding non-symptomatic plants, and stopping irrigation of affected patches in order to prevent movement of infested soil. Fallowing is not an effective strategy unless weeds are controlled, since many weeds are also hosts of the root-knot nematode (Davidson, T. R. and Townshend, J. L. 1967). Tillage practices impact both fungal and nematode pathogens in the soil (Minton, N. A. 1986; Sumner *et al.*, 1981). Reduced tillage procedures may reduce the spread of the pathogens within a field (Minton N. A. 1986). Because of the ability of the fungus to survive in the soil for extended periods of time, tillage is expected to have little impact.

Objective

Since 1990, Fusarium wilt has progressively increased in importance, with novel races of the fungal pathogen emerging. California has recently identified race 4 as inhibiting cotton in fields. Race 4 is considered hyper-virulent and produces a Fusarium wilt which can kill cotton without the presence of nematodes. Recently, in the southern region, Kim *et al*. (2005) discovered races 3 and 8 for the first time in the U.S. outside of California. This group also reported four new novel genotypes of the fungus. The Fusarium wilt fields located on the Plant Breeding Unit of the E. V. Smith Research Center have been utilized nationally to screen cotton breeding lines for Fusarium wilt disease susceptibility for more than half a century. Both pathogens have coexisted in this location and populations of the pathogens have been monitored. However, it is not known what races of FOV exist in our Alabama cotton field; nor do we know the difference of disease susceptibility to cotton cultivars grown in the southern region. Thus, the objectives of this project are to 1) Examine cotton cultivars susceptibility to FOV; 2) Determine cotton cultivar susceptibility to RKN; 3) Conclude yield potential for the cotton cultivars when challenged with RKN and FOV; 4) Identify the FOV race or races present in the E.V. Smith Research Center and; 5) Identify FOV races found on the common commercial cotton cultivars grown in Alabama.

Literature Cited

Allen, S.J., and Kochman, J.K. 2001. Eliminating seed-borne inoculum of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton. Proc. Beltwide Cotton Conf., 1:139-140. Anaheim, CA 9-13 Jan. 2001. National Cotton Council Memphis, TN.

Alves-Santos, F. M., Benito, E. P., Eslava, A. P., and Diaz-Minguez, J. M. 1999. Genetic diversity of *Fusarium oxysporum* strains from common bean fields in Spain. Applied and Environmental Microbiology 65:3335-3340.

Armstrong, J. K., and Armstrong, G. M. 1958. A race of the cotton-wilt Fusarium causing wilt of Yelredo soybean and flue-cured tobacco. Plant Disease Report 42:147-151.

Armstrong, G. M., and Armstrong, J. K. 1960. American, Egyptian, and Indian cotton-wilt Fusaria: Their pathogenicity and relationship to other wilt fusaria. U.S. Department of Agriculture Technology Bulletin 1219

Armstrong, G. M., and Armstrong, J. K. 1978. A new race (race 6) of the cotton-wilt Fusarium from Brazil. Plant Disease Report 2:421-423.

Armstrong, G. M., and Armstrong, J. K. 1981. Formae speciales and races of Fusarium oxysporum causing wilt diseases. In Fusarium: disease, biology, and taxonomy (P. E. Nelson, T. A. Toussoun & R. J. Cook, eds): 391–399. Pennsylvania State University Press, University Park, PN.

Assigbetse, K. B., Fernandez, D., Dubois, M. P., and Geiger, J. P. 1994. Differentiation of *Fusarium oxysporum* f. sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. Phytopathology 84:622-626.

Atkinson, G.F. 1892. Some diseases of cotton. Alabama Polytechnical Institute. Agriculture Experimental Station Bulletin No. 41: 61-65

Bell, A. A., Wheeler, M. H., Liu, J., Stipanovic, R. D., Puckhaber, L. S., and Orta, H. 2003. United States Department of Agriculture—Agricultural Research Service studies on polyketide toxins of *Fusarium oxysporum* f. sp. *vasinfectum*: potential targets for disease control. Pest Management Science, 59: 736–747. doi: 10.1002/ps.713

Ben-Yephet, Y., Stapleton, J. J., Wakeman, R. J., and DeVay, J. E. 1987. Comparative effects of soil solarization with single and double layers of polyethylene film on survival of *Fusarium oxysporum* f. sp. *vasinfectum*. Phytoparasitica 15:181-185.

Bentley, S., Kochman, J. K., Moore, N. Y., Pattemore, J.A., Gulino, L. and O'Neill, W.T. 2000. DNA diagnostics for Fusarium wilt of cotton. In Proceedings of the 10th Australian Cotton Conference, pp. 455–461.

Bridge, P. D., Ismail, M. A., and Rutherford, M. A. 1993. An assessment of aesculin hydrolysis, vegetative compatibility and DNA polymorphism as criteria for characterizing pathogenic races within *Fusarium oxysporum* f. sp. *vasinfectum*. Plant Pathology. 42:264-269

Davidson, T. R., and Townshend, J. L. 1967. Some weed hosts of the southern root-knot nematode, *Meloidogyne incognita*. Nematologica 35:452-458.

Davis, R. D., Moore, N. Y., and Kochman, J. K. 1996. Characterisation of a population of *Fusarium oxysporum* f. sp. *vasinfectum* causing wilt of cotton in Australia. Australian Journal of Agricultural Research. 47:1143-1156.

Davis, R. M., P. D. Colyer, C. S. Rothrock, and Kochman, J. K. 2006. Fusarium Wilt of Cotton: Population Diversity and Implications for Management. Plant Disease 90: 692-703.

DeVay, J. E., Gutierrez, A. P., Pullman, G. S., Wakeman, R. J., Garber, R. H., Jeffers, D. P., Smith, S. N., Goodell, P. B., and Roberts, P. A.1997. Inoculum densities of *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* in relation to the development of Fusarium wilt and the phenology of cotton plants (Gossypium hirsutum). Phytopathology 87:341-346.

Egel, D. S., and Martyn, R. D. 2007. Fusarium wilt of watermelon and other cucurbits. The Plant Health Instructor. DOI: 10.1094/PHI-I-2007-0122-01

Garber, R. H., Jorgenson, E. C., Smith, S. N., and Hyer, A. H. 1979. Interaction of population levels of *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* on cotton. Journal of Nematology 11:133-137.

Gilbert, C. A., Zhang, N., Hutmacher, R. B., Davis, R. M., and Smart, C. D. 2008. Development of a DNA-based macroarray for the detection and identification of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton tissue. Journal of Cotton Science 12:165-170.

Hillocks, R. J. 1992. Fusarium wilt. In: Hillocks RJ, ed. Cotton Diseases. Wallingford, UK: CAB International, 127–60.

Hillocks, R. J., and Kibani, T.H. 2002. Factors affecting the distribution, incidence and spread of Fusarium wilt of cotton in Tanzania. Experimental Agriculture 38, 13–27.

Hyer, A.H., Jorgenson, E.C., Garber, R.H., and Smith, S. 1979. Resistance to root-knot nematode in control of root-knot nematode Fusarium wilt disease complex in cotton *Gossypium hirsutum*. Crop Science. 19:898-901.

Jorgenson, E. C., Hyer, A. H., Garber, R. H., and Smith, S. N. 1978. The influence of soil fumigation on the Fusarium root-knot nematode complex of cotton in California. Journal of Nematology. 10:228-231.

Katan, J., Fisher, G., and Grinstein, A. 1983. Short and long-term effects of soil solarization and crop sequence on Fusarium wilt and yield of cotton in Israel. Phytopathology 73, 1215-19.

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

Kistler, H. C. 1997. Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. Phytopathology 87:474-479.

Koenning, S. R., Barker, H. R., and Bowman, D. T. 2001. Resistance as a tactic for management of *Meloidogyne incognita* on cotton in North Carolina. Journal of Nematology. 33:126-131.

Larkin, R. P., Hopkins, D. L., and Martin, F. N. 1993. Ecology of *Fusarium oxysporum* f. sp. *niveum* in soils suppressive and conducive to Fusarium wilt of watermelon. Phytopathology 83:1105-1116.

Larkin, R.P., and Fravel, D. R. 2002. Effects of varying environmental conditions on biological control of Fusarium wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 92: 1160-1166.

Morrica, S., Kasuga, T., Michelson, K., and Raggazi, A. 1995. *Fusarium oxysporum* f. sp. *vasinfectum* 5.8S rRNA gene and its adjacent ITS1 and ITS2 regions. Fungal Genetics Newsletter. 42:53-55.

Minton, N. A., and Minton, E. B. 1966. Effect of root knot and sting nematodes on expression of Fusarium wilt of cotton in three soils. Phytopathology 56:319–322.

Minton, N. A. 1986. Impact of conservation tillage on nematode populations. Journal of Nematology. 18:135-140.

Namiki, F., Shiomi, T., Kayamura, T., and Tsuge, T. 1994. Characterization of the formae speciales of Fusarium oxysporum causing wilt of cucurbits by DNA fingerprinting with nuclear repetitive DNA sequences. Applied and Environmental Microbiology. 60, 2684–2691.

Neal, D. C. 1954. The reniform nematode and its relationship to the incidence of Fusarium wilt of cotton at Baton Rouge, Louisiana. Phytopathology 44(8) :447-450.

Nelson, P. E., Toussoun, T. A., and Cook, R. J. 1981. Fusarium: Disease, Biology, and Taxonomy. The Pennsylvania State University Press, University Park.

Nelson, P., Burgess, L. W., and Summerall, B. A. 1990. Some morphological and physiological characters of Fusarium species in sections Liseola and Elegans and similar species. Mycologia 82:99-106.

O'Donnell, K., Kistler, H. C., Cigelnik, E., and Ploetz, R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences of the USA 95:2044-2049

Rajaram, B. 1979. Biochemical association between infection of *Hoplolaimus seinhorsti* Luc, 1958 and the development of *Fusarium oxysporum* f. sp. *vasinfectum* (Atk.) Synder & Hansen on cotton. Indian Zoologist 3:135-138.

Skovgaard K., Nirenberg H.I., O'Donnell K., and Rosendahl, S. 2001. Evolution of *Fusarium oxysporum* f.sp. *vasinfectum* races inferred from multigene genealogies. Phytopathology 91, 1231–7.

Smith, S. N., and Snyder, W.C. 1972. Germination of *Fusarium oxysporum* chlamydospores in soils favorable and unfavorable to wilt establishment. Phytopathology 62:273-277.

Smith, S. N., and Snyder, W. C. 1975. Persistence of *Fusarium oxysporum* f. sp. *vasinfectum* in fields in the absence of cotton. Phytopathology 65:190-196

Smith, S. N., DeVay, J. E., Hsieh, W., and Lee, H. 2001. Soil-borne populations of *Fusarium oxysporum* f. sp. *vasinfectum*, a cotton wilt fungus in California fields. Mycologia 93:737-743.

Starr, J. L., Jeger, M. J., Martyn, R. D., and Schilling, K. 1989. Effects of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *vasinfectum* on plant mortality and yield of cotton. Phytopathology 79:640-646.

Sumner, D. R., Doupnik, B., Jr., and Boosalis, M. G. 1981. Effects of reduced tillage and multiple cropping on plant diseases. Annual Review Phytopathology. 19:167-187.

Tharp, W.H. and Young, V.H. 1939. Relation of soil moisture to Fusarium wilt of cotton. Journal of Agricultural Research 58, 47-61.

Toussoun, T.A., and Nelson, P.E. 1968. A Pictorial Guide to the Identification of Fusarium Species. Pennsylvania State University Press, University Park.

Wang, B., Brubaker, C. L., Tate, W., Woods, M. J., Matheson, B. A., and Burdon, J. J. 2006. Genetic variation and population structure of Fusarium oxysporum f. sp. vasinfectum in Australia. Plant Pathology. 55:746- 755.

Wang, B., Brubaker, C. L., Tate, W., Woods, M. J., and Burdon, J. J. 2007. Evolution of virulence in *Fusarium oxysporum* f. sp. *vasinfectum* using serial passage assays through susceptible cotton. Phytopathology 98:296-303.

Wang, C., and Roberts, P.A. 2006 A Fusarium wilt resistance gene in *Gossypium barbadense* and its effect on root-knot nematode-wilt disease complex. Phytopathology 96:727-734.

Zhou, X.G., and Everts, K.L. 2006. Suppression of Fusarium wilt of watermelon enhanced by hairy vetch green manure and partial cultivar resistance. Online Plant Health Progress DOI: 10.1094/PHP-2006-0405-01-RS.

Figure 1.1a. Fusarium wilt interveinal chlorosis and necrosis of cotton foliage.

Figure 1.1b. Older wilt of foliage of cotton plants affected by Fusarium wilt.

Table 1.1. Races of *Fusarium oxysporum* f. sp. *vasinfectum* and response to inoculation of six differential hosts.

	Race **(Origin)**								
Differential Host species and cultivars	1 (USA)	2 (USA)	3 (Egypt)	4 (India)	5 (Sudan)	6 (Brazil & Paraguay)	7 (China)	8 (China)	Australian Biotypes
Gossypium arboreum cv. *Rozi*	R	R	S	S	S	R	S	I	R
G. barbadense cv. *Ashmouni*	S	S	R	R	S	S	…	…	S
G. barbadense cv. *Sakel*	S	S	S	R	S	S	S	I	S
G. hirsutum cv. *Acala* 44	S	S	R	R	R	S	S	I	S
Glycine max cv. *Yelredo*	R*	S	…	…	…	R	I	I	R
Nicotiana tabacum cv. *Gold Dollar*	R*	S	R	R	…	R	R	I	R
Lupinus luteus cv. *Weiko*	S	S	…	…	…	R	…	…	R

R = resistant; S = susceptible; I = intermediate (some plants susceptible); R* = not highly resistant to all isolates of race 1. [See references (Armstrong, G. M., and Armstrong, J. K. 1958), (Assigbetse, K. B. *et al*., 1994), and (Davis, R. D. *et al*., 2006). In some studies, particular cultivars were substituted for the ones shown.]

Figure 1.2a. The root-knot nematode second stage juvenile (RKN), *Meloidogyne incognita.*

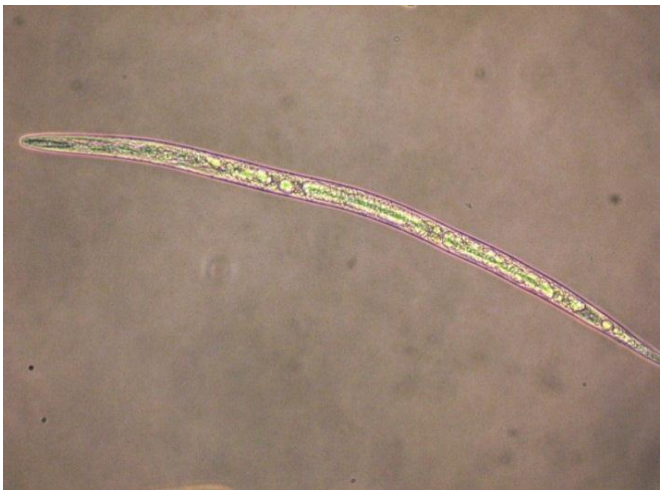


Figure 1.2b. Root infections by *M. incognita* causing gall formation on the root system.

Fusarium Wilt and Root-Knot Nematode Effects on Cotton Cultivars

Abstract

Development of the disease complex Fusarium wilt in upland cotton (*Gossypium hirsutum*) usually entails infections of plants by both the root-knot nematode, *Meloidogyne incognita,* (RKN) and *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). Commercial cotton varieties were examined to determine their response to both pathogens, RKN and FOV. Cotton controls used to compare dissimilarities were the resistant M-315 and susceptible Rowden lines. The 2010 season was environmentally favorable for the pathogens with Fusarium wilt incidence ranging from a high of 16.25% to a low of 0.4%. Phytogen 367 WRF and Stoneville 5458 B2RF displayed the fewest Fusarium wilt symptomatic plants. The populations of root-knot nematodes averaged 2,149 root-knot eggs per gram of root on the susceptible Rowden while the resistant cotton, M-315, supported the fewest with only 88 eggs per gram of root. Phytogen 367 WRF, Phytogen 565 WRF, Stoneville 5458 B2RF, and Stoneville 4288 B2RF cultivars all produced significantly more cotton than Rowden. Canonical analysis indicated Phytogen 367 WRF supported low populations of RKN while producing the greatest yields in the trial, thus it appeared to be resistant to both pathogens. Comparatively, Phytogen 565 WRF appeared tolerant to RKN; this variety supported high populations of RKN while producing high yields. In 2011, Fusarium wilt incidence was similar between all cultivars with a high of 3% FOV incidence. Root-knot nematode numbers were low with Rowden and M-315 averaging 241 and 66 root-knot eggs per gram of root, respectively. Yields were increased two fold in 2011 with low disease incidence.

Introduction

In 1892 Atkinson first identified and described the Fusarium wilt complex of cotton (Atkinson, G.F. 1892). Fusarium wilt, now 120 years later, is still a major economic limiting plant disease in cotton-growing regions of the world. In addition to a worldwide distribution, Fusarium wilt occurs in all four of the domesticated cottons, *Gossypium arboretum* L., *G. barbadense* L., *G. herbaceum* L., and *G. hirsutum* L. (Armstrong, G. M. and Armstrong, J. K. 1960). The causal agent of Fusarium wilt is *Fusarium oxysporum* Schltd.:Fr. f. sp. *vasinfectum* (Atk.) W.C. Snyd. & H.N. Hans. The incidence, rate of development, and severity of Fusarium wilt in cotton can increase in the presence of root-knot nematodes, *Meloidogyne incognita* (Kofoid & White) Chitwood as a disease complex. Atkinson noted that Fusarium wilt of cotton was always more severe when it occurred in a field infested with the root-knot nematode (Atkinson, G.F. 1892). The Fusarium wilt complex infects cotton plants as early as the seedling stage, but the most severed disease symptoms and plant death usually occur during the plants reproductive stages. The *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) grows through the root cortex to the vascular tissues and begins to sporulate when it has invaded the xylem. Systemic spread within the plant occurs by spore transport through the xylem vesicles (Michielse, C.B. *et al*., 2009). Mycelia growth occurs in the vessels and later in the surrounding cortical tissues. The plant responds with tylose formation blocking the vascular system. As the plant becomes fully colonized by the FOV fungus, symptoms of leaf chlorosis, necrosis, and wilting are evident. Water stress in the plant is induced by the combined effect of mycelia growth in the xylem, and vascular obstruction by the host in an attempt to prevent total spread of the fungus. Death of the plant often follows with the production of fungal metabolites and toxins (Skovgaard, K. *et al.*, 2001).

The root- knot nematode creates wounds in the roots as the second stage juvenile penetrates the epidermis, cortex, and begins establishing giant cells in the periderm tissues of the endodermis. The nematode entry wounds allow the FOV fungus to readily penetrate into the vascular tissues of the root system. Though wilt is increased by the occurrence of root- knot, either disorder could occur in the absence of the other (Nelson, P. E. *et al*., 1981). It has been demonstrated that mechanical wounding of the cotton roots alone increased the susceptibility of the plant to Fusarium wilt less than wounding resulting from infection by the root-knot nematode (Thomason, I. J., 1959; Jenkins, W. R., 1957). Giant cells and gall tissues produced by the nematode have been shown to serve as a nutrient sink for nematode feeding; they also support more vigorous hyphal growth of FOV than non-galled tissues (Taylor, C.E. 1979; DeVay, J. E., *et al*., 1997). The root- knot nematode commonly associated with Fusarium wilt of *G. hirsutum* (American upland cotton) is *Meloidogyne incognita*. The root-knot nematode increases the severity of Fusarium wilt. Garber (1979) reported Fusarium wilt symptoms occurred with more than 77, 000 *F. oxysporum* f. sp. *vasinfectum* propagules per gram of soil without root- knot nematodes on root- knot -susceptible Acala SJ-2. Whereas, as few as 650 *F. oxysporum* f. sp. *vasinfectum* propagules caused Fusarium wilt on plants co-inoculated with 50 root knot nematode infective juveniles (J2's) under controlled greenhouse conditions (Garber, R. H. *et al*., 1979; Hyer, A.H., *et al*., 1979). Thus the nematode facilitates entry of the fungal pathogen but also supports fungal growth inside the root system.

Auburn University in its variety testing program evaluates elite breeding lines submitted by cotton seed companies to the National Fusarium Wilt Testing program, to determine Fusarium wilt resistance or susceptibility of the breeding lines in our naturally infested field at the E. V Smith Research and Extension Centers' Plant Breeding Unit located near Tallassee, Alabama. In

2010, the program was expanded to include new cotton cultivars adapted to the southeastern region. Hypothetically, the commercialized cotton cultivars available to growers may have some resistance to the Fusarium wilt complex because the breeding lines were screened at this facility. Specifically, the objectives of this study were to 1) determine cotton cultivars susceptibility to Fusarium wilt by foliar disease expression and FOV re-isolation throughout the growing season; 2) determine cotton cultivar susceptibility to RKN; and 3) determine the combined effect of FOV and RKN on cotton yield.

Materials and Methods

Commercial cotton cultivars most frequently grown in Alabama were studied to determine their tolerance to the soil borne pathogens, *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *vasinfectum* which facilitate and cause the Fusarium wilt complex in cotton. The trial was conducted at the E.V. Smith Research and Extension Center, Plant Breeding Unit, near Tallassee, AL. This location is naturally infested with *Fusarium oxysporum* f sp. *vasinfectum* (FOV) and *Meloidogyne incognita* race 3 (RKN) and has a long history of severe Fusarium wilt disease. The soil type is a Kalmia loams sand (80 % sand, 10 % silt, and 10 % clay). All cotton entries were planted in single 6.10 meter rows on 0.910 meter centers, separated by 1.83 meter alleys. Four replications of the test entries and controls were evaluated in a randomized complete block design with a split plot restriction on randomization. Both the FOV and RKN susceptible line (Rowden) and resistant line (M-315) were included as positive and negative controls to determine the range of pathogen severity in each season and across the field. All plots were maintained throughout the season using standard herbicide, insecticide, and fertility production practices as recommended by the Alabama Cooperative Extension System.

Summers of 2010 and 2011, plots were planted May 14, and May 18 respectively. Initial live plant counts were determined and recorded after crop emergence near 28 days after planting. Plants with visible foliar symptoms of wilt were counted and removed biweekly beginning June 23 through August 26, 2010 and July 7 through August 30, 2011. Fusarium wilt incidence was determined by tabulating the incidence of wilt plants by plot biweekly throughout the season. Cultivar percent susceptibility was calculated by adding the number of diseased plants recorded over the growing season and dividing by the initial number of plants recorded at 4 weeks after planting. Fusarium wilt was confirmed by re-isolation of the fungal pathogen, FOV from the symptomatic plants removed from each plot at each sample date both years. Root hypocotyl sections were surface disinfected in 90% Etoh followed by a minute immersion in a 0.6 % sodium hypochlorite (NaOCl) solution. The outer cortical layer was aseptically removed with a sterile scalpel and the inner vascular tissues were aseptically plated on acidified Potato Dextrose Agar (aPDA) (Sigma-Aldrich, St. Louis, MO) to confirm FOV presence. FOV isolates were recorded by plot and stored on half strength aPDA in test tube slants at 4° C.

Nematode populations were determined near 45 days after planting following a rain event each year. Three random plants per plot were removed by digging up the root systems with paired shovels on opposite sides of each plant on July 22, 2010 and July 27, 2011. Nematode eggs were extracted by agitating the root system for 4 minutes in a 0.6 % NaOCl solution on the rotary shaker at 130 rpm for 4 minutes. RKN J2's and eggs were washed through nested 75µm and 25µm sieves. RKN eggs and J2's were enumerated using a Nikon TS100 inverted microscope at 40 x magnifications, and nematode populations were standardized by volume and fresh root weight. Nematode populations were also determined at cotton plant maturity. Ten soil cores per plot were collected in a zigzag manor, mixed and a 150 cm^3 sub sample was extracted.

RKN J2's were extracted from the soil using combined gravity screening and sucrose (specific gravity = 1.13) centrifugal flotation. RKN J2's were enumerated and recorded.

Seed cotton yields were collected utilizing a plot harvester on October 29, 2010 and on Nov. 1, 2011. The bolls for each cotton cultivar for each replicate were ginned separately immediately following harvest. A 20 seed subsample was taken randomly from the sample and processed to determine if the FOV fungus was present on the cotton seed. Half of the seeds were surface sterilized as previously described while the remaining seeds were not. Both sterile and non-sterilized seed were aseptically plated on aPDA plates. All plates were incubated at room temperature under a laminar flow hood for 5 to 7 days. To confirm the identification of FOV, conidia were examined using the Nikon Eclipse Tí light microscope.

All data was analyzed using Generalized Linear Mixed Models procedures as implemented in SAS PROC GLIMMIX in version 9.1 (SAS Institute, Cary, NC) with a negative binomial distribution function for count variables. Percent wilted plants, RKN, root-knot egg, and seed cotton yield numbers were calculated.

Results

The 2010 season, environmentally, was conducive for the root-knot nematode and Fusarium wilt pathogens with warmer temperatures and higher rainfall amounts during the growing season (Figure 2.1). Fusarium wilt symptoms were initially visible in late June and were expressed with cotton leaf necrosis and wilting. Plant death occurred throughout July and August. Over all the cotton submissions planted in 2010, Fusarium wilt incidence ranged from a high of 16.25% for the susceptible Rowden to a low of 0.4% for the resistant M-315 (Table 2.1). All commercial cotton cultivars except Delta Pine 0949 B2RF exhibited fewer Fusarium wilt

symptomatic plants than the susceptible Rowden. Phytogen 367 WRF and Stoneville 5458 B2RF displayed the fewest symptomatic plants and were followed by Phytogen 375 WRF, Delta Pine 1028 B2RF, Phytogen 485 WRF, Stoneville 4288 B2RF, and Delta Pine 1050 B2RF. The fungal pathogen was readily isolated from Rowden and the symptomatic plants in each of the commercial cultivars except Phytogen 367 WRF, Stoneville 5458 B2RF and the resistant M -315 cotton.

The numbers of root-knot nematodes increased in all the cotton samples submitted. The standard susceptible cotton, Rowden, averaged 2,149 root-knot eggs per gram of root while the resistant cotton, M-315 supported only 88 eggs per gram of root (Table 2.2). All cotton cultivars supported higher populations of the root-knot nematode ($P \geq 0.001$) as compared to M-315 except Phytogen 367 WRF. The Phytogen 367 WRF was the only cultivar less susceptible ($P \geq 0.023$) to the root-knot nematode as compared to the Rowden susceptible control. Three varieties were significantly ($P \leq 0.0001$) more susceptible to the root-knot nematode than the M-315 resistant control. Fiber Max 1740B2F, Phytogen 565 WRF, and Stoneville 4288 B2RF supported nematode populations similar to the susceptible Rowden when compared to the M-315 control.

Seed cotton yield varied from a low of 1289 kg/ha in the susceptible Rowden to a high of 3883 kg/ha in the Phytogen 367 WRF plots (Table 2.3).The Phytogen 367 WRF, Phytogen 565 WRF, Stoneville 4288 B2RF, and Stoneville 5458 B2RF cultivars all produced more seed cotton ($P \geq 0.05$) as compared to Rowden. These four varieties produced an average seed cotton yield of 3356 kg/ha of cotton or a 61% increase in yield over the susceptible Rowden. All of the cotton cultivars produced yields similar to the resistant cotton M-315 except Deltapine 0949

B2RF. The Deltapine 0949 B2RF variety produced a yield that was 58% less that of the M-315 resistant variety but almost identical to the Rowden susceptible variety.

Canonical discriminant analysis (Figure 2.2) indicated the main force driving the differences along the first canonical or CAN 1 was seed cotton yield (r = 0.98). Cotton cultivars separated into three groups. Phytogen 367 WRF, Phytogen 565 WRF, and M-315 were the highest yielding. Phytogen 485 WRF, Deltapine 0949 B2RF and Rowden were the lowest yielding. The remaining cultivars comprised the middle group which could not be distinguished from either the high or low yielding groups. Differences in the second canonical (CAN 2) were driven by the root-knot nematode populations, which are determined by the number of eggs per gram of root. The canonical diagram clearly depicts the relationship between seed cotton yield and root-knot nematode populations. Phytogen 367 WRF produced high yields while supporting fewer root-knot nematodes compared to Phytogen 565 WRF produced high yields and supported a high numbers of nematodes. The canonical analysis visualizes Phytogen 367 WRF as being resistance to RKN as is M-315. Phytogen 565 WRF, however, appears tolerant to RKN. This cultivar produced high yields while supporting high numbers of the nematode.

The 2011 season, environmentally, experienced prolonged periods of drought in May and June which reduced the incidence and severity of the root-knot nematode and Fusarium wilt pathogens compared to 2010. Over all the cotton submissions planted in 2011, Fusarium wilt incidence peaked at a high of 3% for the cultivars Delta Pine 1028 B2RF and Delta Pine 10R052 B2R2 to a low of 0% for Americot 1550 B2RF, Phytogen 485 WRF, Stoneville 5288 B2F, and Stoneville 5458 B2RF (Table 2.4). The average percentage of wilted plants for the susceptible, Rowden, was 2% in 2011 which is 87 % less disease incidence in 2011 as compared to 2010. Although the causal agent, FOV was isolated from 68% of the cotton cultivars planted in 2011

disease expression was minimal. The fungal pathogen was not isolated from the varieties, Delta Pine 0949 B2RF, Phytogen 367 WRF, Phytogen 375 WRF, Phytogen 565 WRF, Croplan Genetics 3787 B2RF, Stoneville 5288 B2F, Americot 1550 B2RF, or the resistant M -315 cotton.

Root-knot nematode numbers increased slowly during the 2011 season. The standard susceptible cotton, Rowden, averaged 241 root-knot eggs per gram of root while the M-315 resistant cotton supported 66 root-knot eggs per gram of root (Table 2.5). None of the cotton cultivars supported fewer numbers of root-knot nematode eggs per gram of root as compared to resistant line M-315 in 2011. The root-knot nematode populations observed in 2011 were less than half of those observed in 2010. All cotton cultivars supported similar populations of root-knot nematodes at 45 days after planting and at harvest in the dry 2011 year. The harvest samples were not able to differentiate any differences in nematode numbers between the cotton cultivars as compared to the resistant M-315 or the susceptible Rowden (Table 2.6).

Seed cotton yields ranged from a high of 5,233 kg/ha for Stoneville 4288 B2F to a low of 1,951 kg/ha for M -315 (Table 2.7). The cotton varieties Croplan Genetics 3787 B2RF, Delta Pine 1028 B2RF, Phytogen 485 WRF, Stoneville 4288 B2F, and Stoneville 5458 B2F produced significantly ($P \leq 0.05$) higher yields than the susceptible Rowden. Seventy five percent of the cotton cultivars produced greater yields than the resistant M-315.

Canonical discriminant analysis (Figure 2.3) indicated the significant force driving the differences along the first canonical or CAN 1 was seed cotton yield (r = 0.98). Cotton cultivars did not separated into groups. Stoneville 4288 B2F and Stoneville 5458 B2RF were the highest yielding cultivars but comprise the middle group which could not be distinguished from either M-315 or Rowden. The lowest yielding cultivars in 2011 were M-315 and Rowden. The

remaining cultivars differences in CAN 2 were determined by the root-knot nematode populations. Stoneville 4288 B2RF produced high yields while supporting few root-knot nematodes compared to Fiber Max 1740 B2F produced high yields and supported a high numbers of nematodes. All cotton cultivars produced higher yields than either Rowden or M-315 control in the dry 2011 season.

Discussion

This study focused on the Fusarium wilt complex and the effects of the pathogens, *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *vasinfectum,* on cotton cultivars. The 2010 growing season proved conducive for both pathogens. Fusarium wilt foliar symptoms and root-knot nematode root galling were pronounced in 2010; Phytogen 367 WRF did respond as a resistant variety to both FOV and to the root knot nematode and did not allow the nematode population to increase over the season. McPherson and Rush (2011) introduced Phytogen 367 WRF as a tolerant cultivar supporting fewer root-knot nematode galls and reduced nematode populations. The incidence of Fusarium wilt in Phytogen 367 WRF was probably low due to the root-knot nematode resistance present in this cultivar. In 2010, Phytogen 367 WRF produced high yields while supporting few root-knot nematodes indicating nematode resistance was present in this cultivar; however, Phytogen 565 WRF produced similar yields to Phytogen 367 WRF but also supporting high numbers of nematodes. Thus Phytogen 565 WRF appeared to be a tolerant cultivar to the root-knot nematode and to Fusarium wilt. This cultivar produced one of the greatest yields and highest nematode populations. Thus a grower could expect a greater level of root-knot nematodes in a field if Phytogen 565 WRF is grown. In this study, cotton entries supporting lower root-knot nematode populations, commonly had highest resistance to Fusarium wilt. All commercial cotton cultivars except Delta Pine 0949 B2RF had fewer Fusarium wilt

symptomatic plants than the susceptible Rowden indicating screening breeding lines in this field has increased FOV resistance in commercial cultivars.

Fusarium wilt is a temperate to tropical disease (DeVay, J. E., *et al*., 1997) and the 2011 season had environmentally poor conditions for the disease complex pathogens FOV and RKN. Temperatures were warm in 2011 with May and June having 53% less rainfall to cultivate the infection, invasion, and spread of both the pathogens than in May and June of 2010. However, seed cotton yield on average were higher than that of the preceding year due to limited pathogen infection and timely rain events which corresponded with plant flowering in late July of 2011. Optimum temperature for Fusarium wilt disease development is between 30 and 32°C, with little disease occurring if temperatures remain below 23°C or above 35°C (Hillocks, R.J, 1992). Cotton plants often recover from the disease and re-grow if the temperature falls below the optimum for disease development after initial infection according to Hillocks. In a study by El Abyad and Saleh (EL-Abyad, M.S. and Saleh, Y.E. 1971), they reported that growth rate of the fungus FOV reached its maximum at 25°C although at 30°C it was not significantly different. In addition, growth rates of the fungus at 10° and 37° were practically negligible in this study. Thus it can be concluded from these findings that the temperature range of 29.4 to 14.1 with an average of 22°C, which is the average temperature for the two weeks after planting in the 2011 test, the FOV pathogen was not at ideal temperatures for germination, infection, sporulation, and growth. Tharp and Young (Tharp, W.H. and Young, V.H. 1939) showed that Fusarium wilt disease was encouraged by moist conditions as well, with an optimum of 85% saturation. With moist soils, the cotton plant may be less able to resist root invasion when soil moisture levels approach field capacity (Ebbels, D.L. 1975). Furthermore, environmental conditions which most favor disease development after invasion of the vascular tissue are probably not the same as

those which favor growth of the pathogen in soil and infection of the root (Hillocks, R.J, 1992). This is demonstrated by 2011 season expressing mild FOV symptom in its hot and dry conditions and 2010 showing acute infections with warm moist conditions.

Root-knot nematodes in the field probably facilitated Fusarium wilt invasion of the vascular system during the 2010 season of with favorable temperatures and moisture conditions. Root-knot nematodes have been reported to increase susceptibility of cotton plants to Fusarium wilt by inducing root galls, giant cells, and damaged tissue. Garrett (Garrett, S.D. 1960) reported that vascular-wilt fungi must have a source of energy for developing a threshold level of inoculum potential before it can overpower a plant's innate defenses and spread systemically. Galls and giant cells in susceptible cotton may provide the fungus with much of the energy it needs for this purpose (Shepherd, R.L. 1986). With this assumption, the more galls, giant cells, and damaged tissue that are induced by RKN in cotton roots, the more energy the fungus would have for invading the plant, overcoming its innate defenses, and causing symptoms of Fusarium wilt. Conversely, with greater RKN resistance, numbers of galls and giant cells and the incidence of wilting will be lower. In 2010, root-knot egg numbers averaged over 1,000 counts per gram of root fresh weight when cotton varieties were averaged together. This resulted in potentially high galling and damaged tissue enabling the fungal pathogen FOV to cause an overall 4.6 percent wilt damage to the varieties; four cultivars surpassed the average wilt injury. This high population of RKN possibly influencing FOV presence can be the described as one of the limiting factor that controls FOV's population growth within the cotton plants.

Concluding from the first season, Phytogen 367 WRF is resistance to RKN and FOV. Based on the prior data and data from this study, it can be argued that most yield loss, suppression of cotton growth, and wilt symptoms caused by FOV are enhanced by *M. incognita*

colonization of the root system. The 2010 season demonstrated Phytogen 367 WRF would be an ideal cultivar for a cotton grower to select if RKN and FOV are causing yield reductions on his acreages. Phytogen 565 WRF supported a large amount of RKN, a high FOV disease incidence, and still managed to be a leading cotton seed producer of 2010. Thus Phytogen 565 WRF could be considered a tolerant variety to RKN. Growers with RKN would need to consider that this cultivar would produce high yields but would also increase the nematode populations in their soils.

Specifically, all current cotton cultivars on the market today are more resistant to FOV than the standard susceptible line Rowden. Resistance to FOV has increased in our cotton cultivars over the last decades. This increase in disease resistance is probably due to the cooperative efforts of Auburn University, USDA, and the commercial companies working together to screen breeding lines in these field over the last few decades. RKN resistance and tolerance appear to be present in the current cotton cultivars. Yields produced are remarkably different when we have severed disease with high levels of RKN and FOV (2010) and with little infection (2011). Thus cultivar selection is very import for the grower and the grower does have good cultivar options.

Literature Cited

Armstrong, G. M., and Armstrong, J. K. 1960. American, Egyptian, and Indian cotton-wilt Fusaria: Their pathogenicity and relationship to other wilt fusaria. U.S. Department of Agriculture Technology. Bulletin. 1219.

Atkinson, G.F. 1892. Some diseases of cotton. Alabama Polytechnical Institute. Agriculture Experimental Station Bulletin No. 41: 61-65

DeVay, J. E., Gutierrez, A. P., Pullman, G. S., Wakeman, R. J., Garber, R. H., Jeffers, D. P., Smith, S. N., Goodell, P. B., and Roberts, P. A.1997. Inoculum densities of *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* in relation to the development of Fusarium wilt and the phenology of cotton plants (*Gossypium hirsutum*). Phytopathology 87:341-346.

Ebbels, D. L. 1975. Fusarium wilt of cotton: A review, with special reference to Tanzania. Cotton Grower Review. 52:295-339.

EL-Abyad, M.S. and Saleh, Y.E. 1971. Studies with *Fusarium oxysporum* f. sp. *vasinfectum*, the cause of cotton wilt in Egypt: germination, sporulation, and growth. Transactions of the British Mycological Society 57, 427-37.

Garber, R. H., Jorgenson, E. C., Smith, S. N., and Hyer, A. H. 1979. Interaction of population levels of *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* on cotton. Journal of Nematology. 11:133-137.

Garrett, S. D. 1960. Biology of Root-Infecting Fungi. Cambridge: University Press. 393pp.

Hillocks, R. J. 1992. Fusarium wilt. In: Hillocks RJ, ed. Cotton Diseases. Wallingford, UK: CAB International, 127–60.

Hyer, A.H., Jorgenson, E.C., Garber, R.H., and Smith, S. 1979. Resistance to root-knot nematode in control of root-knot nematode Fusarium wilt disease complex in cotton *Gossypium hirsutum*. Crop Science. 19:898-901.

Jenkins, W. R., and Coursen, B. W. 1957. The effect of root-knot nematodes, *Meloidogyne incognita* acrita and *M. hapla*, on Fusarium wilt of tomato. Plant Disease Reporter. 4 1: 1 82-86.

McPherson, M., and Rush, D. 2011. Efficacy of PHY367WRF in root-knot infested fields. Proc. Beltwide Cotton Conferences., 1:248. National Cotton Council, Atlanta, GA. Jan. 2011

Michielse, C.B., van Wijk, R., Reijnen, L., Cornelissen, B.J., and Rep, M. 2009. Insight into the molecular requirements for pathogenicity of *Fusarium oxysporum* f. sp. *lycopersici* through large-scale insertional mutagenesis. Genome Biology 10.1: R4.

Nelson, P. E., Toussoun, T. A., and Cook, R. J. 1981. Fusarium: Disease, Biology, and Taxonomy. The Pennsylvania State University Press, University Park.

Shepherd, R.L., and Kappelman, A.J. 1986. Cotton resistance to the root knot-Fusarium wilt complex. I. Relation to Fusarium wilt resistance and its implications on breeding for resistance. Crop Science 26:228-232.

Shepherd, R.L. 1986. Cotton resistance to the root-knot-fusarium wilt complex. II. Relation to root-knot resistance and its implications on breeding for resistance. Crop Science 26:233-237.

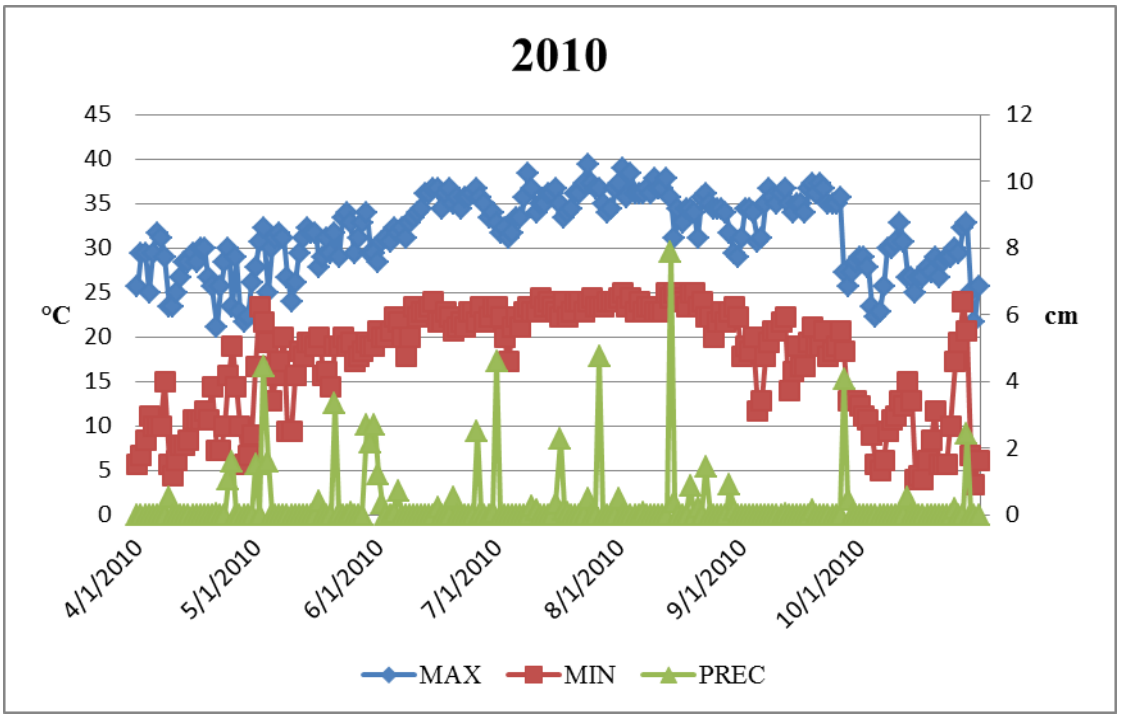
Skovgaard K., Nirenberg H.I., O'Donnell K., and Rosendahl, S. 2001. Evolution of *Fusarium oxysporum* f.sp. *vasinfectum* races inferred from multigene genealogies. Phytopathology 91, 1231–7.

Taylor, C.E. 1979. *Meloidogyne* interrelationships with microorganisms. In: Lamberti, F. and Taylor, C.E. (eds). Root-knot nematodes (*Meloidogyne species*) Academic Press, London, 375-398.

Tharp, W.H. and Young, V.H. 1939. Relation of soil moisture to Fusarium wilt of cotton. Journal of Agriculture Research 58, 47-61.

Thomason, I. J., Erwin, D. C., and Garber, M. J. 1959. The relationship of the root•knot nematode, *Meloidogyne javanica* to Fusarium wilt of cowpea. Phytopathology 49:602-06

Figure 2.1. 2010-2011 average temperature (°C) highs and lows by month and average rainfall amounts (cm) between sampling dates.



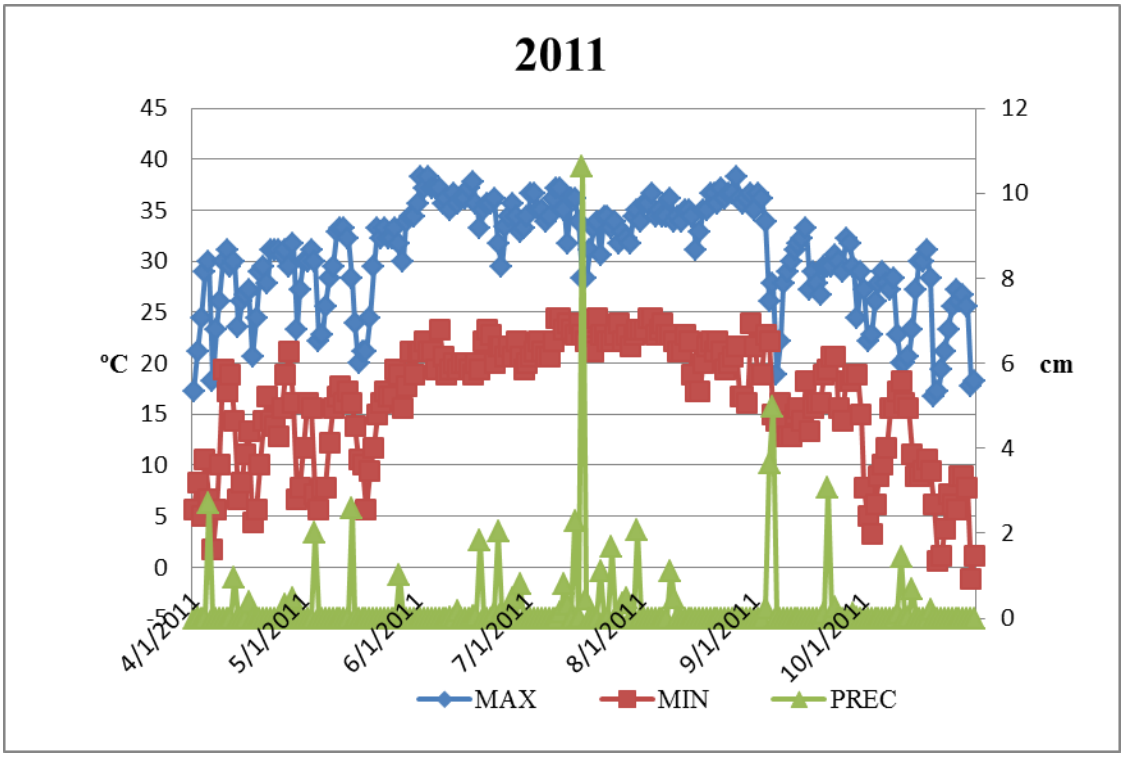


Table 2.1 2010 Average percent of Fusarium wilt incidence for cotton cultivars, confidence intervals, and P-values based on Dunnett's P-value versus the susceptible check Rowden and the resistant check M-315.

	Fusarium wilt	**95 % Confidence Limit**		**Dunnett's P-value vs.**	
Cultivar	**Percent incidence**	**Lower**	**Upper**	**Rowden**	**M-315**
Deltapine 0949 B2RF	7.6	3.7	15.1	0.378	0.044
Deltapine 1028 B2RF	2.6	0.9	7.2	0.013	0.326
Deltapine 1050 B2RF	3.7	1.6	8.1	0.011	0.172
Fiber Max 1740 B2F	5.4	2.9	9.7	0.019	0.080
Phytogen 367 WRF	1.1	0.3	3.6	0.001	0.790
Phytogen 375 WRF	2.5	0.9	7.1	0.011	0.335
Phytogen 485 WRF	3.3	1.4	7.4	0.006	0.207
Phytogen 565 WRF	6.9	4.1	11.5	0.065	0.046
Stoneville 4288 B2RF	3.4	1.6	7.2	0.004	0.188
Stoneville 5458 B2RF	1.6	0.6	4.6	0.001	0.560
Rowden	16.3	11.4	23.0		0.005
M-315	0.4	0.0	3.6	0.013	

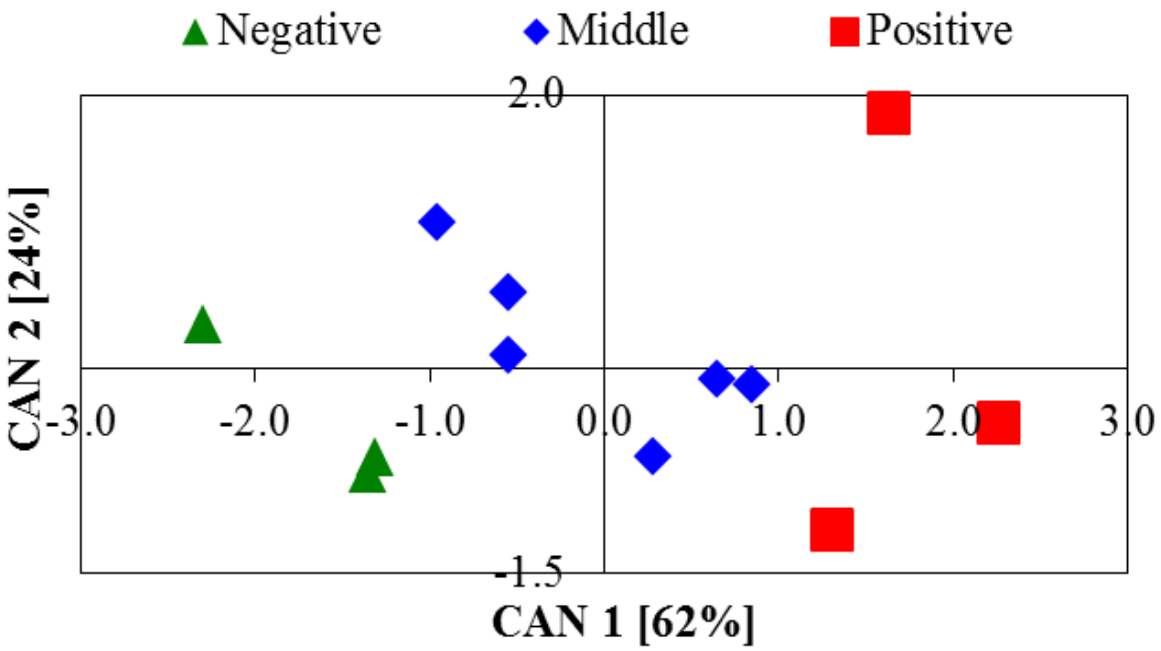
Table 2.2. 2010 Root knot egg numbers (counts per g of root fresh weight), confidence intervals, and *P*-values based on Dunnett's P-value versus the susceptible check Rowden and the resistant check M-315.

	Meloidogyne incognita **95 % Confidence Limit**			**Dunnett's P-value vs.**	
Cultivar	**Mean**	**Lower**	**Upper**	**Rowden**	**M-315**
Deltapine 0949 B2RF	775	347	1732	0.318	0.003
Deltapine 1028 B2RF	1092	488	2440	0.734	0.001
Deltapine 1050 B2RF	693	310	1550	0.225	0.005
Fiber Max 1740 B2F	1852	1176	2916	1.000	<.0001
Phytogen 367 WRF	382	171	853	0.023	0.067
Phytogen 375 WRF	1187	531	2652	0.835	0.000
Phytogen 485 WRF	1170	524	2616	0.819	0.001
Phytogen 565 WRF	1585	1006	2495	0.980	<.0001
Stoneville 42888 B2RF	893	567	1406	0.217	<.0001
Stoneville 5458 B2RF	901	403	2013	0.484	0.002
Rowden	2149	962	4801		<.0001
M-315	88	39	197	<.0001	

Table 2.3. 2010 Seed cotton yield (lbs per acre) for entries and checks, confidence intervals, and P-values based on Dunnett's versus the susceptible check Rowden and the resistant check M-315.

Seed Cotton Yield					
	95% Confidence Limit			**Dunnett's *P* vs.**	
Cultivar	Kg/ha	Lower	Upper	Rowden	M-315
Deltapine 0949 B2RF	1317	544	2090	1.000	0.020
Deltapine 1028 B2RF	1980	1207	2753	0.793	0.272
Deltapine 1050 B2RF	2594	1821	3367	0.150	0.951
Fiber Max 1740 B2F	2020	1248	2793	0.743	0.309
Phytogen 367 WRF	3883	3109	4656	0.0001	0.691
Phytogen 375 WRF	2025	1252	2798	0.738	0.313
Phytogen 485 WRF	1719	946	2493	0.985	0.109
Phytogen 565 WRF	3509	2735	4282	0.003	0.991
Stoneville 4288 B2RF	2915	2141	3688	0.042	1.000
Stoneville 5458 B2RF	3118	2345	3890	0.017	1.000
Rowden	1289	515	2062		0.017
M-315	3110	2337	3883	0.017	

Figure 2.2. Canonical discriminant analysis of cotton cultivar yield and nematode numbers 2010. CAN 1 is seed cotton yield (r = 0.98) thus yield increases moving away from the Y axis. Differences in CAN 2 are driven by root knot egg numbers with higher numbers in the top than the lower red cultivars.



Cultivar	Can1	Middle	Positive	Negative	Lbs./acre
Rowden	-2.29			0.32	1151
Phytogen 485 WRF	-1.35			-0.77	1535
Deltapine 0949 B2RF	-1.31			-0.65	1176
Fiber Max 1740 B2F	-0.95	1.06			1804
Deltapine 1028 B2RF	-0.54	0.09			1768
Phytogen 375 WRF	-0.54	0.55			1808
Deltapine 1050 B2RF	0.28	-0.65			2316
Stoneville 4288 B2RF	0.65	-0.08			2603
Stoneville 5458 B2RF	0.85	-0.13			2784
M-315	1.31		-1.19		2777
Phytogen 565 WRF	1.64		1.85		3133
Phytogen 367 WRF	2.26		-0.41		3467

Table 2.4. 2011 Least squares estimates of the average percent wilted plants for cotton cultivars and checks, confidence intervals, and *P*-values based on Dunnett's versus the susceptible check Rowden and the resistant check

	95% Confidence Limit			Dunnett's *P* vs.	
Cultivar	**Avg**	**LL**	**UL**	**Rowden**	**M315**
Americot 1550 B2RF	0	0	5	0.988	0.957
Croplan Genetics 3787 B2RF	1	0	5	1.000	0.995
Dyna Gro 2570 B2RF	1	0	3	0.993	0.960
Deltapine 0912 B2RF	1	0	3	0.995	0.969
Deltapine 0949 B2RF	1	0	8	1.000	0.998
Deltapine 1028 B2RF	3	1	11	1.000	1.000
Deltapine 1050 B2RF	1	0	5	1.000	0.997
Deltapine 10R052B2R2	3	1	8	1.000	1.000
Deltapine 1137 B2RF	2	0	11	1.000	1.000
Fiber Max 1740 B2F	1	0	3	0.998	0.981
Phytogen 367 WRF	1	0	5	1.000	0.999
Phytogen 375 WRF	1	0	4	0.984	0.943
Phytogen 485 WRF	0	0	7	0.992	0.972
Phytogen 565 WRF	2	1	8	1.000	1.000
Stoneville 4288 B2F	1	0	4	0.997	0.977
Stoneville 5288 B2F	0	0	4	0.975	0.927
Stoneville 5458 B2RF	0	0	4	0.932	0.852
M-315	2	1	8	1.000	
Rowden	2	1	6		1.000

Table 2.5. 2011 Least squares estimate of root knot egg number (counts per g of root fresh weight) at 45 days after planting for cotton cultivars and checks, confidence intervals, and *P*-values based on Dunnett's versus the susceptible check Rowden and the resistant check M-315.

	Meloidogyne incognita **95% Confidence Limit**			**Dunnett's *P* vs.**	
Cultivar	**Mean**	**LL**	**UL**	**Rowden**	**M315**
Americot 1550 B2RF	75	8	726	0.999	1.000
Croplan Genetics 3787 B2RF	57	6	548	0.989	1.000
Dyna Gro 2570 B2RF	161	23	1135	1.000	1.000
Deltapine 0912 B2RF	295	42	2072	1.000	0.969
Deltapine 0949 B2RF	273	28	2636	1.000	0.990
Deltapine 1028 B2RF	125	8	2051	1.000	1.000
Deltapine 1050 B2RF	89	9	860	1.000	1.000
Deltapine 10R052B2R2	85	9	818	1.000	1.000
Deltapine 1137 B2RF	119	7	1951	1.000	1.000
Fiber Max 1740 B2F	1315	187	9249	0.922	0.300
Phytogen 367 WRF	18	2	178	0.609	0.997
Phytogen 375 WRF	1019	145	7166	0.977	0.412
Phytogen 485 WRF	81	5	1323	1.000	1.000
Phytogen 565 WRF	383	40	3703	1.000	0.944
Stoneville 4288 B2F	161	17	1553	1.000	1.000
Stoneville 5288 B2F	37	4	354	0.912	1.000
Stoneville 5458 B2RF	54	6	524	0.985	1.000
M-315	66	9	463	0.991	
Rowden	241	34	1693		0.991

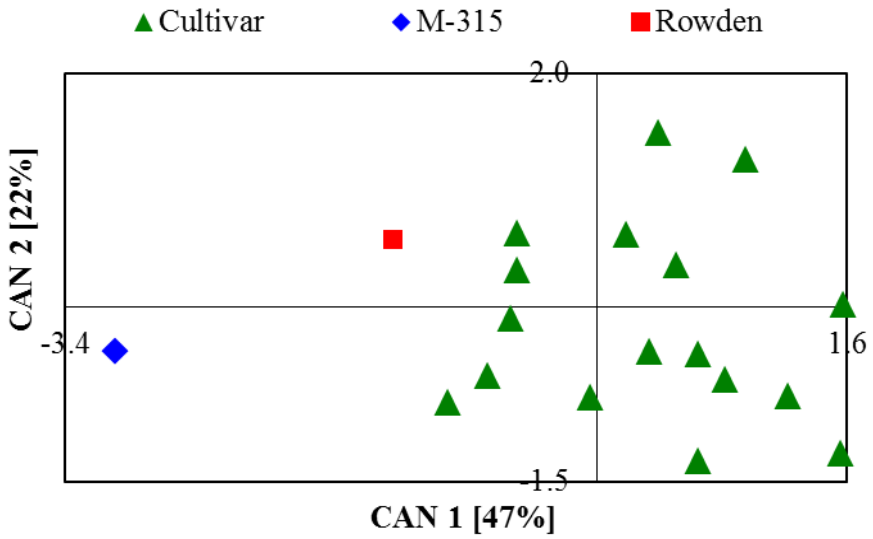
Table 2.6. 2011 Least squares estimate of root knot second stage juvenile numbers (counts per 150 cc) at harvest for cotton cultivars and checks, confidence intervals, and *P*-values based on Dunnett's versus the susceptible check Rowden and the resistant check M-315.

	Meloidogyne incognita **95% Confidence Limit**			**Dunnett's *P* vs.**	
Cultivar	**Mean**	**LL**	**UL**	**Rowden**	**M315**
Americot 1550 B2RF	194	18	2047	1.000	0.537
Croplan Genetics 3787 B2RF	528	50	5583	1.000	0.158
Dyna Gro 2570 B2RF	205	26	1648	1.000	0.397
Deltapine 0912 B2RF	316	39	2536	1.000	0.222
Deltapine 0949 B2RF	281	27	2969	1.000	0.362
Deltapine 1028 B2RF	544	32	9278	1.000	0.282
Deltapine 1050 B2RF	36	3	379	0.843	1.000
Deltapine 10R052B2R2	19	2	205	0.539	1.000
Deltapine 1137 B2RF	185	11	3161	1.000	0.728
Fiber Max 1740 B2F	297	37	2383	1.000	0.244
Phytogen 367 WRF	147	14	1553	1.000	0.681
Phytogen 375 WRF	431	54	3466	1.000	0.137
Phytogen 485 WRF	227	13	3869	1.000	0.637
Phytogen 565 WRF	427	40	4517	1.000	0.213
Stoneville 4288 B2F	26	2	278	0.696	1.000
Stoneville 5288 B2F	24	2	259	0.659	1.000
Stoneville 5458 B2RF	344	33	3642	1.000	0.283
M-315	14	2	111	0.278	
Rowden	270	34	2168		0.278

Table 2.7. Least squares estimate of seed cotton yield (kg/ha) in 2011 for cotton cultivars and checks, confidence intervals, and *P*-values based on Dunnett's versus the susceptible check Rowden and the resistant check M-315

	Seed cotton kg/ ha		**Dunnett's *P* vs.**	
Cultivar	**Mean**	**SE**	**Rowden**	**M315**
Americot 1550 B2RF	4160	422	0.146	0.004
Croplan Genetics 3787 B2RF	4732	422	0.012	0.000
Dyna Gro 2570 B2RF	3639	363	0.585	0.025
Deltapine 0912 B2RF	4016	363	0.165	0.003
Deltapine 0949 B2RF	3320	422	0.978	0.180
Deltapine 1028 B2RF	4592	521	0.065	0.002
Deltapine 1050 B2RF	3629	422	0.705	0.051
Deltapine 10R052B2R2	3425	422	0.925	0.120
Deltapine 1137 B2RF	3991	521	0.435	0.032
Fiber Max 1740 B2F	3631	363	0.597	0.026
Phytogen 367 WRF	3265	422	0.991	0.219
Phytogen 375 WRF	4008	363	0.170	0.003
Phytogen 485 WRF	4536	521	0.080	0.003
Phytogen 565 WRF	3309	422	0.982	0.187
Stoneville 4288 B2F	5233	422	0.001	0.000
Stoneville 5288 B2F	4217	422	0.117	0.003
Stoneville5458 B2RF	4707	422	0.013	0.000
M-315	1951	363	0.756	
Rowden	2736	363		0.756

Figure 2.3. Canonical discriminant analysis of cotton cultivar yield and nematode numbers for 2011. CAN 1 is seed cotton yield (r = 0.98) thus yield increases moving away from the Y axis. Differences in CAN 2 are driven by root knot egg numbers with higher numbers in the top of the x axis.



Class Means on Canonical Variables					
Cultivar	**Can1**	**Cultivar**	**M-315**	**Rowden**	**kg_ha**
M-315	-3.08		-0.39		1951
Rowden	-1.31			0.57	2736
Deltapine 10R052B2R2	-0.95	-0.82			3425
Deltapine 1050 B2RF	-0.70	-0.59			3629
Phytogen 367 WRF	-0.55	-0.11			3265
Deltapine 0949 B2RF	-0.51	0.63			3320
Phytogen 565 WRF	-0.50	0.31			3309
Deltapine 1137 B2RF	-0.04	-0.78			3991
Dyna Gro 2570 B2RF	0.19	0.61			3639
Americot 1550 B2RF	0.34	-0.38			4160
Fiber Max 1740 B2F	0.40	1.49			3631
Deltapine 0912 B2RF	0.51	0.36			4016
Deltapine 1028 B2RF	0.65	-1.33			4592
Stoneville 5288 B2F	0.65	-0.41			4217
Phytogen 485 WRF	0.83	-0.63			4536
Phytogen 375 WRF	0.95	1.26			4009
Croplan Genetics 3787 B2RF	1.23	-0.76			4732
Stoneville 4288 B2F	1.56	-1.26			5233
Stoneville 5458 B2RF	1.58	0.02			4707

Fusarium oxysporum f. sp. _vasinfectum_ Race Identification in Alabama Soils

Abstract

Familiarity of the genetic and pathogenic diversity currently present in *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) populations is essential to successfully establishing resistant cultivars. Fusarium wilt of cotton, caused by FOV, is a widespread disease in the United States with few to no effective management practices. New reports of the disease's development have been publicized since the recent discovery of the race 4, a highly aggressive strain of FOV, in California (Kim, Y. *et al.*, 2005) and the emergence of virulent strains of the fungus in Australia (Wang, B. *et al.*, 2007). Morphologically, isolates obtained from Alabama cotton fields corresponded with common FOV characteristics. Isolates were further characterized by partial sequences of translational elongation factor (EF-1α), beta tubulin (BT), and phosphate permase (PHO) genes. Based on phylogenetic analysis of combined sequences, the Alabama isolates represented three lineages within FOV. The majority of Alabama isolates aligned with lineage II which contains races 1, 2 and 6. Although a few Alabama isolates were in lineage III, containing race 8, and lineage IV, containing race 4. These results indicate that the population of *F. oxysporum* f. sp. *vasinfectum* in Alabama is more diverse than previously reported, and that additional research and survey efforts are needed.

Introduction

Fusarium wilt of cotton occurs in most cotton growing areas of the world. The causal organism, *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *vasinfectum* (Atk.) W.C. Synder & H.N. Hans., invades the host through the tap roots behind the root tip, leading to obstruction of the vascular tissue, and resulting in wilting of the cotton plant. All strains of *F. oxysporum* are successful saprophytes and are able to grow and survive for long periods on organic matter in soil. Thus once this pathogen is introduced into a field it has the capacity for longevity. Recent developments have elevated the status of the disease to international importance. One is the emergence of virulent biotypes of the fungus in Australia (Davis, R. D. *et al*., 1996) and another is the recent discovery of the highly virulent race 4 in California's San Joaquin Valley in the United States (Kim, Y. *et al*., 2005).

Currently, eight races have been described for this wilt pathogen. Races 1 and 2 were described in the United States and Tanzania, race 3 in Egypt, Sudan, China, California, and Israel, race 4 in India, Uzbekistan, and China, race 5 in Sudan, race 6 in Brazil and Paraguay, and race 7 and 8 in China (Armstrong, G.M., and Armstrong, J.K. 1960; Wenji *et al*., 1999, Assigbetse, K.B. *et al*., 1994; Abo, K., *et al.* 2005; Kim, Y. *et al*., 2005). DNA, restriction fragment length polymorphism (RFLP) markers, fatty acid profiles, multigene genealogies, and pathogenicity tests (Holmes, E.A., *et al*., 2009; Assigbetse, K.B. *et al*., 1994; Gilbert, C. A., *et al* 2008) indicate potential redundancies in race designations. Therefore races 1, 2, and 6; 3 and 5; and 4 and 7, are found likely to be identical or nearly so Kim *et al*, 2005. Further assembled the races into lineages would separate into lineage I contained race 3, an independent clade, lineage II contained races 1, 2, and 6, lineage III contained race 8, and lineage IV contained race 4. In

addition to these races, there are two Australian biotypes of FOV, which fall in two vegetative compatibility groups (VCGs), 01111 and 01112 (Zambounis, A.G. *et al*., 2007; Kim, Y. *et al*., 2005). These two biotypes have spread rapidly in Australian and appear to be pathogenically similar to race 6 (Davis, R.M. *et al*., 2006) on cotton cultivars. With the discovery of the novel virulent genotypes in Australia and race 4 in the United States, concern may be reasonable for other genotypes posing considerable threats to current cotton production.

Races of FOV were initially classified based on pathogenicity tests on different cotton species, *Gossypium hirsutum*, *Gossypium barbadense*, and *Gossypium arboretum* (Armstrong, G.M. and Armstrong, J.K. 1958, 1978; Ibrahim, F.M. 1966), and other plant hosts including okra, alfalfa, and tobacco to distinguish eight races. However, current detection and identification of species of the genus *Fusarium* is based on a combination of diverse molecular markers to identify various races within *formae specialis*, in addition to pathogenicity assays (Baayen, R.P. *et al*., 2000; O'Donnell, K. *et al*., 2009). This system has further characterized forma specialis *vasinfectum* into races categorized into five major lineages of FOV strains worldwide: lineages I, II, III, IV, and V (Skovgaard, K. *et al*., 2001).

Partial sequences of translational elongation factor (EF-1α), nitrate reductase, phosphate permase, and the mitochondrial small subunit rDNA genes were sequenced by Skovgaard, K., *et al.* in 2001 to compare *F. oxysporum* f. sp. *vasinfectum* strains and determine evolutionary origins. Likewise, Kim, Y., *et al*. (2005) used partial sequences of translational elongation factor (EF-1α), phosphate permase, beta-tubulin genes, and restriction digests of the intergenic spacer region of nuclear rDNA (IGS) to investigate the genetic diversity of FOV in California. In these studies, the isolates were acceptably characterized by the partial sequences of translational elongation factor (EF-1α), which proved to be the most informative gene.

The objective of this study is to identify what races or genotypes of FOV exist in Auburn University's cotton breeding fields, which are naturally infested with the Fusarium wilt fungus, by using current classification procedures. This will be accomplished by objective 4: identify the FOV race or races present in the Auburn University's Fusarium wilt fields; and objective 5, identify FOV races found in the common commercial cotton cultivars grown in Alabama.

Materials and Methods

[1]Fungal isolation and sequence analysis

A total of 94 isolates of *F. oxysporum* f. sp. *vasinfectum* collected from the E.V. Smith Research and Extension Center, Plant Breeding Unit, near Tallassee, AL in 2010 and 2011 were examined. Isolates were obtained from cotton plants exhibiting symptoms typical of Fusarium wilt: stunted growth, vascular discoloration, wilt, chlorosis, and necrosis of leaves. In 2010, isolates were acquired from commercial cultivars, Phytogen 375 WRF, Phytogen 565 WRF, Phytogen 485 WRF, Deltapine 1050 B2RF, Deltapine 0949 B2RF, Stoneville 4288 B2RF, and the susceptible cotton line Rowden. In 2011, isolates were acquired from commercial cultivars Deltapine 1028 B2RF, Deltapine 1050 B2RF, Deltapine 0949 B2RF, Deltapine 10R052B2R2, Deltapine 1137 B2RF, Stoneville 4288 B2RF, Stoneville 5458 B2RF, Croplan Genetics 3787 B2RF, Fibermax 1740 B2F and the susceptible cotton line Rowden. Fungi were isolated from the hypocotyl and upper tap roots of symptomatic cotton plants and grown on acidified Potato Dextrose Agar (aPDA) at 27°C for 7 days. Cultures were identified and confirmed to be FOV and then maintained on ¼-strength PDA slants and stored at 4°C for later use.

To obtain DNA from cultures, *F. oxysporum* f. sp. *vasinfectum* isolates were grown for 4 to 5 days at room temperature in 1/2-strength Potato Dextrose Broth (PDB) and placed on a

rotary shaker (130 rpm).The mycelium was harvested by centrifuging, decanting the broth, and rinsing in sterile distilled water. To obtain total genomic DNA, 200 mg of rinsed mycelia was ground into a powder in liquid nitrogen. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) following the manufacturer's protocol, and stored at -20°C prior to use

The intergenic spacer (IGS) region of nuclear rDNA was amplified with the conserved primers LR12R and CNS1, with priming sites at the 3′ end of the 28S gene and the 5′ end of the 18S gene, respectively, to prescreen the isolates for potential novel genotypes. All isolates were further examined by amplifying portions of additional nuclear genes with the following primers, translation elongation factor primers (EF-1α), EF-1 and EF-2, β-tubulin (BT) primers, BT-3 and BT-5, and phosphate permase (PHO) primers, PHO-1 and PHO-6 [(Kim, Y., *et al.* 2005); Table 3.1]. PCR amplification was conducted in 50-µl reactions containing 3 µl of template DNA, 25 µl of EconoTaq Plus Green 2x Master Mix (Lucigen Corporation, Middleton, WI; 0.1 units/µl of EconoTaq DNA polymerase, reaction buffers (pH 9.0), 400 µM each of dATP, dCTP, dGTP, and dTTP, and 3 mM MgCl2), 1 µM each of forward and reverse primers, and 20 µl of nuclease-free water. Cycling conditions for IGS was previously described in (Kim, Y., *et al*., 2005). Additional reactions were conducted in a thermal cycler under the following parameters: 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and a final extension of 5 min at 72°C for EF-1α; 35 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min 30 s for BT; 97°C for 1 min of initial denaturing followed by 35 cycles of 96°C for 30 s, 53°C for 1 min, and 72°C for 1 min followed by a final extension time of 10 min at 72°C for PHO (Kim, Y. *et al*., 2005). Two negative controls of no template DNA were included in each PCR reaction. One control was a water control lacking DNA, and the other consisted of all reagents minus the primer. Amplification

products were visualized under UV light on 1.5% agarose gels stained with ethidium bromide. The PCR products were purified with QIAquick PCR Purification Kits (Qiagen, Inc.) using the manufacturer's protocol. Sequencing was conducted at the Eurofins MWG Operon DNA Sequencing Lab (Huntsville, AL).

Phylogenetic analyses were conducted on individual genes and the concatenated three-gene alignments for the 2010 and 2011 isolates obtained from commercial cotton lines and known susceptible cotton line. The software program, Geneious, version 5.6 (Drummond, A. J., *et al*. 2011.), was used to trim low-quality sequence end reads, create consensus gene sequences, translate sequences, and align sequences using the Geneious ClustalW plug-in. To reconstruct the evolutionary relationships of the *F. oxysporum* isolates, phylogenies were generated for each individual gene alignment and for the concatenated sequence alignment. Bayesian phylogenetic (BP) analyses were conducted using the MrBayes program, version 3.1.2 (Ronquist, F. and Huelsenbeck, J.P. 2003), with two chains of 2 million generations each. Trees were sampled every 200 generations, and the first 5,000 trees (10%) were discarded as burn-in for each chain, prior to generating the extended-majority-rule consensus tree. Maximum parsimony (MP) analyses were performed using the MEGA version 5.05 program and statistical support for branches was obtained by running1, 000 bootstraps and tree bisection with reconnection branch swapping. Gaps were treated as missing data. Trees were rooted in both programs with ATCC *Fusarium oxysporum* isolate 1502 as the outgroup. Previously published EF-1α sequences of *F. oxysporum* f. sp. *vasinfectum* (Skovgaard *et al*., 2001; Holmes *et al*., 2009; O'Donnell *et al*., 2000; 2009) were downloaded from GenBank for comparison to the newly sequenced isolates. These included sequences from reference isolates ATCC-16421 (race 1), ATCC-16611 (race 2),

ATCC-16612 (race 3), ATCC-16613 (race 4), 31665 (race 8), and AUST16 and 19 (Australian races).

[2]Greenhouse pathogenicity

The pathogenicity of the Alabama *F. oxysporum* f. sp. *vasinfectum* isolates representing races 1, 4, and 8 were obtained to compare the virulence among genotypes. Four trials with treatments of five cotton cultivars, three *Fusarium* isolates, plus a water control were tested, with a single potted plant within each block serving as the experimental unit for each of the 15 combinations of cultivar and fungal isolate. One Upland cultivar (*G. hirsutum*) Phytogen 367 WRF, two Pima cultivars (*G. barbadense*) Phytogen 800 and Delta Pine 744, and two Fusarium wilt control cultivars, resistant M-315 and susceptible Rowden, were planted in 12-cm-diameter plastic pots filled with sterilized potting mix. Alabama fungal isolates representing races 1, 4, and 8 were acquired from symptomatic plants obtained at the E.V. Smith Research and Extension Center, Plant Breeding Unit, near Tallassee, AL. Spore suspensions were prepared from week-old cultures grown in potato dextrose broth, spores were filtered through two layers of cheesecloth and blended with water to 1×10^5 conidia per ml, and inoculated into week old cultivars. Each trial was replicated four times in a randomized complete block design. Since the FOV pathogen is more virulent in the presence of nematodes, 2,000 J2 stage *M. incognita* in 5 ml of water was inoculated for potential disease incidence increase in each treatment of two trials. Greenhouse temperatures ranged between 20 and 25°C. Foliar symptoms and vascular discoloration were measured approximately 6 weeks after inoculation. Individual plants were rated for vascular necrosis based on a 0 to 5 scale: 0 = no discoloration, 1 = subtle brown vascular discoloration, 2 = light brown, streaky vascular discoloration, 3 = brown vascular discoloration, 4 = dark brown vascular discoloration, 5 = darkest brown to black discoloration

(Figure3.10). Plant height was measured in centimeters. Response variables of plant height and necrosis were each examined using mixed models (PROC GLIMMIX, SAS release 9.3 ed.; SAS Institute, Cary, NC). In both analyses, fixed effects included cultivar, fungal isolate, and their interaction.

Results

[1]Cultural characteristics and phylogenetic analyses

Morphologically, isolates obtained from Alabama cotton fields in 2010 and 2011 corresponded with common FOV characteristics. Colonies from these isolates appeared white and loosely floccose, with the purple pigments on the reverse side intensifying over time (Figure 3.1). Conidiogenous cells were monophialidic, short, and single. Microconidia were abundant, ellipsoid, 1-celled, and averaged 10.09-2.58 µm. Macroconidia were falcate with tapering apical and basal cells, had 3 to 5 septa, and averaged 18.40-4.86 µm. Chlamydospores were rough-walled, sub-globose, and 8.73-3.4 µm in. Polymerase chain reaction (PCR) product of 2010 *Fusarium oxysporum* f. sp. *vasinfectum* using the intergenic spacer (IGS) region of nuclear rDNA to prescreen FOV and give an initial banding pattern revealed polymorphisms useful in distinguishing isolates as FOV (Figure 3.2).

From the 95 isolates obtained in 2010 and 2011, all sites and parsimony-informative sites (in parentheses), the consistency index for individual genes EF, BT, and PHO was 1.0 (1.0), the retention index was 1.0 (1.0), and the composite index was 1.0 (1.0). Amplicons were approximately 680 base pair (bp) for the EF-1α gene from isolates collected. A total of 630 nucleotides were analyzed with 97% pairwise residues and 75.9% identical alignment, with 62 variable sites; 12 were phylogenetically informative. The evolutionary history was inferred using

maximum parsimony of the EF-1α gene data, and produced a consensus tree inferred from 470 most parsimonious trees, with five clades each. Of the 12 isolates collected in 2010, eight were genotypically identical to race 1 belonging to lineage II, three were identical to race 8 belonging to lineage III, and one isolate was identical to race 4 belonging to lineage IV (Figure 3.3).

Additional analysis of 2010 and 2011 isolates with beta tubulin gene data yielded sorted topology and parsimonious trees with clades identical in position. Amplification of the BT gene yielded a PCR product with an approximate length of 600 base pairs. A total of 394 nucleotides were analyzed with 99.6% pairwise residues and 97.2% identical alignment with 9 variable sites; two were phylogenetically informative. The evolutionary history inferred using MP in MEGA 5.05 revealed a single consensus tree inferred from 350 most parsimonious trees with two clades. Eight Alabama isolates were genetically similar to lineage II, while all other isolates and known FOV biotypes were grouped into an additional clade (Figure 3.4). Amplification of all isolates collected with the PHO gene yielded a PCR product with an approximate length of 900 base pairs. A total of 513 nucleotides were analyzed with 99.3% pairwise residues and 92% identical alignment, with 18 variable sites; three were phylogenetically informative. The evolutionary history inferred using the MP analysis yielded one most parsimonious tree with a single clade (Figure 3.5).

Concatenated sequences from the isolates collected were 1,595 bp in length with 98.4% pairwise residues and 86.5% identical alignment. All positions containing gaps and missing data were eliminated. The evolutionary history inferred using MP revealed a consensus tree inferred from 480 most parsimonious trees. Branches corresponding to partitions reproduced in less than 50% trees are collapsed. The consistency index was 1.0 (1.0), the retention index was 1.0 (1.0), and the composite index was 1.0 (1.0) for all sites (and parsimony-informative sites). When all

three genes were analyzed as a combined data set, parsimony analysis revealed one most parsimonious tree with five distinct clades. In 2010, 67% of isolates were found to be genotypically identical to race 1. The four cultivars that produced these isolates were Phytogen 565 WRF, Phytogen 485 WRF, Deltapine 1050 B2RF, and Rowden. Three cultivars had isolates genotypically identical to race 8, Stoneville 4288 B2RF, Phytogen 565 WRF, and Deltapine 0949 B2RF. Only one cultivar had an isolate to be genotypically similar to race 4 in 2010, Phytogen 375 WRF (Figure 3.6). In 2011, 75% of isolates were genotypically identical to race 4. These isolates were obtained from cultivars Deltapine 1050 B2RF, Deltapine 0949 B2RF, Deltapine 10R052B2R2, Deltapine 1137 B2RF, Stoneville 4288 B2RF, Stoneville 5458 B2RF, Croplan Genetics 3787 B2RF, Fibermax 1740 B2F, and Rowden. Three cultivars had isolates genotypically identical to race 1, Deltapine 1028 B2RF, Deltapine 1137 B2RF, and Rowden. Only one cultivar had an isolate to be genotypically similar to race 8 in 2011, Deltapine 1028 B2RF (Figure 3.7 and 3.8).

A separate alignment with isolates obtained in 2011 from the Fusarium wilt susceptible line Rowden was performed to see if different races could be found inhabiting one cultivar variety. Amplification of EF-1α, BT, and PHO on isolates was analyzed to show a 98.4% pairwise residue and 74.6% identical alignment in MrBayes. When all three genes were analyzed as a combined data set, parsimony analysis revealed one most parsimonious tree with five distinct FOV clades and the bulk of isolates belonging to the lineage II. The placement of two isolates above all known FOV biotypes was unresolved, and positioned above the five clades. No isolates obtained were genetically similar to race 3 or the Australian biotypes (Figure 3.9).

[2]Greenhouse pathogenicity

All isolates representing lineages II, III, and IV were virulent on the cotton cultivars although there was a range of symptoms and level of aggressiveness. Symptoms included leaf chlorosis and necrosis, plant wilt and stunt, and occasional plant death. Aggressive isolates, caused a cessation of growth; veins darkened, leaves became partly chlorotic and sometimes detached. Vascular discoloration usually occurred in the tap root and in the lower part of the stem approximately 1 to 2 cm above the soil level. Relatively less aggressive isolates often caused a slight yellowing of cotyledons and light vascular discoloration. There was an overall substantial difference in the cultivars with root knot nematode inoculum, than without RKN inoculation. Over all, Race 1 with the presence of nematodes averaged a significantly lower plant height than races 4 and 8. Differentiating by necrosis, overall race 4 without the presence of nematodes had significantly higher disease rating than race 8 but not race 1. Resistant Fusarium wilt complex cultivar M-315 isolated with race 4 had significantly higher plant heights than the susceptible cultivar, Rowden, isolated with race 1 ($P \leq 0.05$). M-315 had significantly higher necrosis rating with race 4 isolate, compared to with race 1 isolate. M-315 also averaged the lowest mean plant height when inoculated with race 1 and RKN (Table 3.2); conversely, M-315 also averaged the highest mean plant height when inoculated with race 4 and RKN. Pima commercial cultivar Deltapine 744 is a highly susceptible variety to FOV race 4; it is known to suffer substantial crop damage without the presence of nematodes (Kim, Y., *et al*, 2005). Deltapine 744 inoculated with Alabama isolates representing race 1, 4, and 8 revealed race 1 significantly reduced plant heights more than race 4 and 8, with and without the presence of RKN; root necrosis rating for Deltapine 744 with and without nematodes were similar.

Discussion

Presently, there are few options for managing Fusarium wilt in cotton. With detailed knowledge of the genetic variation and evolutionary origins and relationships within *Fusarium oxysporum* f. sp. *vasinfectum,* a framework for developing more effective disease control strategies and breeding programs can be developed. Diverse lineages of the FOV fungus were identified in this study based on differences in EF-1α, BT, and PHO sequences. The most informative gene, EF-1α, identified isolates collected in 2010as belonging to three known lineages, lineages II, III and IV. Bennett, R.S., *et al.,* (2011) reported similar results from isolates obtained in Alabama during 2010. This study used data from the EF-1α gene to show the 15 Alabama isolates tested were genetically diverse, and a few of those isolates appeared to be closely related to race 4 isolates from California and Asia.

Although environmental conditions in 2010 were more conducive to Fusarium wilt, isolates collected in 2011 were genetically similar to those collected in 2010. The isolates collected in 2011 also clustered among three lineages, lineage II, III, and IV, with majority of the isolates belonging to lineage IV (race 4). Neither the 2010 nor 2011 cotton seasons produced isolates from lineages including race 3 (lineage I) or the Australian biotypes (lineage V). Sequence analysis from the Fusarium wilt susceptible line Rowden proved one cultivar could accommodate diverse FOV races; lineages II, III, and IV were all observed inhabiting the cultivar Rowden.

The most significant finding from these data is that isolates genetically similar to race 4 are present in Alabama. However, based on symptoms found on cotton, these isolates were not as virulent as FOV race 4 isolates in California. Race 4-like isolates from Alabama were identical to race 4 isolates from California with the EF-1α gene (Bennett, R.S., *et al.,* 2011). However, subsequent comparisons of the nuclear ribosomal DNA intergenic spacer region (IGS rDNA)

indicated differences between Alabama race 4-like isolates and California race 4 isolates (R. Bennett, personal communication). Conflicting EF-1α and IGS phylogenies were previously reported in *F. oxysporum* (O'Donnell, K. *et al*., 2009). In 2009, O'Donnell *et al*. presented a study using differences in phylogenies obtained from EF-1α and IGS rDNA to develop a two-locus database for identifying *Fusarium oxysporum* forma specialis (O'Donnell, K. *et al*., 2009). The study illustrated the homoplastic evolutionary history of the IGS rDNA locus, and the utility of combined EF-1 α and IGS rDNA dataset for identifying *Fusarium oxysporum* genotypes (O'Donnell, K. *et al*., 2009). Consequently, with Alabama and California FOV race 4 being genotypically similar but performing alternatively, it would be in the best interest of researchers to sequence both EF-1 α and IGS rDNA, to accurately identify genotypes of FOV.

Although Alabama isolates were weak pathogens on the varieties tested by Bennett, R.S. (personal communication), isolates obtained in this study were isolated from symptomatic field plants and were able to cause disease on cotton grown in the greenhouse. *Fusarium oxysporum* f. sp. *vasinfectum* isolates obtained from symptomatic cotton plants, which were molecularly identical to race 1at the EF-1 α gene, was significantly more aggressive on the cotton than isolates of races 4 and 8. While all isolates successfully colonized the cotton cultivars, most isolates in the three lineages tested caused symptoms but were not virulent to cause plant death. In earlier studies, the ability of race 4 to cause disease varied with its cotton host. Race 4 in California was shown to be highly virulent on Pima varieties tested (Kim, Y. *et al*., 2005), and known for its potent disease loss without the presence of nematodes; however, there was no significant differences in Pima and Upland cultivars necrosis when inoculated with the race 4 strain from Alabama. Likewise Phytogen 367 WRF, an Upland cultivar that is known for its resistance to Fusarium wilt complex because of its resistance to RKN (McPherson, M. and Rush,

D. 2011), produced no significant difference in disease ratings between the three races. This leads to the conclusion that although Alabama isolates are genetically similar at the EF-1α locus to California isolates of race 4, pathogenicity test reveal isolates to be contrasting. It is possible that isolates lost pathogenicity during storage (Nelson, P. E., *et al*., 1981).

In 1892, *F. oxysporum* f. sp. *vasinfectum* was first identified in the United States in Alabama cotton fields by Atkinson (Armstrong, G. M. and Armstrong, J. K. 1981). Since this time, countless cotton lines around the world have been tested for Fusarium wilt resistance within this filed, with little knowledge of what races inhabit the soil. These same fields are where the FOV isolates for this study were collected. Responding to the objectives of this study discovered that FOV within Alabama fields grouped into lineage II (race 1), III (race 8), and IV (race 4) based on partial sequence differences in EF-1α, BT, and PHO genes. These different races were collected from all cotton varieties with no preference by cotton variety. Pathogenically the Alabama race 4 isolate did not perform as the virulent race 4 California strain. Because race 4 and the Australian biotypes, unlike the other known races, are highly virulent on certain cultivars of cotton, the rapid identification of specific races is desired for management of the disease. However, in Alabama FOV isolates belonging to race 1 are more common and seem to be more virulent in the field. In future studies, the attempt to discriminate lineages further by screening additional *Gossypium* as well as analyzing more isolates of *F. oxysporum* f. sp. *vasinfectum* using additional markers need to be performed.

Literature Cited

Abo, K., Klein, K. K., Edel-Hermann, V., Gautheron, N., Traore, D, Steinberg C. 2005. High genetic diversity among strains of *Fusarium oxysporum* f.sp. *vasinfectum* from cotton in Ivory Coast. Phytopathology 95:1391–6.

Armstrong, J. K., and Armstrong, G. M. 1958. A race of the cotton-wilt *Fusarium* causing wilt of Yelredo soybean and flue-cured tobacco. Plant Disease Report 42:147-151.

Armstrong, G. M., and Armstrong, J. K. 1960. American, Egyptian, and Indian cotton-wilt Fusaria: Their pathogenicity and relationship to other wilt fusaria. U.S. Department of Agriculture Technical Bulletin. 1219.

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

Armstrong, G. M. & Armstrong, J. K. 1981. Formae speciales and races of Fusarium oxysporum causing wilt diseases. In *Fusarium*: disease, biology, and taxonomy (P. E. Nelson, T. A. Toussoun & R. J. Cook, eds), pages 391–399. Pennsylvania State University Press, University Park, PN.

Assigbetse, K. B., Fernandez, D., Dubois, M. P., and Geiger, J.-P. 1994. Differentiation of *Fusarium oxysporum* f. sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. Phytopathology 84:622-626.

Baayen, R. P., O'Donnell, K., Waalwijk, C., Bonants, P. J. M., Cigelnik, E., Kroon, P. N. M., and Roebroeck, E. J. A. 2000. Gene genealogies and AFLP analyses within the *Fusarium oxysporum* complex identify monophyletic and non-monophyletic *formae speciales* causing wilt and rot disease. Phytopathology 90:891-900.

Bennett, R., *et al.,* 2011. Progress report on a contemporary survey of the Fusarium wilt fungus in the United States. Proceedings. Beltwide Cotton Conferences. 1:267. National Cotton Council, Atlanta, GA. Jan. 2011

Davis, R. D., Moore, N. Y., and Kochman, J. K. 1996. Characterisation of a population of *Fusarium oxysporum* f. sp. *vasinfectum* causing wilt of cotton in Australia. Australian Journal of Agriculture Research. 47:1143-1156.

Davis, R. M., P. D. Colyer, C. S. Rothrock, and J. K. Kochman. 2006. Fusarium Wilt of Cotton: Population Diversity and Implications for Management. Plant Disease. 90:692-703.

Drummond, AJ, *et al*,. 2011. Geneious, version 5.4. Geneious, Auckland, New Zealand.

Gilbert, C. A., Zhang, N., Hutmacher, R. B., Davis, R. M., and Smart, C. D. 2008. Development of a DNA-based macroarray for the detection and identification of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton tissue. Journal of Cotton Science. 12:165-170.

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

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

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

McPherson, M., and Rush, D. 2011. Efficacy of PHY367WRF in root-knot infested fields. Proc. Beltwide Cotton Conferences., 1:248. National Cotton Council, Atlanta, GA. Jan. 2011

Nelson, P. E., Toussoun, T. A., and Cook, R. J. 1981. *Fusarium*: Disease, Biology, and Taxonomy. The Pennsylvania State University Press, University Park.

O'Donnell, K., Kistler, H. C., Tacke, B. K., and Casper, H. H. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proceeding. National. Academy of Science. USA 97:7905-7910

O'Donnell, K., *et al*., 2009. A two locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. Fungal Genetics of Biology. 46:936-948.

Ronquist, F, and Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Skovgaard, K., Nirenberg, H.I., and O'Donnell, K., and Rosendahl S., 2001. Evolution of *Fusarium oxysporum* f.sp. *vasinfectum* races inferred from multigene genealogies. Phytopathology 91, 1231–7.

Wang, B., Brubaker, C. L., Tate, W., Woods, M. J., and Burdon, J. J. 2007. Evolution of virulence in *Fusarium oxysporum* f. sp. *vasinfectum* using serial passage assays through susceptible cotton. Phytopathology 98:296-303.

Wenji, S., Liang, J.G., Ying, C.Q., Sun, W.J., Jian, G.L., and Chen, Q.Y. 1999. Study on monitoring of physiological race of cotton Fusarium wilt in China. Science Agriculture Sinica. 32:51–57.

Zambounis, A.G., Paplomatas, E., and Tsaftaris, A.S. 2007. Intergenic spacer-RFLP analysis and direct quantification of Australian *Fusarium oxysporum* f. sp *vasinfectum* isolates from soil and infected cotton tissues. Plant Disease. 91:1564–73.

Table 3.1. Primers used in this study.

Locus	**Primer sequence**
Translational elongation factor (EF-1α)	
EF-1	ATGGGTAAGGAAGACAAGAC
EF-2	GGAAGTACCAGTGATCATGTT
Phosphate permase (PHO)	
PHO 1	ATCTTCTGGCGTGTTATCATG
PHO 6	GATGTGGTTGTAAGCAAAGCCC
Beta-tubulin (BT)	
BT 3	CGTCTAGAGGTACCCATACCGGCA
BT 5	GCTCTAGACTGCTTTCTGGCAGACC
Intergenic spacer (IGS) region	
LR12R	CTGAACGCCTCTAAGTCAGAA
CNS1	GAGACAAGCATATGACTAC

Figure 3.1. Morphology of *Fusarium oxysporum* f. sp. *vasinfectum* colony growing on potato dextrose agar, with white, loosely floccose mycelium, and purple pigments on reverse side.

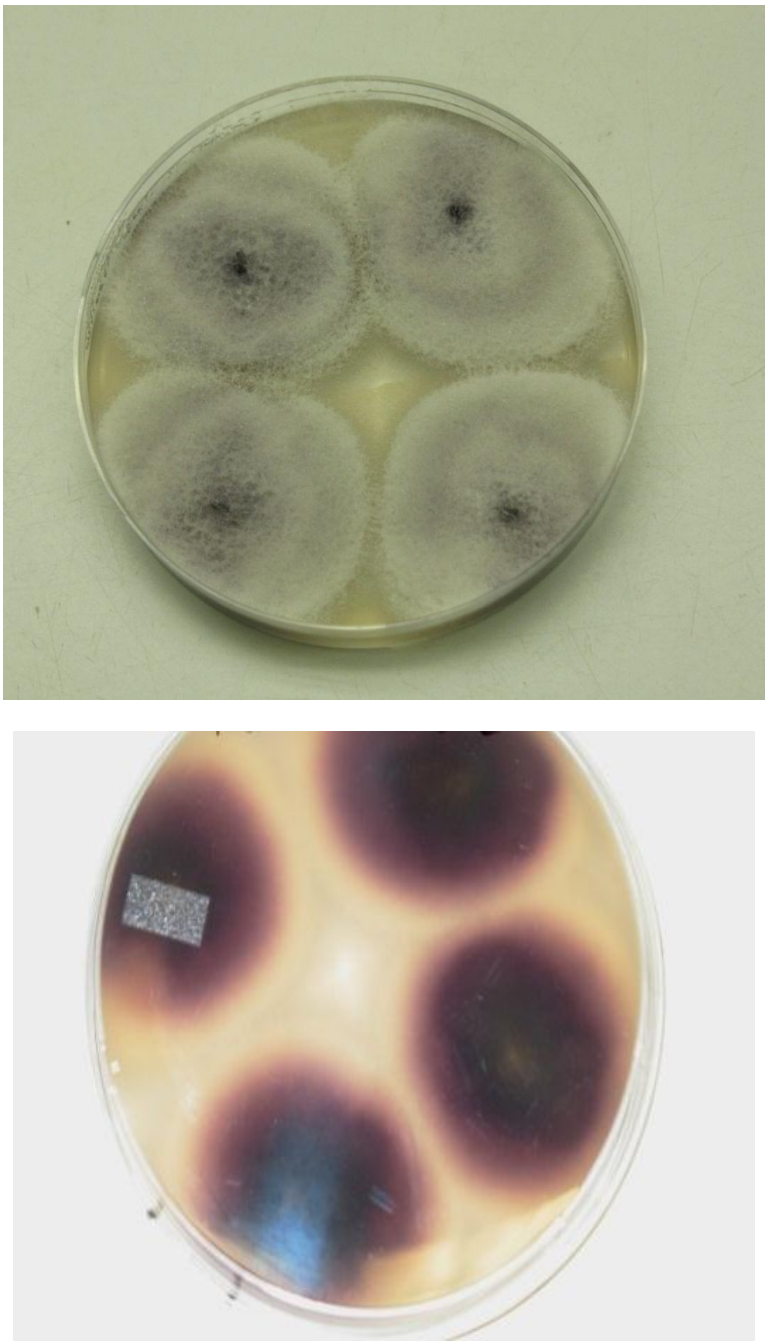
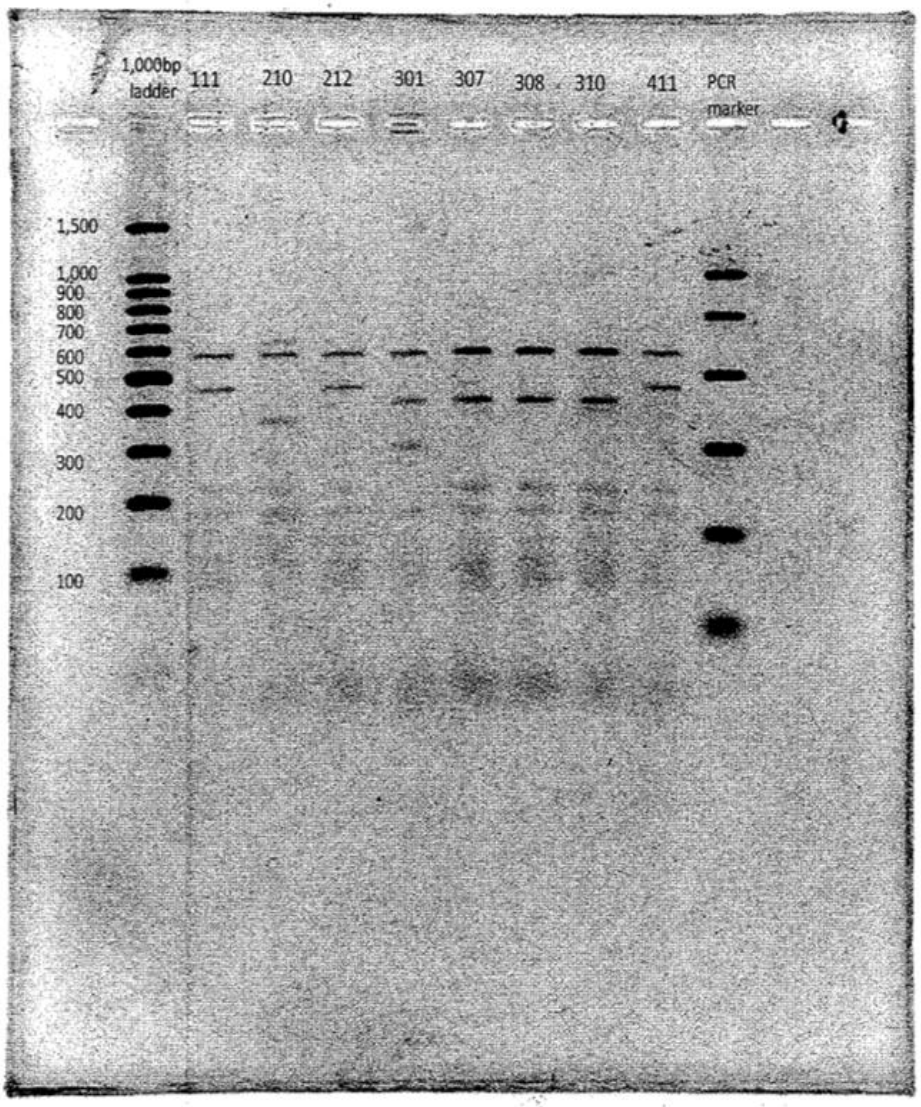


Figure 3.2. Amplified intergenic spacer (IGS) polymerase chain reaction (PCR) product of 2010 *Fusarium oxysporum* f. sp. *vasinfectum* isolates showing the diversity of PCR product migration of isolates. Lanes 1to 8 of commercial cultivar (synonymous molecular ID race): PHY 565 (race 1), PHY 375 (race 4), PHY 485 (race 1), DP 1028 (NA), FM 1740 (NA), PHY 565 (race 8), DP 0949 (race 8), PHY 485 (NA). Fragment sizes in kb.



NA= sequence results not applicable

Figure 3.3. Consensus of two most parsimonious trees inferred from the EF-1α dataset of 2010. Numbers above nodes indicate bootstrap intervals from 1000 replicates.

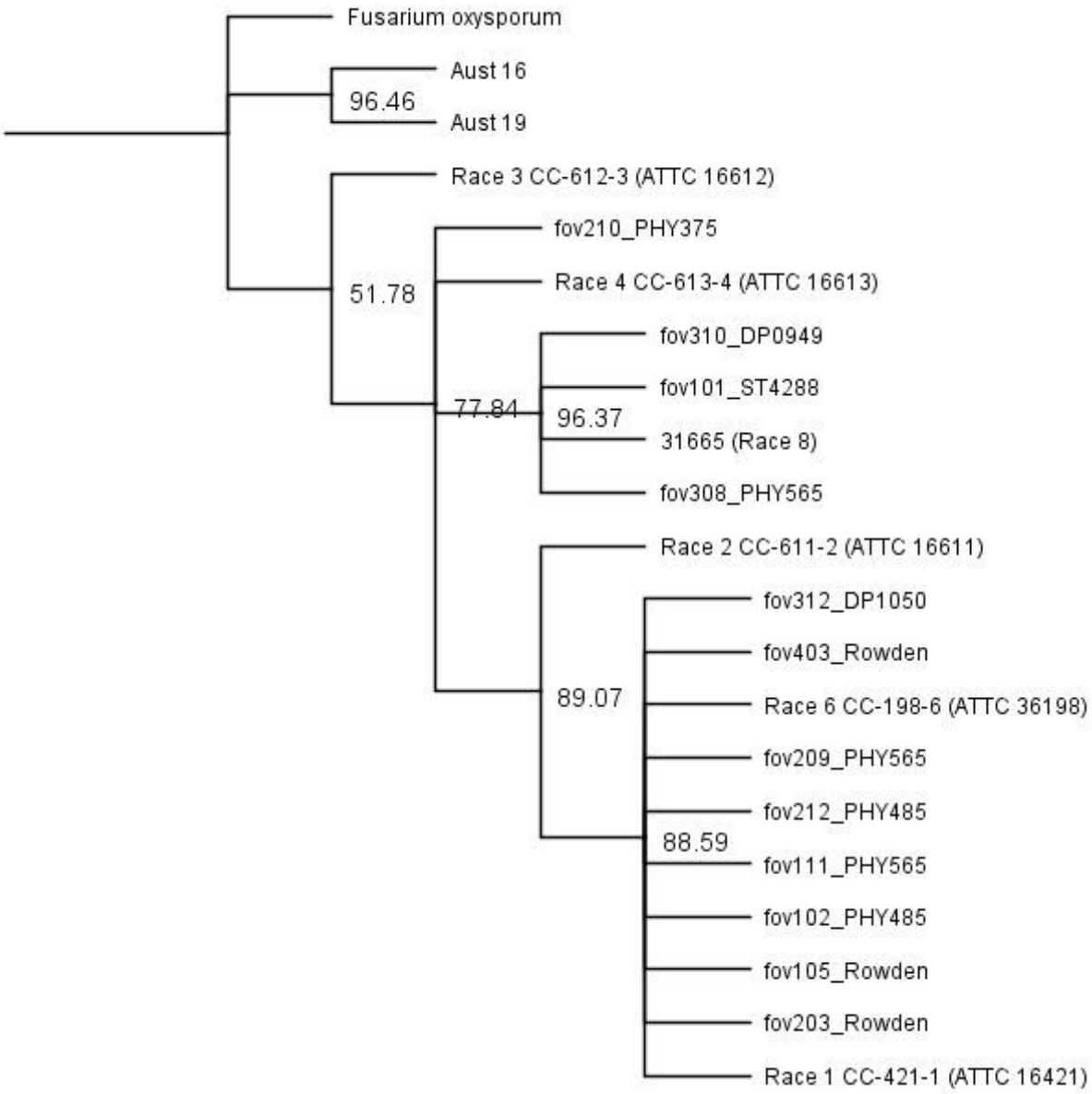


Figure 3.4. Single most parsimonious tree generated from analysis of the beta tubulin gene on the isolates collected in 2010. Numbers above nodes indicate bootstrap intervals from 1000 replicates.

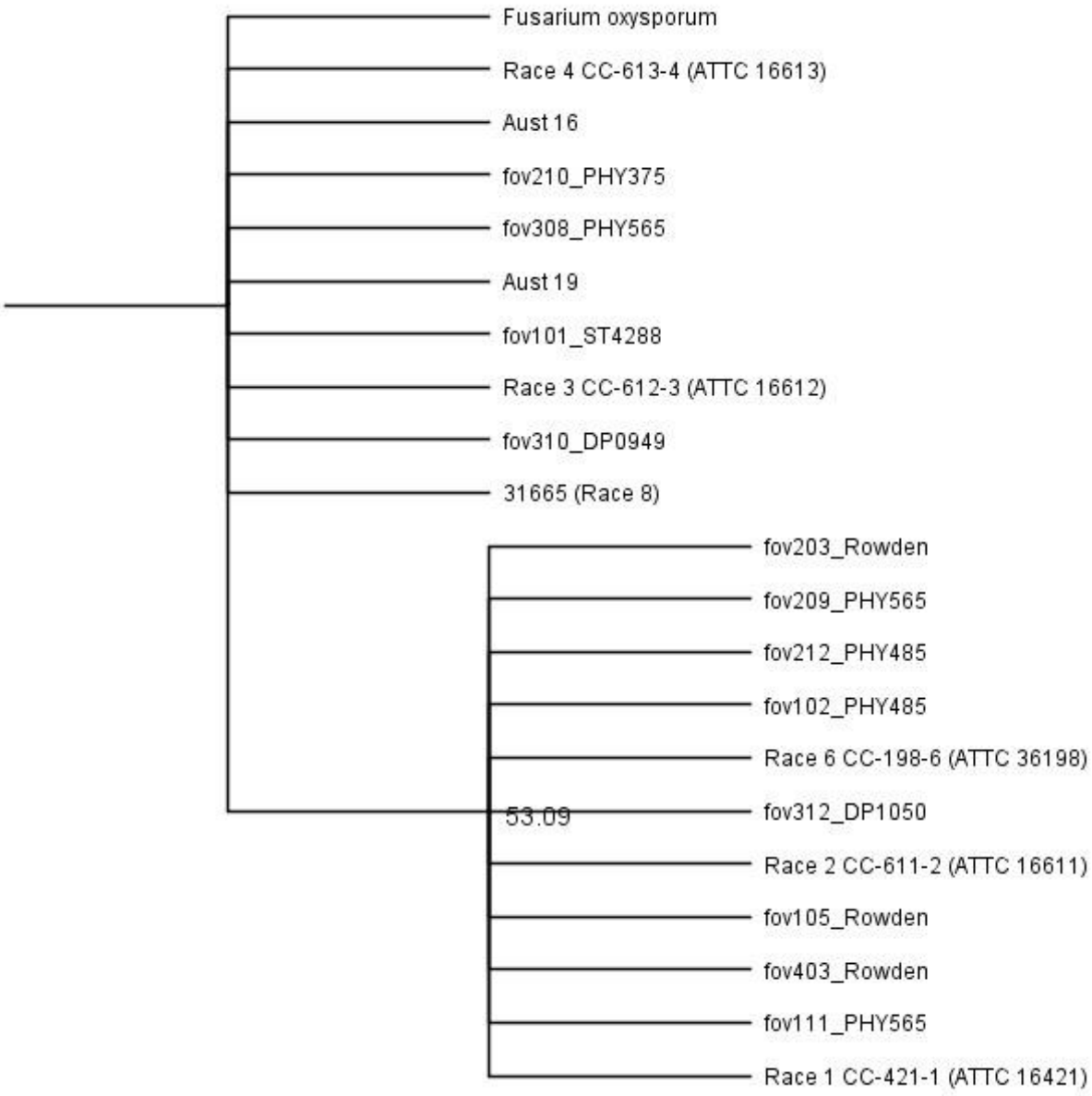


Figure 3.5. Single most parsimonious tree generated from analysis of the phosphate permase gene on the 2010 isolates. Numbers above nodes indicate bootstrap intervals from 1000 replicates.

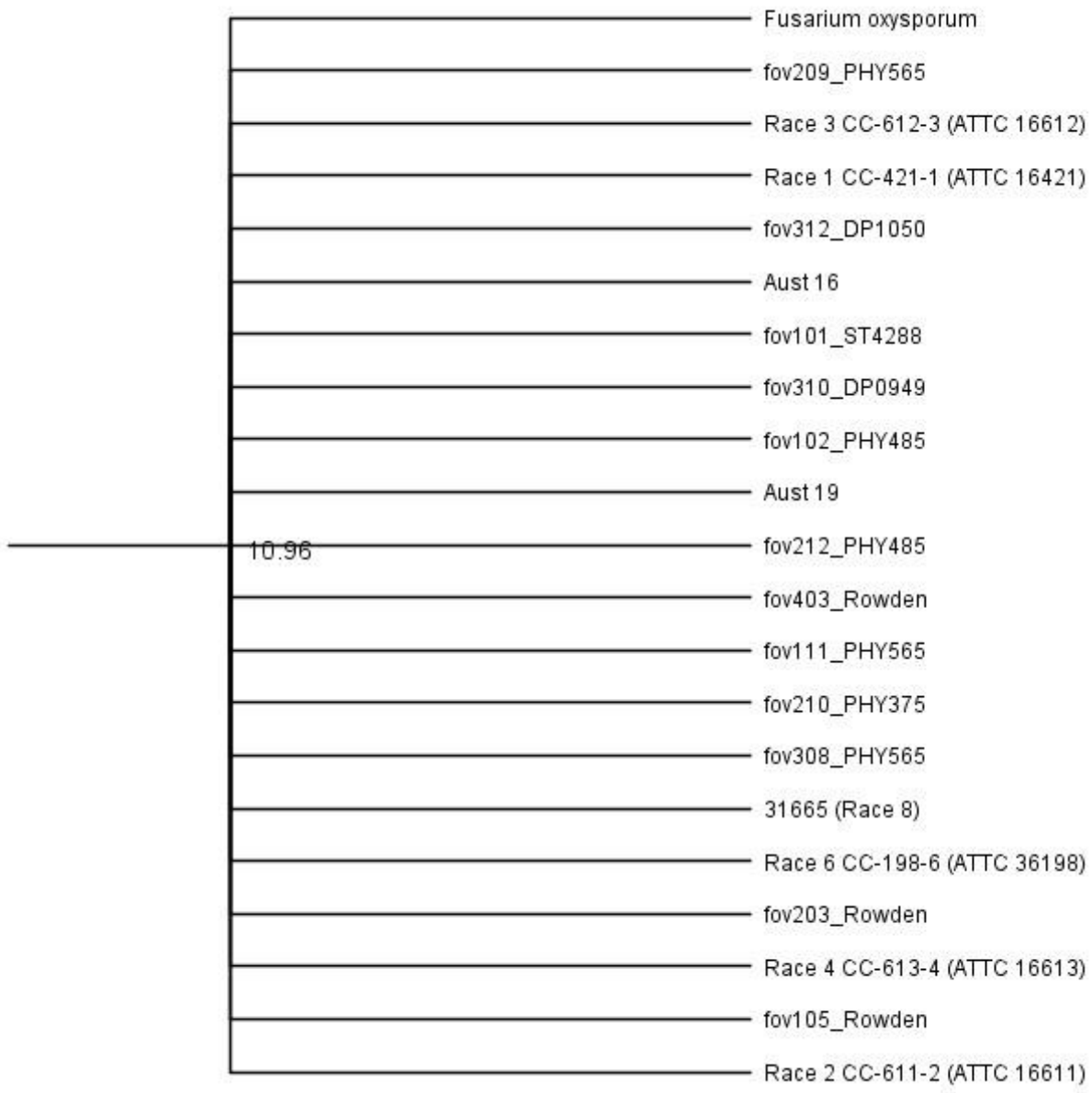


Figure 3.6. Sorted topology tree on the 2010 isolates from MrBayes run. Tree generated from combined analysis of translational elongation factor, beta tubulin, and phosphate permase gene sequences. Five lineages of FOV races are identified by brackets. Bootstrap frequencies from 1,000 replications are noted beside branches.

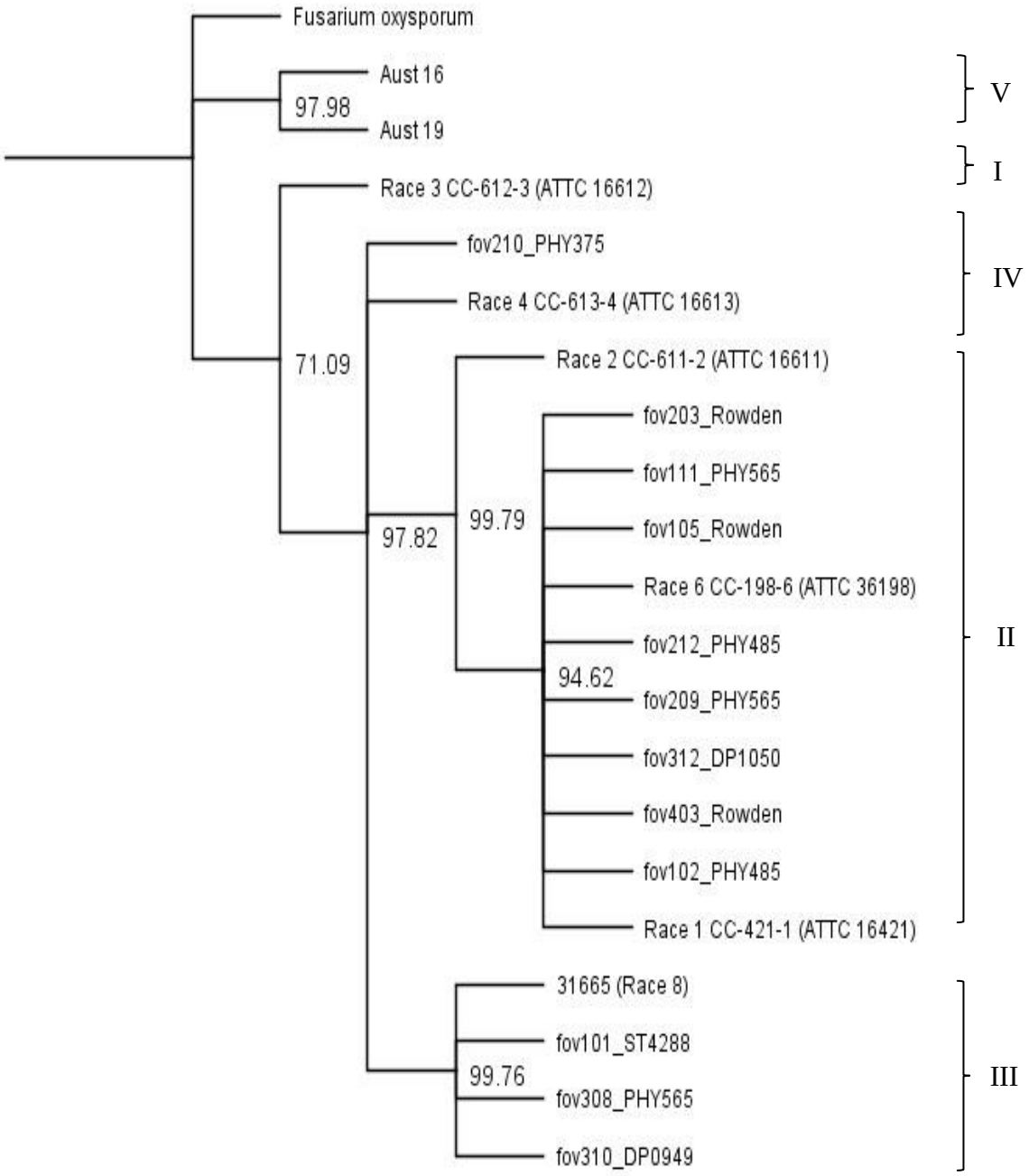


Figure 3.7. Consensus of most parsimonious trees inferred from the EF-1α dataset of 2011. Numbers above nodes indicate bootstrap intervals from 1000 replicates.

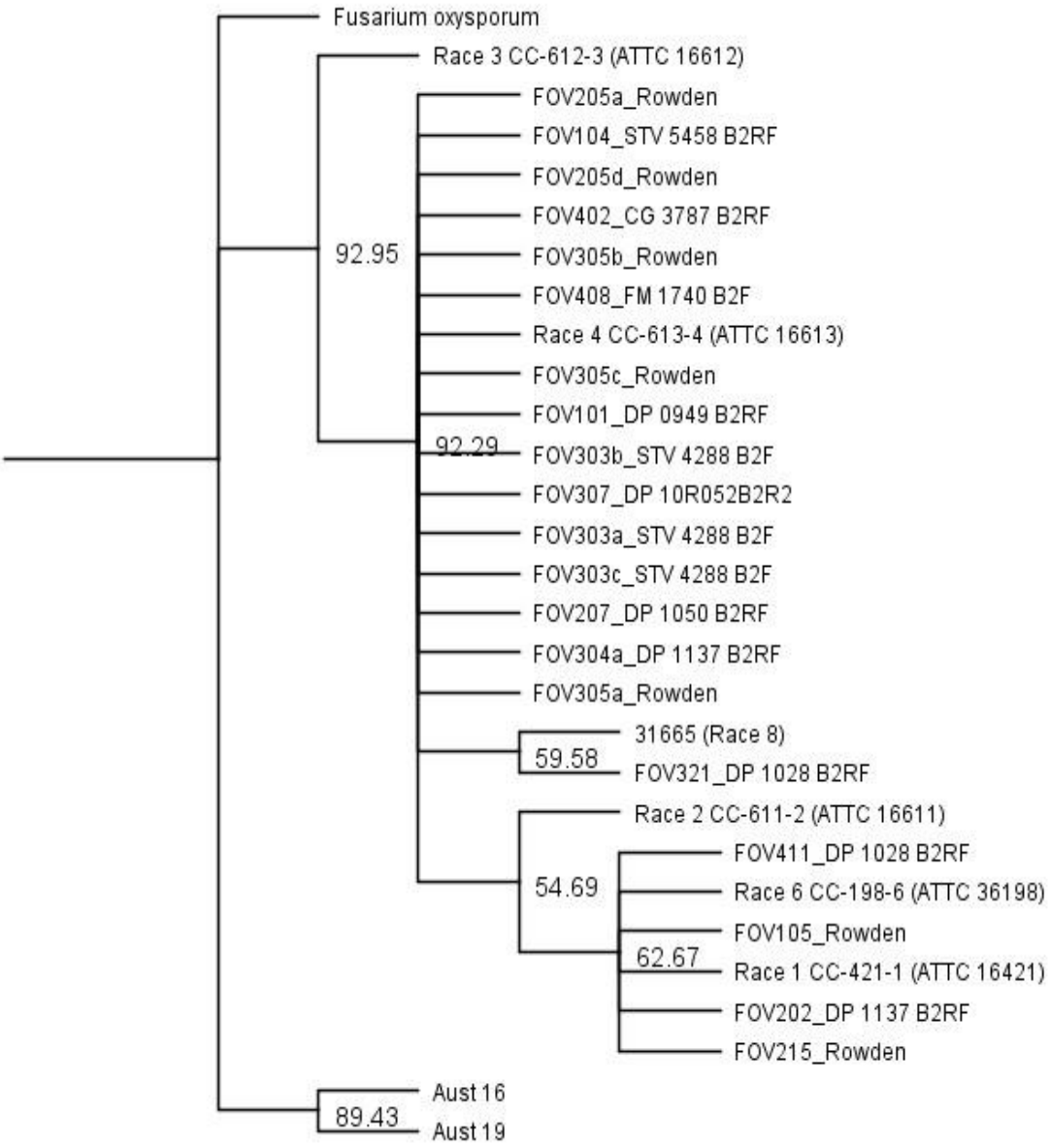


Figure 3.8. Sorted topology tree on the 2011 commercial cotton isolates from MrBayes run. Tree generated from combined analysis of translational elongation factor, beta tubulin, and phosphate permase gene sequences. Five lineages of FOV races are identified by brackets. Bootstrap frequencies from 1,000 replications are noted beside branches.

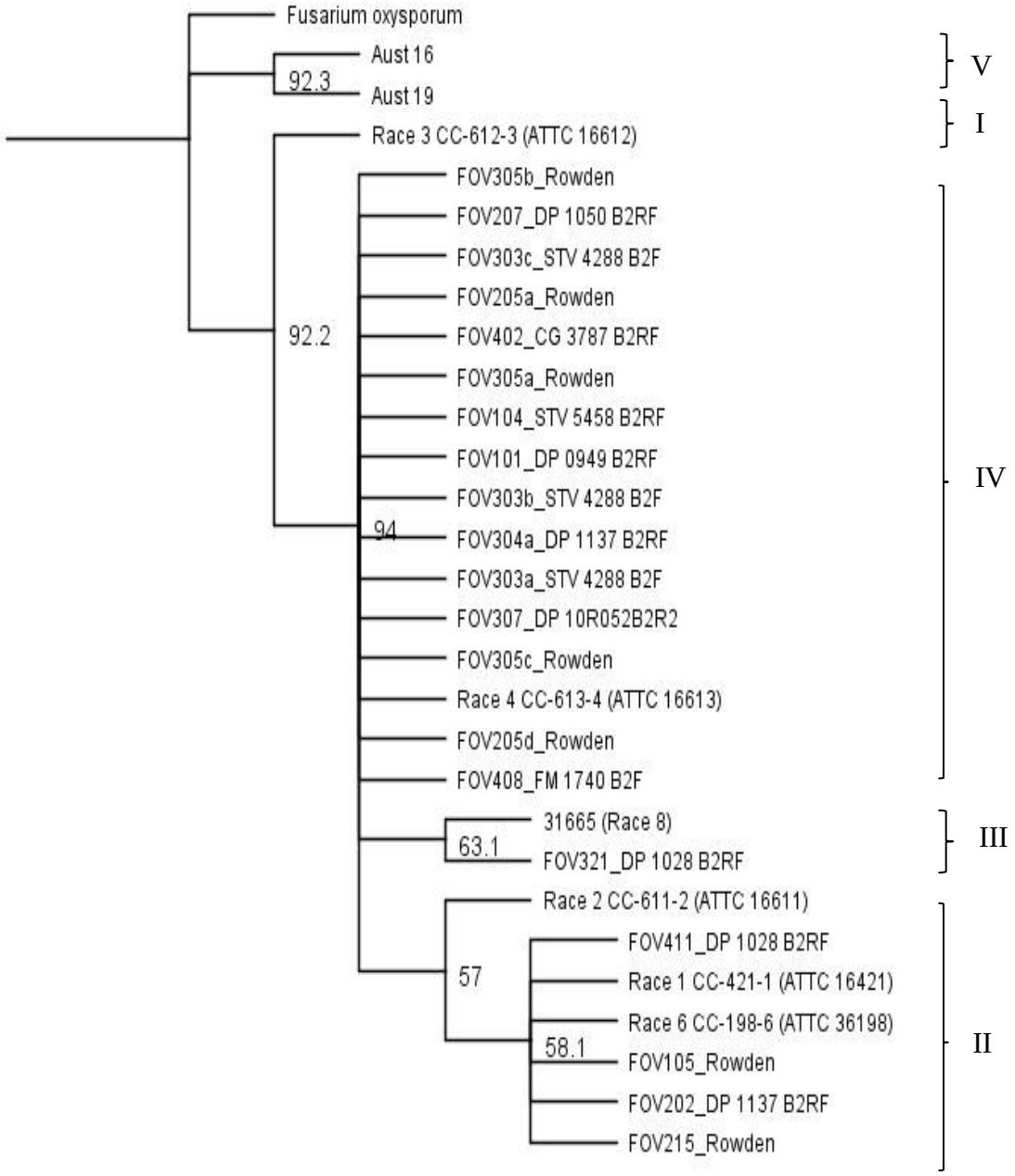


Figure 3.9. Sorted topology tree on the 2011 control cotton isolates, Rowden, from MrBayes run. Tree generated from combined analysis of translational elongation factor, beta tubulin, and phosphate permase gene sequences. Bootstrap frequencies from 1,000 replications are noted beside branches.

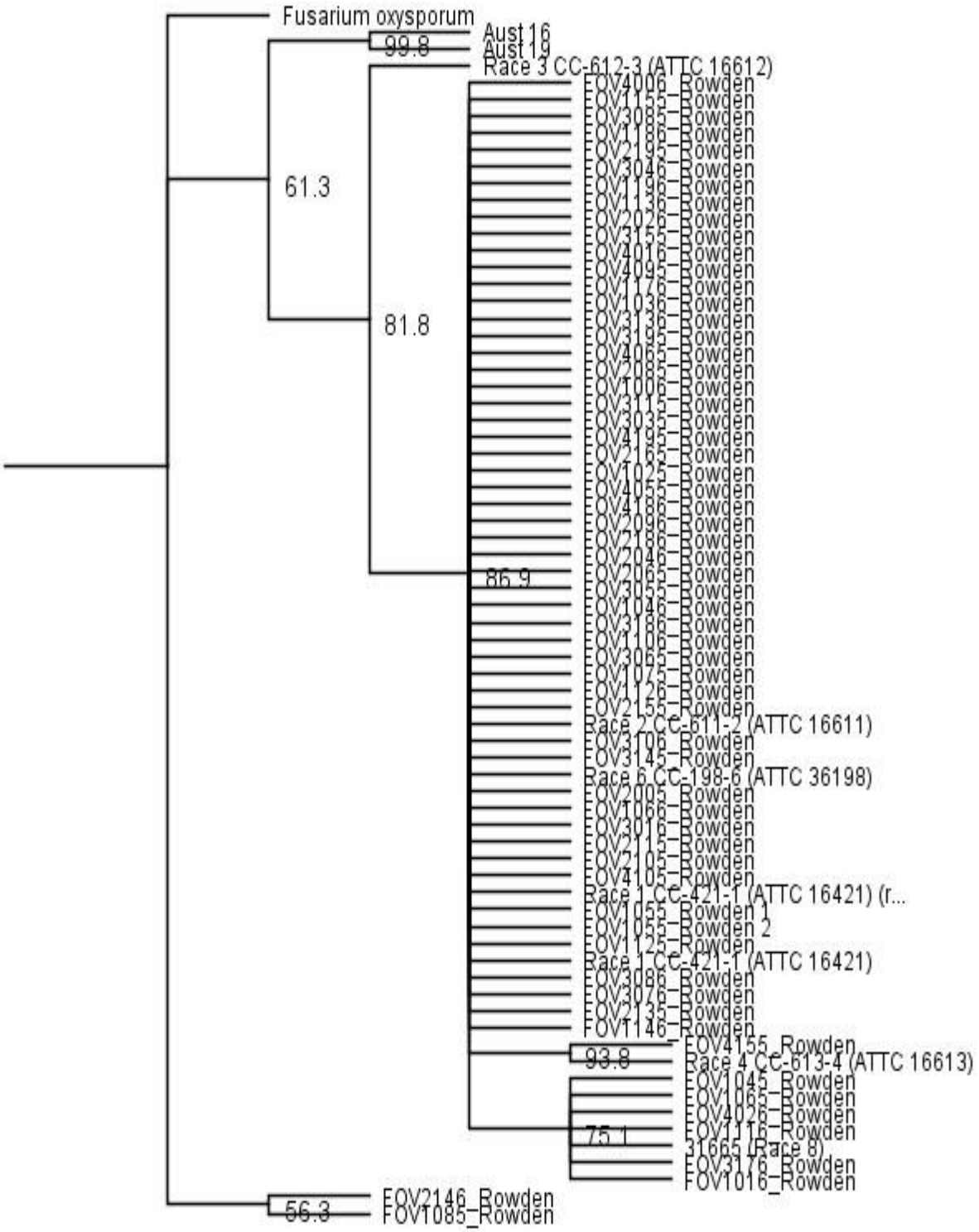


Figure 3.10. Disease rating image of cotton roots on a 0 to 5 scale.

Table 3.2. Alabama isolate pathogenicity comparison on [a]cultivars with and without the presence of root-knot nematodes						
Cultivar	**Isolate**	**RKN**	**[b]Plant Height**		**[b]Necrosis**	
Deltapine 744	Race1	yes	15.6	bcd	3.5	ab
	Race1	no	10.7	def	2.3	cd
	Race4	yes	17.2	abcd	3.5	ab
	Race4	no	15.8	bcd	3.0	abc
	Race8	yes	17.3	abc	2.8	bc
	Race8	no	18.1	abc	3.0	abc
Phytogen 800	Race1	yes	16.9	abcd	3.5	ab
	Race1	no	18.0	abc	3.0	abc
	Race4	yes	16.6	bcd	3.5	ab
	Race4	no	19.3	ab	3.0	abc
	Race8	yes	16.8	abcd	3.0	abc
	Race8	no	18.7	abc	3.3	abc
Phtogen 367 WRF	Race1	yes	12.3	cdef	3.0	abc
	Race1	no	15.4	bcd	3.0	abc
	Race4	yes	14.5	bcd	2.8	bc
	Race4	no	18.1	abc	3.0	abc
	Race8	yes	17.2	abcd	3.0	abc
	Race8	no	18.1	abc	3.0	abc
M-315	Race1	yes	13.6	bcde	3.0	abc
	Race1	no	6.5	f	0.8	e
	Race4	yes	19.9	ab	4.0	a
	Race4	no	23.3	a	3.0	abc
	Race8	yes	14.6	bcd	2.5	bcd
	Race8	no	18.1	abc	3.3	abc
Rowden	Race1	yes	13.6	bcde	3.0	abc
	Race1	no	7.9	ef	1.5	ed
	Race4	yes	15.3	bcd	3.0	abc
	Race4	no	17.3	abcd	3.0	abc
	Race8	yes	16.5	bcd	3.0	abc
	Race8	no	17.4	abc	3.0	abc
[c]LSD = $P \leq 0.05$			6.59		1.00	

[a] Individual plants were rated for vascular necrosis based on a 0 to 5 scale: 0 = no discoloration, 1 = subtle brown vascular discoloration, 2 = light brown, streaky vascular discoloration, 3 = brown vascular discoloration, 4 = dark brown vascular discoloration, 5 = darkest brown to black discoloration

[b] Values are means of four replications.

[c] LSD-means with the same letter are not significantly different