Identification of resistant or tolerant commercial cotton cultivars to the Fusarium wilt root-knot nematode disease complex and the identification of *Fusarium oxysporum* f. sp. *vasinfectum* races in Alabama.

by

Amber Leigh Smith

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
May 9, 2015

Approved by

Kathy S. Lawrence, Chair, Professor of Plant Pathology
C. Dale Monks, Professor of Crop, Soil, and Environmental Sciences
David Held, Associate Professor of Entomology
Edzard van Santen, Professor of Crop, Soil, and Environmental Sciences
Leonardo De La Fuente, Associate Professor of Plant Pathology

Abstract

Fusarium oxysporum Schltd.:Fr. f. sp. *vasinfectum* (Atk.) W.C. Snyd. and H. N. Hans (FOV) is the causal agent of Fusarium wilt in cotton. This causal agent is a soil-dwelling fungus that can remain dormant for many years in soil and has proven difficult to manage. There is an association between this disease-causing fungi and the Southern root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood (RKN). The nature of this association is not understood, but the fungal spores gain entry to the plant via wounds in the root systems caused by nematodes' stylets. Symptoms of Fusarium wilt in cotton include plant wilting throughout the season, interveinal chlorosis and necrosis, leaf abscission, yield reduction, and death. Commercial cotton cultivar trials were conducted in 2013 and 2014 to screen commercially available cotton lines for resistance or tolerance to the Fusarium wilt root-knot nematode disease complex. One trial in 2013 and two trials in 2014 were set up as single row, completely randomized block designs and analyzed for Fusarium wilt disease incidence, *M. incognita* population density, and yield. In 2013, FiberMax 1944 GLB2 had the highest yield, lowest Fusarium wilt incidence, and the lowest nematode density of all cultivars tested. Phytogen 339 WRF and Phytogen 499 WRF yielded significantly higher than the resistant check and had low wilt incidence; Phytogen 499 WRF had low nematode egg density and Phytogen 339 WRF had high nematode egg density. Stoneville 4747 GLB2, first included in this study in 2014, was the highest yielding cultivar with low wilt incidence and low nematode egg density. Stoneville 4946 GLB2, Phytogen 499 WRF, and Phytogen 427 WRF were also significantly

higher yielding than the resistant check. Deltapine 1454NR B2RF, a new cultivar marketed as *M. incognita*-resistant, had the lowest nematode population density, but higher Fusarium wilt incidence. Cultivars with exceptional performance in 2013 also performed well in 2014. Isolates of the fungus were taken from symptomatic plants throughout the seasons and were extracted for DNA. Sequences were amplified by PCR for race identification. A total of 123 samples were identified to race. Races detected in 2013 were 1, 8, LA 108, LA 110, and LA 127/140. In 2014, the same races were detected with the exception of LA 110, which was not found. Races 3, 4, and LA 112 were not detected in either year. Race 1 was the predominate race isolated in both years.

Acknowledgments

I would like to express my humble appreciation to my major professor, Dr. Kathy Lawrence, for her patience and guidance she has shown throughout my completion of this degree. I would like to extend gratitude to Kathy Glass; her help made the field part of this project much easier than it could have been. Thank you to Julia Hu for guiding and instructing me along the molecular path of this project. Thank you to the Alabama Cotton Commission and Cotton, Incorporated for their contributions to the funding of this project. To my graduate committee members, Dr. Dale Monks, Dr. David Held, Dr. Edzard van Santen, and Dr. Leonardo De La Fuente, thank you for your time contributions and guidance during this project. Thank you to my graduate peers – Justin Luangkhot, Ni Xiang, and Caroline Land, and to the undergraduate students – Andrew Sikkens, Stephen Till, and Presley Townsend, and colleagues Roelof Sikkens and Lam Duong for any and all help I received towards the completion of this project.

I would like to extend a very special thank you to my colleague and friend, Justin, for his constant support and willingness to help whenever I asked. This would not have been possible without you. To my parents and sister, I appreciate your support, encouragement, and prayers you have provided me along this journey.

Table of Contents

Abstract …….. ii

Acknowledgements ……... iv

List of Tables ……... vii

List of Figures ……. viii

Chapter I: Review of Literature ……...…….. 1

Fusarium oxysporum f. sp. *vasinfectum* …… 1

Southern root-knot nematode ……. 4

Disease complex …… 5

Current disease management strategies ……. 7

Literature cited ……... 9

Chapter II: Commercial Cotton Cultivar Trial ……...…….. 14

Abstract …… 14

Introduction …….. 15

Materials and Methods ……. 15

Results …….. 20

Discussion …… 23

Literature cited ……. 26

Chapter III: *Fusarium oxysporum* f. sp. *vasinfectum* race identification ……. 28

Abstract …… 28

Introduction …….. 29

Materials and Methods ……. 29

Results …….. 33

Discussion …… 34

Literature cited ……. 36

Overall Conclusion …….. 38

Appendix …….. 39

List of Tables

Table 1. Cultivars of the commercial cotton Fusarium wilt trials by year. ……. 39

Table 2. Fusarium wilt (FOV) incidence percentages, *M. incognita* eggs per gram of root, and seed cotton yield in kg/ha with confidence intervals and Dunnett's *P* vales for comparison to susceptible (Rowden) and resistant (M-315) checks (2013). …..…… 40

Table 3. Fusarium wilt (FOV) incidence percentages, *M. incognita* eggs per gram of root, and seed cotton yield in kg/ha with confidence intervals and Dunnett's *P* vales for comparison to susceptible (Rowden) and resistant (M-315) checks (2014). ..……...…….. 41

Table 4. Summary of FOV isolates taken at first and final disease evaluations (2014). ……..… 42

List of Figures

Figure 1. *M. incognita* damage on cotton. ……..……..…….. 43

Figure 2. Fusarium wilt symptoms on cotton. ……. 43

Figure 3. Fusarium wilt chlorotic and necrotic symptoms on cotton. ……..……...…… 43

Figure 4. Chlorotic Fusarium wilt symptoms on cotton. ……..……... 43

Figure 5. Vascular discoloration of cotton caused by Fusarium wilt. ……..……... 43

Figure 6. Isolated culture of FOV from symptomatic cotton plants (APDA). ……..…….. 43

Figure 7. Isolated culture of FOV from symptomatic cotton plants (APDA). ……...……..… 43

Figure 8. Minimum and maximum temperatures (C) and precipitation (cm) during the 2013 growing season ……. 44

Figure 9. Minimum and maximum temperatures (C) and precipitation (cm) during the 2014 growing season. …….……... 44

Figure 10. Canonical discriminant analysis of cotton cultivar yield, FOV incidence, *M. incognita* eggs per gram of root, and total nematode egg counts in 2013/2014. CAN 1 is driven by root-knot egg numbers (r = 0.98) thus nematodes increase moving away from the Y axis. Differences in CAN 2 are driven by seed cotton yield and FOV incidence with lower yield and higher wilt in the top half of the chart. ……... 45

Figure 11. Canonical discriminant analysis of cotton cultivar yield, FOV incidence, *M. incognita* eggs per gram of root, and total nematode egg counts for 2014. CAN 1 is driven by seed cotton yield (r = 0.94) and has a negative correlation with wilt incidence (r = .0.83) thus yield increases and FOV incidence decreases from left to right. Differences in CAN 2 are driven by nematode variables (r = 0.98) with nematode numbers increasing from bottom to top. …….…… 46

Figure 12. Maximum likelihood evolutionary tree for *Fusarium oxysporum f. sp. vasinfectum* isolated in 2013. Races 1, 8, LA 108, LA 110, and LA 127/140 were detected. Races 3, 4, and LA 112 were not detected. ……... 47

Figure 13. Disease evaluation at 33 DAP in 2014. Race 1 was the prominent race detected. Races 8, LA 108, and LA 127/140 were also detected. ……..... 48

Figure 14. Disease evaluation at 93 DAP in 2014. Race 1 was the prominent race detected. Races LA 108 and LA 127/140 were also detected. …….. 49

Chapter I: Review of Literature

Fusarium oxysporum* f. sp. *vasinfectum

Fusarium oxysporum Schltd.:Fr. f. sp. *vasinfectum* (Atk.) W.C. Snyd. and H. N. Hans (FOV) was first discovered as the causal agent of Fusarium wilt on cotton (*Gossypium hirsutum* L.) in Alabama by Atkinson in 1892 (Atkinson, 1892). Atkinson noticed and described a relationship between FOV and root-knot nematodes, noting that in the presence of the root galling (Figure 1) caused by *Meloidogyne incognita* (Kofoid and White) Chitwood (RKN) the wilt infection seemed to be more severe (Atkinson, 1892). Atkinson documented symptoms and signs that were present within affected cotton plants (Figures 2, 3, 4, and 5). Symptoms of Fusarium begin at any crop development stage, with leaves first wilting or becoming chlorotic. Infected plants are stunted. Cotton plants tend to wilt gradually early in the season, but a rapid escalation of wilting may occur mid-season or at flowering following rain events (Colyer, 2001). A typical leaf symptom of chlorosis begins at the leaf margins following the main veins. These areas become necrotic and abscission of affected leaves occurs. Abscission usually begins with the lowest leaves and moves up the plant. Wilting of the entire plant may be followed by plant death (Colyer, 2001). Initially these disease symptoms were referred to as "Frenching" (Atkinson, 1892) and today we refer to the symptoms as Fusarium wilt (Colyer, 2001). Observations via microscope led Atkinson to discover the fungal hyphae (Figures 6 and 7) that caused vascular discoloration and xylem blockage that induced plant wilting (Atkinson, 1892). Re-isolation of the hyphae present in the stem of plants led him to the conclusion that the pathogen was of the *Fusarium* species, but was different from saprophytic types already known. He proposed the species name *"Fusarium oxysporum* f. sp. *vasinfectum*" (Atkinson, 1892).

According to the National Cotton Council, in 2011-2013 there were 4010, 8300, and 3100 bales of cotton lost due to Fusarium wilt alone in Alabama (Lawrence et al., 2014).

FOV produces larger, fusiform, curved macroconidia that are multi-cellular in addition to smaller, simpler one- to two-celled microconidia on phialides (Domsch et al., 1980). Chlamydospores are also produced and are very hardy, essential for surviving in harsh soil conditions (Domsch et al., 1980; Nelson et al., 1983). Chlamydospores can germinate into infection hyphae, which may cause the initial infection in a plant or, if the environmental conditions change, reform into chlamydospores; in this way the fungi can continue a cycle of proliferation. Macro- and microconidia are often found in the xylem and cortex of the cotton plant. Macroconidia may germinate directly, producing hyphae or forming new chlamydospores. In this way FOV is extremely tolerant to adverse conditions, and can thrive in a variety of pH levels, soil types, and temperatures (Domsch et al., 1980).

There are distinct races of FOV that affect each of the four domesticated cotton species differently, in addition to parasitizing a variety of other species of host plants (Armstrong and Armstrong, 1948; Armstrong and Armstrong, 1958; Armstrong and Armstrong, 1960; Armstrong and Armstrong, 1975; Davis et al., 2006). American races 1 and 2 had similar effects across 29 cotton cultivars and breeding lines, but could be differentiated by an increased virulence of soybean (*Glycine max* L. Merr.) and tobacco (*Nicotiana* L.) from race 2 (Armstrong and Armstrong, 1958). The Brazilian race 6 (Armstrong and Armstrong, 1978) was also similar to races 1 and 2 when comparing cotton pathogenicity tests, but alfalfa (*Medicago sativa* L.), soybean, tobacco, and lupin (*Lupinus luteus* L.) did not show wilt symptoms as with races 1 and

2. Egyptian (race 3) and Indian (race 4) isolates were different from the American isolates in the fact that neither induced wilt symptoms on alfalfa, Yelredo soybean, okra (*Hibiscus esculentus* L.), Kentucky 5 burley tobacco, or Gold Dollar flue-cured tobacco (Armstrong and Armstrong, 1960).

In 2009, Holmes identified four novel genotypes of FOV that were present in the southeastern United States (Holmes et al., 2009). Novel genotypes LA 127/140, 108, 110, and 112 were discovered to be genetically different than all previously identified races (Holmes et al., 2009). Scott et al. (2011) confirmed races 1, 2, 4, and 8 in Alabama in 2010. Bennett et al. (2011) confirmed these races to be present in the southeastern United States in the multi-state Fusarium wilt survey. Some isolates from Alabama and Mississippi were identified as isolates of Lineage IV (race 4-like isolates) (Bennett et al., 2011; Scott et al., 2011). The pathogenic race 4 was first identified in the United States in 2001 in the San Joaquin Valley of California (Holmes et al., 2009) and its origin is currently unknown, although speculations have indicated that Asia or Australia could have been the source. This pathogenic strain of FOV race 4 has proven to be damaging to cotton crops without the presence of *M. incognita*, unlike other races that infest cotton (DeVay et al., 1997; Bennett et al., 2013). This strain is present in Australia and currently California, USA, but the race 4-like isolates found in the southeastern United States are distinguishable from the pathogenic race 4 strain by genetic separation using the intergenic spacer (IGS) nuclear region of rDNA (Kim et al., 2005). Scott et al., (2011) placed the AL race 4-like isolates on susceptible Pima cotton and were not able to confirm the same severe pathogenicity as race 4 is known to possess in the CA isolates. Thus the AL race 4-like isolates did not possess the same pathogenicity as the CA isolates. Further testing by Bennett et

al. (2011) confirmed the AL and MS race 4-like isolates did not match the CA race 4 when using the IGS nuclear rDNA comparisons. One source of genetic resistance to the pathogenic race 4 found in California has been identified in *G. barbadense* Pima-S6 cotton, and this germplasm line is being used to breed resistant Pima and Upland cotton varieties (Ulloa et al., 2013).

Southern root-knot nematode

Four different species of root-knot nematode, *Meloidogyne hapla, M. incognita, M. javanica,* and *M. arenaria*, are some of the most economically important plant pests in the world and attack 99% of all crops affected by *Meloidogyne* species (Mai and Abawi, 1987). *Meloidogyne* spp. is a sedentary pest and an endoparasitic nematode (Mai and Abawi, 1987; Koenning et al., 2004). *Meloidogyne incognita* (Kofoid and White) Chitwood, also known as the southern root-knot nematode (Mai and Abawi, 1987), thrives in light (sandy) soils, increasing to high population densities and is inversely related to clay and/or silt content in soils (Koenning et al., 1996; Koenning et al., 2004; Mai and Abawi, 1987). *Meloidogyne incognita* thrives in tropical to sub-tropical regions (Mai and Abawi, 1987) which are the same regions in which cotton is grown. The southern root-knot nematode has a broad host range that includes over 700 species, including most cultivated ornamentals and crops. There are four known host races recognized among populations of *M. incognita* (Mai and Abawi, 1987; Koenning et al., 2004). Host races 3 and 4 are known to cause symptoms (Figure 1) in cotton (Koenning et al., 2004).

Life Cycle

The life cycle of *M. incognita* begins as an egg (Koenning et al., 2004; Mai and Abawi, 1987; Taylor and Sasser, 1978). As the nematode develops in the egg, it will molt from a first stage juvenile to a second stage juvenile and hatch from the egg after repeated thrusting with a

stylet. The second stage juvenile (J2) will then begin searching for a root to infect, and detection of root exudates will lead the nematode to the root tip. The J2 is the infective stage of this nematode (Thomas and Kirkpatrick, 2001). After infection, the nematode migrates intercellularly and intracellularly toward the vascular system of the plant. The J2 will begin feeding on parenchyma cells near the developing vascular cylinder by gaining entry to the plant using a stylet (Mai and Abawi, 1987). The nematode's feeding causes the host's cell numbers to increase (hyperplasia) and to swell (hypertrophy) into what is known as "giant cells", or feeding cells (Taylor and Sasser 1978; Koenning et al., 2004). Feeding cells become a nutrient sink and the nematode remains in this location in the root, feeding from these cells. After two additional molts, the male juveniles exit the root to potentially mate and die. The female also molts twice more becoming a young mature female and begins to produce eggs. Her body swells and takes on a lemon-shaped appearance inside the root tissue creating a root swelling or "gall." As the female produces eggs, she lays them in a gelatinous matrix creating an "egg sac" on the root surface of smaller roots or within the root of larger galls. The optimum temperature of 25-30°C will induce egg hatching; otherwise, the eggs will remain dormant for extended periods of time.

Disease complex

There was recognition of an association between the Fusarium wilt and the *M. incognita* pathogens by Atkinson during his description of the disease in 1892. Davis et al. (2006) also notes other nematodes associated with increased severity of Fusarium wilt: the reniform (*Rotylenchulus reniformis* Linford & Oliveira), sting (*Belonolaimus gracilis* Steiner and *B. longicaudatus* Rau), lance (*Hoplolaimus seinhorsti* Luc) and lesion (*Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans-Stekhoven). Although studies have shown relationships

between other nematodes and Fusarium wilt, none have such a profound effect on Fusarium wilt as *M. incognita* (Davis et al., 2006). Where the *M. incognita* infection was more intense, so was the Fusarium wilt severity. Influence of nematodes on incidence and severity of wilt was demonstrated in several studies involving the use of sterilized and/or fumigated soil (Hyer et al., 1979; Jorgenson et al., 1978; Smith, 1948; Taylor et al., 1940). The association was originally thought to be strictly mechanical; the fungal spores would use the wound caused by the nematode stylet as an entry way into the plant below the surface of the ground. However, not all plant parasitic nematodes have the same effect on Fusarium wilt infection. Martin et al. (1956) demonstrated *M. incognita* increasing Fusarium wilt levels in both wilt-susceptible and resistant cotton varieties. The nematode genera *Trichodorus*, *Tylenchorhynchus*, and *Heliocotylenchus* did not increase Fusarium wilt while genera *Rotylenchulus* and *Praytlenchus* increased Fusarium wilt in wilt-susceptible but not in wilt-resistant cultivars. Higher inoculation levels of the fungal and nematode pathogens cause greater severity of wilt symptoms (Garber, 1979; Starr et al., 1989; DeVay et al., 1997).

Garber (1979) performed an experiment to understand how the presence of a *M. incognita* population affected the severity of the Fusarium wilt disease in cotton. He found 77,000 *Fusarium* conidia per gram of soil to be the threshold for visual foliar wilt symptom expression if there was no *M. incognita* nematode presence. In sharp contrast, only 650 conidia per gram of soil produced visual symptoms if 50 *M. incognita* were present. Fusarium wilt disease incidence was directly correlated to the increase of one or both pathogens. Nematodes did not produce visual symptoms of Fusarium wilt if no *Fusarium* conidia were present. Davis et al., (2006) discussed how many studies have been conducted concerning the relationship

between these two pathogens; however, the relationship is still not fully understood. It was believed to be strictly mechanical (Davis et al., 2006, Michell & Powell 1972) as the nematode provides a wound for the fungi to enter, but is now believed to be more complex to include root exudates or other attractants (Martin et al., 1956). It has been proven that other nematode genera also influence the severity of Fusarium wilt (Cooper and Brodie, 1963; Davis et al., 2006; Holdeman and Graham, 1954; Jones et al., 1959; Michell and Powell, 1972; Minton and Minton, 1966; Neal, 1954), but none have as much of an effect as *M. incognita*.

Current disease complex management strategies

Management of this disease complex has proven to be difficult. There are no cotton cultivars that are immune to Fusarium wilt available, but some current cultivars show promising resistance and may be vital for future FOV management. Chemical fungicides provide minimal disease reductions at best; most fungicides are ineffective (Hutmacher et al., 2011). The fumigants 1, 3 dichloropropene and metam sodium are the only fumigants currently labeled for cotton nematode control, but both are costly and difficult to apply requiring optimum soil moisture and temperature and a waiting period between application and planting (Davis et al., 2006). Cultural practices such as crop rotations are also ineffective due to long survival of FOV chlamydospore presence in the soil (Hutmacher et al., 2011). Chlamydospores are hardy and can remain dormant in the soil for many years. The most successful ways to manage this disease complex are 1) manage nematode populations utilizing resistant cultivars, nematicides, and crop rotations; 2) remove infested plant material from fields to prevent inoculum spread; 3) limit water, equipment, and personnel movement through infected fields; and 4) production and utilization of quality, disease-free seed (Davis et al., 2006; Hutmacher et al., 2011).

The overall hypothesis of this study is that some commercial cultivars tested will show promising Fusarium wilt tolerance in comparison to the resistant and susceptible control varieties and that *M. incognita* density will be correlated to wilt incidence; in addition, a plethora of Fusarium races will be detected in the soil at PBU where the commercial trial is located. The objectives of this study are: 1) Observe commercial cultivars disease susceptibility or resistance to Fusarium wilt and *M. incognita*; 2) Determine yield in selected cotton cultivars when challenged with this disease complex; and 3) Identify races of *Fusarium oxysporum* f. sp. *vasinfectum* currently present in Alabama. The overall goals of this project are to identify potentially resistant cotton cultivars to the Fusarium wilt root-knot nematode disease complex – or to identify tolerant cotton cultivars which will produce acceptable yield under disease pressure – and to profile the soil for FOV races present in the Fusarium wilt trial field at the Plant Breeding Unit of the E. V. Smith Research Center.

Literature Cited

Armstrong, G. M., and Armstrong, J. K. 1948. Non susceptible hosts as carriers of wilt Fusaria. Phytopathology 38:808-826.

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

Armstrong, G. M., and Armstrong, J. K. 1975. Reflections on the wilt Fusaria. Phytopathology 13:95-103.

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

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

Atkinson, G. F. 1892. Some diseases of cotton. Alabama Agricultural Experiment Station Bulletin 41.

Bennett, R. S., Bell, A. A., Woodward, J. E., Lawrence, K. S., Rothrock, C. S., Kirkpatrick, T. L., Lawrence, G. W., Colyer, P. D., Davis, R. M. Progress report on a contemporary survey of the Fusarium wilt fungus in United States. Proceedings of the 2011 Beltwide Cotton Conferences, Atlanta, GA, January 4-7, 2011; pages 267-274. National Cotton Council of America, Memphis, TN. <http://www.cotton.org/beltwide/proceedings>

Bennett, R. S., Scott, T. Z., Lawrence, K. S., and Lawrence, G. W. 2013. Sequence characterization of race 4-like isolates of *Fusarium oxysporum* from Alabama and Mississippi. The *Journal of Cotton Science 17:1–6 (2013)* <http://journal.cotton.org>

Cooper, W. E., and Brodie, B. B. 1963. A comparison of Fusarium-wilt indices of cotton

varieties with root-knot and sting nematodes as predisposing agents. Phytopathology 53:1077-1080.

Colyer, P. D. 2001. Fusarium wilt. Pages 27-28 in Cotton Compendium. eds. Kirpatrick and Rothrock. American Phytopathological Society Publications, St. Paul, Minnesota.

Davis, M., Colyer, P. D. , Rothrock, C. S., Kochman, J. D. 2006. Fusarium wilt of Cotton: Population diversity and implications for management. Plant Disease Vol. 90:6.

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 Vol. 87, No. 3: 341-346.

Domsch, K. H., Gams, W. and Anderson, T. H. 1980. Fusarium. Pages 305-341 in Compendium of Soil Fungi. Academic Press, London.

Garber, R. H., Jorgenson, E. C., Smith, S., and Hyer, A. H. 1979. Interaction of population levels of *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* on cotton. Journal of Nematology 11(2): 133-137.

Holdeman, Q. L., and Graham, T. W. 1954. Effect of sting nematode on expression of Fusarium wilt in cotton. Phytopathology 44:683-685.

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 Dis. 93:1298-1304.

Hutmacher, R. B., Ulloa, M., Wright, S. D., Davis, R. M., Keeley, M. P., Delgado, R., Banuelos,

G., Marsh, B. H., Munk, D. S. 2011. Fusarium race 4: management recommendations for growers. Proceedings of the 2011 Beltwide Cotton Conference Vol. 1: 224-229. National Cotton Council of America, Memphis, TN. http://www.cotton.org/beltwide/proceedings

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. Crop Sci. 19:898-901.

Jones, J. E., Newson, L. D., and Finley, E. L. 1959. Effect of reniform nematode on yield, plant characters, and fiber properties of upland cotton. Agron. J. 51:353-356.

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. J. Nematol. 10:228-231.

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

Koenning, S. R., Walters, S. A., and Barker, K. R. 1996. Impact of soil texture on the reproductive and damage potentials of *Rotylenchulus reniformis* and *Meloidogyne incognita* on cotton. Journal of Nematology 28(4): 527-536.

Koenning, S. R., Kirkpatrick, T. L., Starr, J. L., Wrather, J. A., Walker, N. R., Mueller, J. D. 2004. Plant parasitic nematodes attacking cotton in the United States: Old and emerging production challenges. APS Plant Dis. Vol. 88 No. 2: 100-113.

Lawrence, K., Olsen M., Faske, T., Hutmacher, R., Muller, J., Mario, J., Kemerait, R., Overstreet, C., Sciumbato, G., Lawrence, G., Atwell, S., Thomas, S., Koenning, S., Boman, R., Young, H., Woodward, J., and Mehl, H. 2014. Cotton disease loss estimate committee report, 2013. Proceedings of the 2014 Beltwide Cotton Conference Vol. 1:

247-248. National Cotton Council of America, Memphis, TN. <http://www.cotton.org/beltwide/proceedings>

Mai, W. F., and Abawi, G. S. 1987. Interactions among root-knot nematodes and Fusarium wilt fungi on host plants. Annual Review of Phytopathology 25:1, 317-338.

Martin, W. J., Newson, L. D., and Jones, J. E. 1956. Relationship of nematodes to the development of Fusarium wilt in cotton. Phytopathology 46:285-289.

Michell, R. E., and Powell, W. M. 1972. Influence of *Pratylenchus brachyurus* on the incidence of Fusarium wilt in cotton. Phytopathology 62:336-338.

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.

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

Nelson, P. E., Toussoun, T. A., and Marasas, W. F. O. 1983. Section Elegans. Pages 142-145 in Fusarium Species: An Illustrated Manual for Identification. The Pennsylvania State University Press, University Park and London.

Scott, T. Z., Lawrence, K. S., Castillo, J. D., and Glass, K. 2011. Fusarium wilt identification and root-knot nematode effects on commercial cotton cultivars in 2010. Proceedings of the 2011 Beltwide Cotton Conference Vol. 1: 224-229. National Cotton Council of America, Memphis, TN. <http://www.cotton.org/beltwide/proceedings>

Smith, A. L. 1948. Control of cotton wilt and nematodes with a soil fumigant. Phytopathology 38:943-947.

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.

Taylor, A. L., and Sasser, J. N. 1978. Morphology and development in *Meloidogyne* species. Pages 4-11 in Biology, identification and control of root-knot nematodes. North Carolina State University Graphics, North Carolina.

Taylor, A. L., Barker, H. D., and Kime, P. H. 1940. Further observations on the nematode Fusarium-wilt experiments at Lumberton, North Carolina. Phytopathology 30:710.

Thomas, S. H., and Kirkpatrick, T. L. 2001. Root-knot nematodes. Pages 40-42 in Cotton Compendium. eds. Kirkpatrick and Rothrock. American Phytopathological Society Publications, St. Paul, Minnesota.

Ulloa, M., Hutmacher, R. B., Roberts, P. A., Wright, S. D., Nichols, R. L., Davis, R. M., and Burke, J. 2013. Discovery of Fusarium wilt race 4 resistance in cotton. Proceedings of the 2013 Beltwide Cotton Conference Vol. 1:991-1001. National Cotton Council of America, Memphis, TN. <http://www.cotton.org/beltwide/proceedings>

Chapter II: Evaluation of Commercial Cotton Cultivars for Resistance to *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita*

Abstract

Fusarium oxysporum Schltd.:Fr. f. sp. *vasinfectum* (Atk.) W.C. Snyd. and H. N. Hans (FOV) is the causal agent of Fusarium wilt in cotton. There is an association with *Meloidogyne incognita* (Southern root-knot nematode) that enhances the severity of Fusarium wilt infections. Commercial cotton cultivars were tested in 2013 and 2014 for resistance or tolerance to the Fusarium wilt root-knot nematode disease complex in Alabama and were compared to both resistant and susceptible checks. One trial in 2013 and two trials in 2014 were set up as single row, randomized complete block designs and analyzed for Fusarium wilt disease incidence, *M. incognita* population density, and yield. In 2013, FiberMax 1944 GLB2 had the highest yield, lowest Fusarium wilt incidence, and the fewest nematodes of all cultivars tested. Phytogen 339 WRF and Phytogen 499 WRF were also high-yielding cultivars. Stoneville 4747 GLB2, a cultivar first introduced to both the public and this study in 2014, was the highest-yielding cultivar with low wilt incidence and a low nematode egg population in 2014. Stoneville 4946 GLB2, Phytogen 499 WRF, and Phytogen 427 WRF were also significantly higher yielding than the resistant check. Deltapine 1454NR B2RF was also a new cultivar tested in 2014 that is marketed as a root-knot nematode-resistant cultivar. This cultivar had the lowest nematode density, but had higher Fusarium wilt incidence. Cultivars with exceptional performance in 2013 also performed well in 2014.

Introduction

Fusarium oxysporum f. sp. *vasinfectum* (FOV) is a soil-borne fungal disease that causes wilting and reduced yield in cotton crops across the world (Domsch et al., 1980; Smith et al., 1981). Foliar symptoms of Fusarium wilt include season-long wilting, chlorosis, necrosis, yield reduction, and plant death (Atkinson, 1892; Colyer, 2001). There is an absence of chemical control available for this disease on cotton (Hutmacher et al., 2011). *Meloidogyne incognita* is a sedentary endoparasitic nematode and the female swells with eggs causing root galling (Koenning et al., 2004; Mai and Abawi, 1987; Taylor and Sasser, 1978). It is believed the fungal spores use the nematode wound to gain entry into the plant (Davis et al., 2006; Michell and Powell, 1972). The objectives of this study are to: 1) identify commercial cotton cultivars resistance or susceptibility to Fusarium wilt (FOV) based on foliar disease symptoms; 2) evaluate the effect of *M. incognita* population density on the cotton cultivars; and 3) determine the overall yield of these cotton cultivars when challenged with FOV and *M. incognita*. The overall hypothesis of this study is that cotton cultivars with resistance to Fusarium wilt and *M. incognita* with optimum yield productions will be identified from a set of selected commercially available cultivars recommended for the southeastern cotton belt region.

Materials and Methods

Field trials

In 2013 and 2014, experimental trials were implemented to determine potential tolerance of selected cotton cultivars to the Fusarium wilt root-knot nematode disease complex. Three field trials were conducted in 2013 and 2014 at the Plant Breeding Unit (PBU) of the E.V. Smith Research Center near Tallassee, Alabama. The field locations at PBU are naturally infested with

both *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* race 3. The soil texture class in these fields is a Kalmia loamy sand soil (% sand, silt, clay at 80-10-10, pH 6.5). In 2013, the trial was located on the "South" (32°29'22"N, -85°53'00"W) end of PBU while in 2014, two identical trials were planted, again one on the "South" side and an addition test on the "North" side (32°30'5"N, -85°53'34"W). Cultivars included in 2013 and 2014 were selected by yield potential in Alabama (Table 1). The controls included Rowden and M-315 as the standard FOV susceptible and resistant cotton lines. Each cultivar seed were treated with the standard fungicides and insecticides applied by each company. Fibermax and Stoneville cultivars were treated with the fungicides thiram at 0.002 mg a.i./seed, metalaxyl at 0.0003 mg ai/seed, and ipconazole at 0.0001 mg a.i./seed plus the insecticide imidacloprid 0.34 mg a.i./seed (Bayer CropScience, Research Triangle Park, NC). Deltapine cultivars were treated with the Acceleron FI system (Monsanto Company, St Louis, MO); Phytogen cultivars were treated with fungicides azoxystrobin, fludioxonil, and mefenoxam at 0.03 mg a.i./seed, plus the insecticide thiodicarb at 0.375 mg a.i./seed (Syngenta, Greensboro, NC). Trials in each year were set up as randomized complete block designs with four replications for a total of 48 and 128 experimental units in 2013 and 2014, respectively. Each single row plot was six meters long with one meter row spacing, and 1.8 meter alleys separated each replication. One hundred seed per row from each of the cultivars were planted with a John Deere Maxemerge planter with Almaco cone planters attached (Nevada, Iowa). Trials were planted May 23, 2013 and May 19, 2014.

Cotton cultivar parameters evaluated were initial *M. incognita* population density at planting, cotton plant stand or survival, Fusarium wilt disease incidence, nematode egg density, final plant survival percentage, and seed cotton yield.

Fusarium wilt incidence

Disease evaluations were made by inspecting every cotton plant for symptoms of Fusarium wilt disease, including wilting, chlorosis and/or necrosis, leaf abscission, and death (Figures 2-5). Four disease evaluations were made throughout the 2013 season on July 2, July 8, August 7, and August 22 (39, 45, 76, and 91 DAP, respectively). Five disease evaluations were made for each trial throughout the 2014 season on June 20, July 1, July 16, July 30, and August 20 (33, 43, 58, 73, and 93 DAP, respectively). For each evaluation, diseased plants were removed from the trial, counted, and stored in plastic zippered bags. Diseased plants were transported to a lab on campus at Auburn University and the causal agent was isolated onto half-strength acidified potato dextrose agar (APDA) (VWR, Radnor, PA). This process consisted of each hypocotyl being cut away from the shoot and root of the plant using a scalpel and slicing a 0.5 cm layer of the hypocotyl away for surface sterilization. After sterilizing for 30 seconds in 95% alcohol followed by two minutes in 0.625% NaOCl, the piece of hypocotyl was placed in APDA. Wilt percentages were calculated for each cultivar by dividing the number of confirmed diseased plants by the initial stand count and multiplying by 100 to convert to a percentage format.

Meloidogyne incognita population density

In both 2013 and 2014, plots were evaluated for *M. incognita* nematode population density. Initial *M. incognita* population density was determined at planting by taking ten 2.5 x 20 cm composite soil cores from each rep, mixing the soil, and extracting a 150 cm^3 sub-sample. Nematodes were extracted from the soil using the gravity sieving and sucrose centrifugation methods. Nematode egg density was determined at midseason to determine levels of nematodes

reproducing on each cotton cultivar. Samples were taken July 23 in 2013, and July 21 in 2014 (61 and 63 DAP, respectively). Three random composite plant samples, including root systems, were excavated from throughout each row with shovels 10 cm from the cotton stalk. The root systems were cut from the shoot and combined to make a composite sample for the whole plot. The root samples were transported to the lab where 5-g subsamples consisting of small fibrous roots were cut randomly from each composite sample. Eggs were extracted by placing in 0.625% NaOCl, shaking for 4 minutes at 140 rpm, followed by a water rinse. Eggs were centrifuged at 1400 rpm for 1 minute in sucrose (sp. gravity 1.14). Eggs were collected on a 25µm pore sieve, enumerated using the Nikon TSX inverted microscope, and reported as eggs per gram of root fresh weight.

Yield

In 2013 and 2014, entire plots were machine harvested on October 20 (150 DAP) and September 30 (134 DAP), respectively. Individual plots were weighed and data was converted from pounds of seed cotton yield per plot to kilograms seed cotton yield per hectare.

Statistical analyses

Data for 2013 and 2014 were analyzed using generalized linear mixed models procedures as implemented in SAS 9.3 PROC GLIMMIX (SAS Institute, Inc. Cary, NC). The binomial distribution function with the canonical logit link function was used to analyze the proportion of plants showing wilt symptoms. *M. incognita* egg count per gram of root weight was modeled with a negative binomial distribution function and canonical log link. The ilink option in the LSMEANS statement of the abovementioned procedure was used to express mean and 95%

confidence limits on the data scale. Yield was analyzed as a normally-distributed variable. Response data from 2013 and 2014 were analyzed together when similar cultivars were present. Response data from 2014 was pooled and analyzed jointly when no interactions were detected between repeated trials. The original mean values are presented in the tables with Dunnett's *P* values to determine statistical differences in comparison to susceptible and resistant checks.

The multivariate techniques canonical discriminant analysis (CDA) was utilized to explain differences among cultivars by jointly utilizing all response variables rather than one response variable at a time. Counts were log-transformed to achieve approximate normality. For the 2013 data I used the following four response variables: RK_egg, RKgmroot, Wilt, and Yield_kgha. For the 2014, each response variable by location (North or South) was considered a separate response, hence there were eight response variables: N_RKeggs, S_RKeggs, N_RKgmroot, S_RKgmroot, N_Wilt, S_Wilt, N_Yield_kgha, and S_Yield_kgha. The combined analysis across years had 12 response variables. Multivariate centroid means were calculated for the 1st and 2nd canonical variates. The phenotypic correlation between original variables and canonical variates (the BETWEEN structure in SAS PROC CANDISC) was used to explain the variables that drive the differences among cultivars. An online tool (http://vassarstats.net/rsig.html) was used to determine if a correlation was significantly different from zero at $P = 0.05$ (one-sided). There were nine and 18 cultivars in 2013 and 2014, respectively. Hence the lowest absolute r that was significantly different from zero was 0.59 and 0.42, in 2013 and 2014, respectively. The combined analysis across year had nine entries and hence, significance of correlations identical to 2013.

Results

Fusarium wilt disease incidence varied between the two years of the study. Disease incidence was very light in 2013 followed by a year with high disease incidence in 2014. Monthly average maximum temperatures in 2013 from pre-planting in April through harvest in October were 23.7, 26.3, 30.6, 29.7, 30.3, 29.9, and 24.7°C with average minimum temperatures of 10.3, 13.4, 19.9, 20.5, 20.2, 17.3, and 11.1°C, respectively. Rainfall accumulation for each month was 97, 51, 180, 164, 131, 46, and 12 mm with a total of 681 mm over the entire season (Figure 8). In 2014, monthly average maximum temperatures for the growing season from April through harvest in October were 23.4, 27.7, 25.2, 25.2, 25.9, 24.4, and 19.6°C while average minimum temperatures were 10.5, 14.1, 19.4, 19.1, 19.5, 18.5, and 12.1°C, respectively. Rainfall accumulations for each month were 223, 113, 107, 89, 75, 50, and 67 mm respectively with a total of 725 mm over the entire season (Figure 9). The 2013 season was less conducive for Fusarium wilt than the 2014 season (Tables 2 and 3). The 2014 season consisted of a wet spring with 56% more rainfall in April and May of 2014 than 2013, which was conductive for disease development.

A canonical discriminant analysis was conducted in order to compare cultivars that were evaluated in both 2013 and 2014 (Figure 10). The 1st canonical variate accounted for 58% of the multivariance and differences along the x-axis were driven by *M. incognita* egg populations (r = 0.98). The positive correlation with between *M. incognita* populations and the 1st canonical variate (CAN 1) means that populations increase along the X-axis from left to right. Canonical variate 2 (CAN 2) (represented along the Y-axis) accounted for 28% of the total multivariance and was driven by Fusarium wilt (r=0.93), hence Fusarium wilt disease incidence increases with

increasing Y-axis values. Seed cotton yield was negatively correlated with CAN 2 (-0.84) indicating yield decrease with increasing Y-axis values or increasing Fusarium wilt incidence. Stoneville cultivars tended to have higher yield along with higher nematode density. All cultivars yielded higher than the susceptible check Rowden and most averaged numerically higher yield than the resistant check M-315, with the exception of two Deltapine cultivars.

A canonical discriminant analysis was also used to analyze the combined response variables from the North and South fields for 2014 (Figure 11). Canonical variate 1 represented 49% of the multivariance, and CAN 2 35%. Differences along CAN 1 were driven by yield (r = 0.94 North, r = 0.67 South), therefore yield increases moving left to right along the X-axis. There was a significant negative correlation for the CAN 1 (r = -0.83 North, r = -0.81 South) meaning Fusarium wilt disease incidence percentage decreased along the X-axis moving from left to right. Canonical variate 2 (Y-axis) was driven by both total *M. incognita* eggs and *M .incognita* eggs per gram of root (r = 0.98 for the South field, and r = 0.73 for the North field). The canonical diagram clearly depicts the relationship between seed cotton yield and *M. incognita* populations. Stoneville 4747 GLB2 produced high yield while supporting fewer *M. incognita* compared to Stoneville 6448 GLB2 that produced high yield and supported high numbers of nematodes. Phytogen 427 WRF supported lower nematodes than Stoneville 4747 GLB2, and also had slightly lower yield. The canonical analysis visualizes Stoneville 6448 GLB2 as being tolerant to *M. incognita* because of its ability to produce high yield in the presence of the nematode. Deltapine 1454NR B2RF is marketed as resistant to *M. incognita* (Monsanto 2014, St. Louis, MO), and it supported fewer nematodes than M-315. Over all, cotton yield increased as Fusarium wilt incidence and *M. incognita* population density decreased.

Fusarium wilt incidence

Fusarium wilt disease incidence was low in 2013 when compared to 2014 (Tables 2 and 3). The average wilt percentage for the susceptible check Rowden in 2013 was 11.8% over the entire season (Table 2). The resistant check M-315 performed as expected with an average of only 0.9% of the plants with wilt symptoms. Seven of the cotton cultivars displayed Fusarium wilt symptoms similar to the resistant standard M-315 with a range of 0.3 to 3.7 % wilt, respectively. In 2014, a 70% increase in Fusarium wilt incidence was observed above 2013. All cultivars tested in 2014 had significantly lower wilt incidence than the susceptible check Rowden, which had 30% wilt incidence (Table 3). Twelve varieties were similar to the resistant check M-315 and had wilt incidence percentages ranging from 1-9%.

M. incognita reproduction factors

In both years, nematode population density was calculated as eggs per gram of root fresh weight. In 2013, *M. incognita* populations were lower across cultivars than the following season, ranging from a high of 999 to a low of 86 eggs per gram of root. The susceptible control Rowden averaged 510 eggs per gram of root (Table 2) while the resistant check M-315, supported only 86 eggs per gram of root. Two cotton cultivars supported *M. incognita* populations similar ($P \leq 0.05$) to the resistant check M-315; these were Deltapine 12R242 B2RF and FiberMax 1944 GLB2. Deltapine 1137 B2RF, Deltapine 1321 B2RF, Phytogen 339 WRF, and Phytogen 375 WRF maintained *M. incognita* populations similar to the susceptible standard Rowden. *M. incognita* nematode population density was higher in 2014 than in 2013. The average *M. incognita* eggs per gram of root for 2013 were 352 eggs, and in 2014 the average was 1289 eggs per gram of root, an increase of 366% in 2014.

Yield

Yield varied among cultivars in 2013 (Table 2). Three cultivars yielded similarly to the resistant check M-315: FiberMax1944 GLB2, Phytogen 339 WRF and Phytogen 499 WRF. Yield in 2013 was 172% higher in the South field than in 2014. The average yield for 2013 was 3848 kg per hectare, and the average yield for 2014 was 2236 kg per hectare. All cultivars tested in 2014 yielded higher than the susceptible check Rowden. Four cultivars yielded statistically more than the resistant check M-315: Phytogen 499 WRF, Phytogen 427 WRF, Stoneville 4747 GLB2, and Stoneville 4946 GLB2 with 2842, 3033, 3214, 2826 kg per hectare respectively (Table 3).

The susceptible check Rowden would have produced a return profit of $667 per acre with the 2013 price of $0.85 per pound of lint (pounds of lint*0.85). The highest yielding cultivar, FiberMax 1944 GLB2, would have produced an economic gross income of $1802 per acre. The susceptible check Rowden would have yield value of $164 per acre at the end of the 2014 season at $0.60 per pound of lint. The highest yielding cultivar for 2014, Stoneville 4747 GLB2, would have yield value of $688 per acre at the same price. This demonstrates that cultivar selection can make profound impacts upon profit return in the presence of Fusarium wilt and *M. incognita*.

Discussion

This study focused on the Fusarium wilt complex on cotton and the effects of pathogens *Fusarium oxysporum* f. sp. *vasinfectum* and *Meloidogyne incognita* on commercially available cotton cultivars that are commonly grown in the southeastern United States. The 2013 growing season supported little FOV because Fusarium wilt is a temperate to tropical disease (DeVay et al., 1997), but both years proved conducive for *M. incognita* (Tables 2 and 3). A wet spring in

2014 (Figure 9) favored the development of Fusarium wilt (Tharp and Young, 1939). Fusarium wilt foliar symptoms and *M. incognita* root symptoms were pronounced in 2014. Because of the lack of FOV disease pressure in 2013 (Table 1), the overall driving factor for differences among cultivars across both trial years according to the canonical discriminant analysis was the nematode egg total egg count and the egg number per gram of root (Figure 10). In 2014, the driving forces were yield and wilt (Figure 11). Weather patterns in 2014 could have induced higher Fusarium wilt incidences than those observed during the 2013 season (Figures 8 and 9). Cooler temperatures combined with high rainfall are favorable environmental conditions for Fusarium wilt (Ebbels, 1975; Hillocks, 1992). Higher rainfall at the beginning of the 2014 season could have contributed to the higher Fusarium wilt incidence seen across different cultivars in comparison to 2013.

Eleven of the twelve cultivars tested in 2013 were repeated in 2014 (CG 3787 B2RF, Phytogen 375 WRF, Phytogen 499 WRF, Phytogen339 WRF, FiberMax1944 GLB2, Deltapine 1050 B2RF, Deltapine 1137 B2RF, Deltapine 1252 B2RF, Deltapine 1321 B2RF, Stoneville 4946 GLB2, Deltapine 6448 B2RF). In addition, five cultivars were added in 2014 (Phytogen 427 WRF, Phytogen 575 WRF, Deltapine 1133 B2RF, Stoneville 4747 GLB2, and Deltapine 1454NR B2RF). FiberMax1944 GLB2 had the lowest wilt incidence percentage, lowest nematode egg population density, and greatest yield in 2013. In 2014, FiberMax1944 GLB2 had average wilt percentages, high nematode egg numbers, and average yield. Phytogen 499 WRF and Phytogen 339 WRF had significantly high yield in 2013, and both yielded well in 2014, though only Phytogen 499 WRF was significantly higher in comparison to the resistant check M-315 ($P \leq 0.05$). Phytogen 339 WRF had high nematode population density during both years,

which would indicate tolerance to the nematode pathogen (Davis and May, 2003). Stoneville 6448 GLB2 also has this indication of tolerance with very high nematode eggs each year and average yield (Davis and May, 2003). Egg population density and wilt percentage increased in both Phytogen 339 WRF and Phytogen 499 WRF from 2013 to 2014, which was the normal trend across cultivars. Phytogen 427 WRF was the overall best performing cultivar in the 2014 trials with low wilt, low nematode numbers, and high yield. Deltapine 1454NR B2RF is a newly released cultivar for 2014 with genes for *M. incognita* resistance (Monsanto 2014, St. Louis, MO). This cultivar had the lowest nematode egg reproduction for 2014, but only produced an average yield. This cultivar is not drought tolerant (Drew Schrimsher personal communication, 2014) and the late season drought probably is responsible for the low yield in this location. Deltapine 1454NR B2RF also had high Fusarium wilt incidence. This cultivar would be appropriate to grow in fields with nematode problems, but not necessarily Fusarium wilt problems.

Literature cited

Atkinson, G. F. 1892. Some diseases of cotton. Alabama Agricultural Experiment Station Bulletin 41.

Colyer, P. D. 2001. Fusarium wilt. Pages 27-28 in Cotton Compendium. eds. Kirpatrick and Rothrock. American Phytopathological Society Publications, St. Paul, Minnesota.

Davis, M., Colyer, P. D., Rothrock, C. S., Kochman, J. D. 2006. Fusarium wilt of cotton: Population diversity and implications for management. Plant Disease Vol. 90(6): 692-703.

Davis, R. M., and May, O. L. 2003. Relationships between tolerance and resistance to *Meloidogyne incognita* in cotton. Journal of Nematology 35(4): 411-416.

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.

Domsch, K. H., Gams, W. and Anderson, T. H. 1980. Fusarium. Pages 305-341 in Compendium of Soil Fungi. Academic Press, London.

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

Hillocks, R. J. 1992. Fusarium wilt. Pages 127-160 in Cotton Diseases. Ed. Hillocks. Wallingford, UK: CAB International.

Hutmacher, R. B., Ulloa, M., Wright, S. D., Davis, R. M., Keeley, M. P., Delgado, R., Banuelos,

G., Marsh, B. H., Munk, D. S. 2011. Fusarium race 4: management recommendations for growers. Proceedings of the 2011 Beltwide Cotton Conference Vol. 1: 224-229. National Cotton Council of America, Memphis, TN. http://www.cotton.org/beltwide/proceedings

Koenning, S. R., Kirkpatrick, T. L., Starr, J. L., Wrather, J. A., Walker, N. R., Mueller, J. D. 2004. Plant parasitic nematodes attacking cotton in the United States: Old and emerging production challenges. Plant Disease Vol. 88(2): 100-113.

Mai, W. F., and Abawi, G. S. 1987. Interactions among root-knot nematodes and Fusarium wilt fungi on host plants. Annual Review of Phytopathology 25(1): 317-338.

Michell, R. E., and Powell, W. M. 1972. Influence of *Pratylenchus brachyurus* on the incidence of Fusarium wilt in cotton. Phytopathology 62:336-338.

Monsanto Company 2014. New nematode-resistant cotton a step up in yield potential. <http://www.aganytime.com/newsroom/news/Pages/New-Nematode-Resistant-Cotton-a-Step-Up-in-Yield-Potential.aspx> Accessed March 17, 2015.

Smith, S. N., Ebbels, D. L., Garber, R. H., and Kappelman, Jr. A. J. 1981. Fusarium wilt of cotton. Pages 29-38 in Fusarium: Diseases, biology, and taxonomy. Eds. Nelson, Toussoun, and Cook. The Pennsylvania State University Press, University Park and London.

Taylor, A. L., and Sasser, J. N. 1978. Morphology and development in *Meloidogyne* species. Pages 4-11 in Biology, identification and control of root-knot nematodes. North Carolina State University Graphics, North Carolina.

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

Chapter III: *Fusarium oxysporum* f. sp. *vasinfectum* race identification

Abstract

Fusarium oxysporum f. sp. *vasinfectum* is a parasitic soil-dwelling fungus that causes Fusarium wilt of cotton. During 2013 and 2014, isolates of this fungus were cultured from Fusarium wilt symptomatic cotton plants and resulting cultures were extracted for DNA. Sequences were amplified by PCR for race identification using a specific partial portion of the translation elongation factor region DNA, or EF-1α, and a portion of the beta tubulin DNA sequence. A total of 123 samples were identified to race. Races detected in 2013 were 1, 8, LA 108, LA 110, and LA 127/140. In 2014, similar results were detected with the exception of LA 110. Races 3, 4, and LA 112 were not detected in either year. A higher quantity of FOV samples was obtained earlier in the seasons, but there was no difference in the races found throughout the season.

Introduction

Fusarium oxysporum Schltd.:Fr. f. sp. *vasinfectum* (Atk.) W.C. Snyd. and H. N. Hans (FOV) was first discovered in an Alabama cotton field by Atkinson in 1892 (Atkinson, 1892) and now plagues the entire cotton belt region (Bennett et al., 2011; Kappelman, 1983; Lawrence et al., 2014). Several distinct races of FOV are known to exist and were previously defined by their ability to infect different domesticated cotton lines in addition to other plants (Armstrong and Armstrong, 1948; Armstrong and Armstrong, 1958; Armstrong and Armstrong, 1960; Armstrong and Armstrong, 1975; Davis et al., 2006), but molecular techniques are utilized now in race distinction and identification. The term "race" refers to genetically differentiated fungi that affect different cultivars of a species in different ways. Holmes et al. (2009) identified novel genotypes of FOV that are believed to have originated in Louisiana and are distinct from all previously known races (LA 127/140, 108, 110, and 112). Some of these races were confirmed to exist in the southeastern United States (Bennett et al., 2011). Several races have been known to exist in Alabama (Bennett et al., 2011; Scott et al., 2011), but a complete profiling of races present has never been conducted until now. The objectives of this study are to: 1) identify via molecular techniques which races of FOV are present at the Fusarium wilt cotton genotype screening field location at the Plant Breeding Unit of Auburn University, 2) determine whether races are more diverse at the beginning or the end of the season, and 3) confirm or deny detection of race 4 in Alabama at this time.

Materials and Methods

Plants displaying foliar symptoms of Fusarium wilt and suspected of infection by FOV were counted for incidence determination and removed from the field trial four times during

2013 (39, 45, 76, and 91 DAP) and from both field trials five times during 2014 (33, 43, 58, 73, and 93 DAP). Removed plants were kept separate by plot using 7.5-liter sized plastic zippered bags. Plants were transported to the laboratory and the hypocotyl was cut away from the shoot and root sections of the plant using clippers or a scalpel. A 0.5 cm thin slice of the hypocotyl was excised using a scalpel, surface sterilized using 95% ETOH for 30 seconds and 0.625% NaOCl for 2 minutes, and aseptically plated onto half-strength acidified potato dextrose agar (APDA) (VWR, Radnor, PA). After 3-5 days of growth, isolates were morphologically confirmed as FOV and incidence recorded (Figures 6 and 7).

A single individual isolate per plot was transferred to a clean half-strength APDA plate in order to increase isolate growth in preparation for DNA extraction. After 3-5 days of growth, a 3-mm plug of the isolate was transferred to a 60-mm plate and grown for 7 days on potato dextrose broth (PDB) (VWR, Radnor, PA). The resulting mycelium was transferred into an autoclaved mortar, frozen with liquid nitrogen for cell lysing and ground. The sample was transferred into a 1.5ml centrifuge tube for frozen storage for up to two weeks. Samples were removed from storage and DNA was isolated using a DNeasy® Plant Mini Kit from Qiagen, Inc. (Maryland, USA). Extracted DNA concentrations were verified using a Nanodrop 2000 and then diluted to a 10% solution using DNA and RNA free PCR water. PCR reactions were conducted in 8-tube strips with 50µl reactions according to Kim et al. (2005). Each reaction included 3µl of concentrated DNA, 2µl each of the forward and reverse primers, 18µl of PCR water, and 24µl of MasterMix (VWR, Radnor, PA). Two sets of primer sequences were used to differentiate between races. The first set was from a specific partial portion of the translation elongation factor region DNA, or EF-1α. Forward primer used was EF1 (5'-

ATGGGTAAGGAAGACAAGAC-3') and reverse primer used was EF2 (5'-GGAAGTACCAGTGATCATGTT-3'). The second set of primers was a portion of the beta tubulin DNA sequence. The forward primer was BT3 (5'-CGTCTAGAGGTACCCATACCGGCA-3') and the reverse primer was BT5 (5'GCTCTAGACTGCTTTCTGGCAGACC-3'). Amplification for the translation elongation factor was carried out in a thermocycler as follows: 95 °C for 2 minutes for initial denaturation; 40 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute; and a final extension of 72°C for 5 minutes. The beta tubulin amplification was carried out using the same thermocycler as follows: initial denaturation at 94°C for 5 minutes followed by 35 cycles of 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 1.5 minutes. After amplification, the PCR products were sent to Lucigen, Corp. (Middleton, WI) for sequencing. Sequence results were aligned using BioEdit Sequence Alignment Editor (Hall, 1999) and compared to previously published reference sequences downloaded from GenBank (Holmes et al., 2009). Phylogenetic and molecular evolutionary analyses were conducted using MEGA software version 6 (Tamura et al., 2013). Maximum likelihood, neighbor joining, and minimum evolutionary analyses were conducted. A bootstrap method with 1000 replicates was used to determine branching pattern in each analysis.

For the 2013 analysis (Figure 12), the evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-2556.9192) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix

of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 49 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 798 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

To analyze the first disease evaluation in 2014 (33 DAP, Figure 13), the evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-889.3654) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 67 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 522 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

To analyze the final wilt evaluation in 2014 (93 DAP, Figure 14), the evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (-

1264.3068) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 40 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 763 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

Results

In 2013, forty FOV isolates were sequenced and analyzed for race identification (Figure 12). Of all isolates detected, 45% of isolates were identified as race 1, 12.5% of isolates were race 8, 32.5% of isolates were LA 108, 2.5% of isolates were LA 110, and 7.5% of isolates were LA 127/140. Races 3, 4, and LA 112 were not detected in 2013. In 2014, 83 isolates were sequenced and identified. Race 1 included 80.7% of isolates; race 8 7.3% of isolates, LA 108 6% of isolates, and LA 127/140 6% included isolates. Races 3, 4, LA 110 and LA 112 were not detected in 2013.

In 2014, five disease evaluations were conducted throughout the season. Samples from the first and fifth evaluations were identified to race in order to determine the variability among races at different sampling dates in the season (Figures 13 and 14). The first wilt evaluation was taken 33 DAP. Fifty-eight isolates were examined and races 1 (76.3%), 8 (9%), LA 108 (7%),

and LA 127/140 (5.4%) were detected. The fifth and final disease evaluation was taken 93 DAP and 31 isolates were identified as race 1 (89.6%), LA 108 (3.8%), and LA 127/140 (7.7%). Race 1 was the most prevalent race detected at both points in the growing season. There were fewer isolates overall and one less race detected later in the season. Race 4 was not detected in either year in this study.

Discussion

It is necessary to identify the races of FOV present in Alabama to understand the diversity of this important disease-causing fungus. A survey for FOV races in the United States had not been conducted since 1983 when Kappelman (1983) collected isolates of FOV from across the country and conducted greenhouse pathogenicity tests on several cotton and two tobacco differentials and determined races 1 and 2 were present in the United States; he also determined that race 2 was more distributed than originally thought (Kappelman, 1983). This was the latest FOV race identification study until a comprehensive survey of the cotton belt was initiated in 2010 (Bennett et al., 2011). Scott et al. (2011) contributed to this process in 2010 by identifying in Alabama races 1, 8, and 4 from lineages II, III, and IV, respectively using partial portions of the EF1-α, BT, and PHO sequences of the fungal DNA. Bennett et al. (2011) confirmed the presence of these races in addition to LA 108 and LA 110. This was the first indication that races 8, LA 108, and LA 110 were present in Alabama (Bennett et al., 2011). The current study confirmed the presence of these races in test plots in Alabama, in addition to other novel genotypes identified by Holmes et al. (2009). Race 1 was discovered in Alabama in 1958 and has a wide distribution across the country (Armstrong and Armstrong, 1958). Race 1 was the most prevalent race detected in this study throughout the season. Race 3 was not detected in

this study, nor was it detected in the study by Bennett et al. (2011). Race 3 has indications of low pathogenicity and is thought to have little impact on commercial cotton production (Kim et al., 2005). LA 108, 110, and 127/140 were detected in 2013, and LA 108 and 127/140 were identified in 2014. LA 112 was not detected in either year and LA 110 was not detected in 2014. The previously detected race 4-like isolates in Alabama (Bennett et al., 2011; Scott et al., 2011) were further tested with pathogenicity studies and further investigation led to the conclusion that these race 4-like isolates had genetically distinct intergenic spacer (IGS) nuclear DNA when compared to race 4 isolates in the San Joaquin Valley of California (Bennett et al., 2013). To our knowledge, race 4 is contained within California in the United States. The quantity of isolates found at the beginning of the season (33 DAP) in 2014 was higher than that found at the end of the season (93 DAP), but the same races were detected throughout the season (Figures 13 and 14). The susceptible check Rowden was infected with all races of FOV, but no specific conclusions could be drawn about FOV races being more infective towards a certain commercial cultivar over another.

Literature Cited

Armstrong, G. M., and Armstrong, J. K. 1948. Non susceptible hosts as carriers of wilt Fusaria. Phytopathology 38:808-826.

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. 1975. Reflections on the wilt Fusaria. Phytopathology 13:95-103.

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

Atkinson, G. F. 1892. Some diseases of cotton. Alabama Agricultural Experiment Station Bulletin 41.

Bennett, R. S., Bell, A. A., Woodward, J. E., Lawrence, K. S., Rothrock, C. S., Kirkpatrick, T. L., Lawrence, G. W., Colyer, P. D., Davis, R. M. 2011. Progress report on a ontemporary survey of the Fusarium wilt fngus in United States. Proceedings of the 2011 Beltwide Cotton Conferences, Atlanta, GA, January 4-7, 2011; pages 267-274. National Cotton Council of America, Memphis, TN. <http://www.cotton.org/beltwide/proceedings>

Bennett, R. S., Scott, T. Z., Lawrence, K. S., and Lawrence, G. W. 2013. Sequence characterization of Race 4-like isolates of *Fusarium oxysporum* from Alabama and Mississippi. The Journal of Cotton Science 17:1–6 (2013).

Davis, M., Colyer, P. D., Rothrock, C. S., Kochman, J. D. 2006. Fusarium wilt of cotton: Population diversity and implications for management. Plant Disease Vol. 90: 692-703.

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 Dis Vol. 93:1298-1304.

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

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

Scott, T. Z., Lawrence, K. S., Castillo, J. D., Glass, K. 2011. Fusarium wilt identification and root-knot nematode effects on commercial cotton cultivars in 2010. Proceedings of the 2011 Beltwide Cotton Conference Vol. 1: 224-229. National Cotton Council of America, Memphis, TN. <http://www.cotton.org/beltwide/proceedings>

Tamura, K. and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512-526.

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

Overall Conclusion

The main purposes of this project were to 1) screen and identify potentially resistant or tolerant commercial cultivars to the Fusarium wilt *M. incognita* disease complex and 2) identify strains of FOV to race in order to determine what races are present at the Fusarium wilt testing site in Alabama. Although no cultivars have been proven to be completely resistant, several show promising results with very little Fusarium wilt incidence and low *M. incognita* populations. There were five FOV races found to be naturally present in Alabama at the field testing site, in addition to a healthy natural *M. incognita* population, making this particular site a good location to continue execution of these trials in the future. One significant find was there was no FOV race 4 detected in either year. FOV race 4 has proven to be extremely pathogenic without the presence of the *M. incognita* and is currently only found in the San Joaquin Valley in California. This particular race can cause total devastation for a cotton crop, so it is important to keep this race isolated so it does not invade other areas of the country.

Appendix

Table 1. Cultivars of the Commercial Cotton Fusarium Wilt Trials by Year.[z]

2013	2014
*Susceptible check: Rowden	*Susceptible check: Rowden
*Resistant check: M-315	*Resistant check: M-315
*Croplan 3787 B2RF	*Croplan 3787 B2RF
*Deltapine 1050 B2RF	*Deltapine 1050 B2RF
*Deltapine 1137 B2RF	*Deltapine 1137 B2RF
*Deltapine 1252 B2RF	*Deltapine 1252 B2RF
Deltapine 12R242 B2R2	Deltapine 1133 B2RF
*Deltapine 1321 B2RF	*Deltapine 1321 B2RF
*Fibermax 1944 GLB2	Deltapine 1454 NR B2RF
*Stoneville 4946 GLB2	*Phytogen 339 WRF
*Stoneville 6448 GLB2	*Phytogen 375 WRF
*Phytogen 339 WRF	*Phytogen 499 WRF
*Phytogen 375 WRF	Phytogen 427 WRF
*Phytogen 499 WRF	Phytogen 575 WRF
	*Fibermax 1944 GLB2
	*Stoneville 4946 GLB2
	*Stoneville 6448 GLB2
	Stoneville 4747 GLB2

[z] Cultivars indicated by an asterisk were tested in both years.

Table 2. Fusarium wilt (FOV) incidence percentages, *M. incognita* eggs per gram of root, and seed cotton yield in kg/ha with confidence intervals and Dunnett's *P* vales for comparison to susceptible (Rowden) and resistant (M-315) checks (2013).

Cultivar	FOV %	95% CL	Dunnett's *P* M-315	Dunnett's *P* Rowden	*M. i.* eggs/g root	95% CL	Dunnett's *P* M-315	Dunnett's *P* Rowden	Yield kg/ha	95% CL	Dunnett's *P* M-315	Dunnett's *P* Rowden
CG 3787 B2RF	2.5	(1.1, 5.7)	0.186	0.004	169	(73, 390)	0.869	0.332	3700	(2078, 5322)	1.000	0.748
DP 1050 B2RF	5.8	(3.3, 10.1)	0.032	0.123	141	(61, 325)	0.982	0.176	3114	(1492, 4736)	1.000	0.987
DP 1137 B2RF	3.7	(1.8, 7.2)	0.281	0.012	409	(178, 943)	0.055	1.000	3252	(1631, 4874)	1.000	0.963
DP 1252 B2RF	7.4	(4.3, 12.2)	0.001	0.485	252	(109, 580)	0.362	0.841	2130	(508, 3752)	0.923	1.000
DP 12R242 B2R2	4.3	(2.3, 7.8)	0.010	0.013	111	(48, 255)	1.000	0.064	4257	(2635, 5879)	0.999	0.365
DP 1321 B2RF	1.0	(0.3, 3.2)	1.000	0.001	409	(177, 942)	0.055	1.000	2781	(1159, 4403)	1.000	1.000
FM 1944 GLB2	0.3	(0.0, 2.6)	0.998	0.010	111	(48, 257)	1.000	0.066	5936	(4314, 7558)	0.164	0.008
PHY 339 WRF	0.3	(0.0, 2.3)	0.891	0.007	681	(295, 1569)	0.004	1.000	5277	(3655, 6899)	0.350	0.045
PHY 375 WRF	1.1	(0.4, 3.5)	0.999	0.001	602	(261, 1389)	0.008	1.000	4716	(3094, 6338)	0.791	0.157
PHY 499 WRF	0.9	(0.2, 3.0)	1.000	0.001	183	(79, 422)	0.778	0.424	5383	(3761, 7005)	0.511	0.034
ST 4946 GLB2	0.8	(0.2, 3.0)	1.000	0.001	264	(115, 609)	0.308	0.891	5074	(3452, 6696)	0.481	0.072
ST 6448 GLB2	0.9	(0.2, 3.1)	1.000	0.002	999	(434, 2304)	0.000	0.877	2708	(1086, 4330)	1.000	1.000
M-315 (R check)	0.9	(0.3, 2.6)		0.000	86	(43, 170)		0.006	3353	(2004, 4701)		0.873
Rowden (S check)	11.8	(7.5, 18.1)	0.000		510	(256, 1016)	0.006		2198	(850, 3547)	0.826	

Table 3. Fusarium wilt (FOV) incidence percentages, *M. incognita* eggs per gram of root, and seed cotton yield in kg/ha with confidence intervals and Dunnett's *P* vales for comparison to susceptible (Rowden) and resistant (M-315) checks (2014).

Cultivar	FOV %	95% CL	Dunnett's *P* M-315	Dunnett's *P* Rowden	*M. i.* eggs/g root	95% CL	Dunnett's *P* M-315	Dunnett's *P* Rowden	Yield kg/ha	Standard Error	Dunnett's *P* M-315	Dunnett's *P* Rowden
CG 3787 B2RF	13	(4, 17)	0.0024	<.0001	940	(0, 2138)	0.1232	0.4339	1866	321.64	0.6224	0.0006
PHY 375 WRF	9	(0, 12)	0.0684	<.0001	2156	(957, 3354)	0.0005	0.2226	1617	321.64	0.2079	0.0068
PHY 499 WRF	5	(0, 9)	0.4453	<.0001	1078	(0, 2276)	0.0774	0.5792	2842	321.64	0.0121	<.0001
PHY 339 WRF	2	(0, 6)	0.9087	<.0001	1372	(174, 2570)	0.0251	0.9439	2592	321.64	0.0800	<.0001
PHY 427 WRF	1	(0, 5)	0.5929	<.0001	636	(0, 1834)	0.2958	0.2002	3033	321.64	0.0021	<.0001
PHY 575 WRF	7	(0, 10)	0.2529	<.0001	1670	(471, 2868)	0.0067	0.6739	1805	321.64	0.4956	0.0011
DP 1321 B2RF	3	(0, 7)	0.8485	<.0001	1675	(477, 2873)	0.0065	0.6671	2238	321.64	0.5077	<.0001
DP 1133 B2RF	15	(6, 19)	0.0003	<.0001	823	(0, 2022)	0.1763	0.3304	1859	321.64	0.6091	0.0006
DP 1252 B2RF	7	(0, 11)	0.1590	<.0001	639	(0, 1837)	0.2935	0.2020	1605	321.64	0.1949	0.0075
DP 1050 B2RF	6	(0, 10)	0.3403	<.0001	788	(0, 1986)	0.1954	0.3025	2031	321.64	0.9845	<.0001
DP 1137 B2RF	11	(2, 15)	0.0114	<.0001	654	(0, 1852)	0.2824	0.2108	2016	321.64	0.9796	0.0001
DP1454NR B2RF	17	(8, 21)	<.0001	<.0001	433	(0, 1631)	0.4761	0.1070	1569	321.64	0.1588	0.0104
ST 4747 GLB2	1	(0, 5)	0.5929	<.0001	1302	(104, 2500)	0.0333	0.8534	3214	321.64	0.0003	<.0001
ST 4946 GLB2	2	(0, 6)	0.7891	<.0001	935	(0, 2133)	0.1250	0.4296	2826	321.64	0.0139	<.0001
ST 6448 GLB2	5	(0, 9)	0.5163	<.0001	3061	(1862, 4259)	<.0001	0.0074	2366	321.64	0.2899	<.0001
FM 1944 GLB2	6	(0, 10)	0.3214	<.0001	2176	(977, 3374)	0.0066	0.2105	2291	321.64	0.4093	<.0001
M-315 (R ck)	2	(0, 6)		<.0001	270	(0, 1167)		0.0072	2024	278.54		<.0001
Rowden (S ck)	30	(21, 33)	<.0001		1601	(563, 2638)	0.0027		767	321.64	<.0001	

Table 4. Summary of FOV isolates taken at first and final disease evaluations (2014).

	20-Jun-14	20-Aug-14
Race	No. of isolates	No. of isolates
Race 1	36	27
Race 8	5	0
LA 127/140	4	2
LA 108	4	1
Total no. of isolates	49	30
Total no. of plants with wilt symptoms	49	30
Total plants examined for wilt	144	144

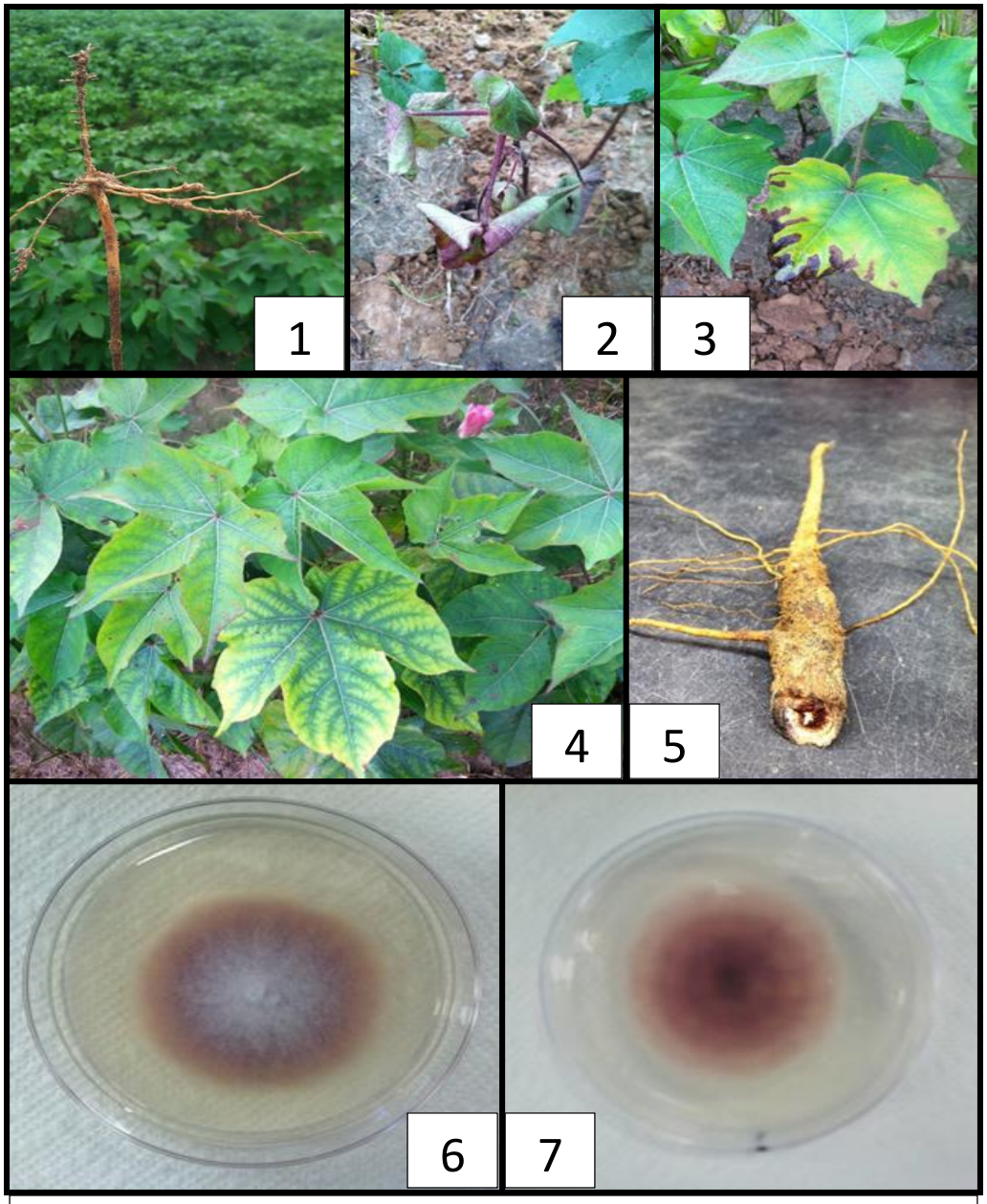


Figure 1. *M. incognita* damage on cotton.
Figure 2. Fusarium wilt symptoms on cotton.
Figure 3. Fusarium wilt chlorotic and necrotic symptoms on cotton.
Figure 4. Chlorotic Fusarium wilt symptoms on cotton.
Figure 5. Vascular discoloration of cotton caused by Fusarium wilt.
Figure 6. Isolated culture of FOV from symptomatic cotton plants (front view, APDA).
Figure 7. Isolated culture of FOV from symptomatic cotton plants (rear view, APDA).

Figure 8. Minimum and maximum temperatures (C) and precipitation (cm) during the 2013 growing season. Hot, dry conditions early in the season discouraged FOV pressure.

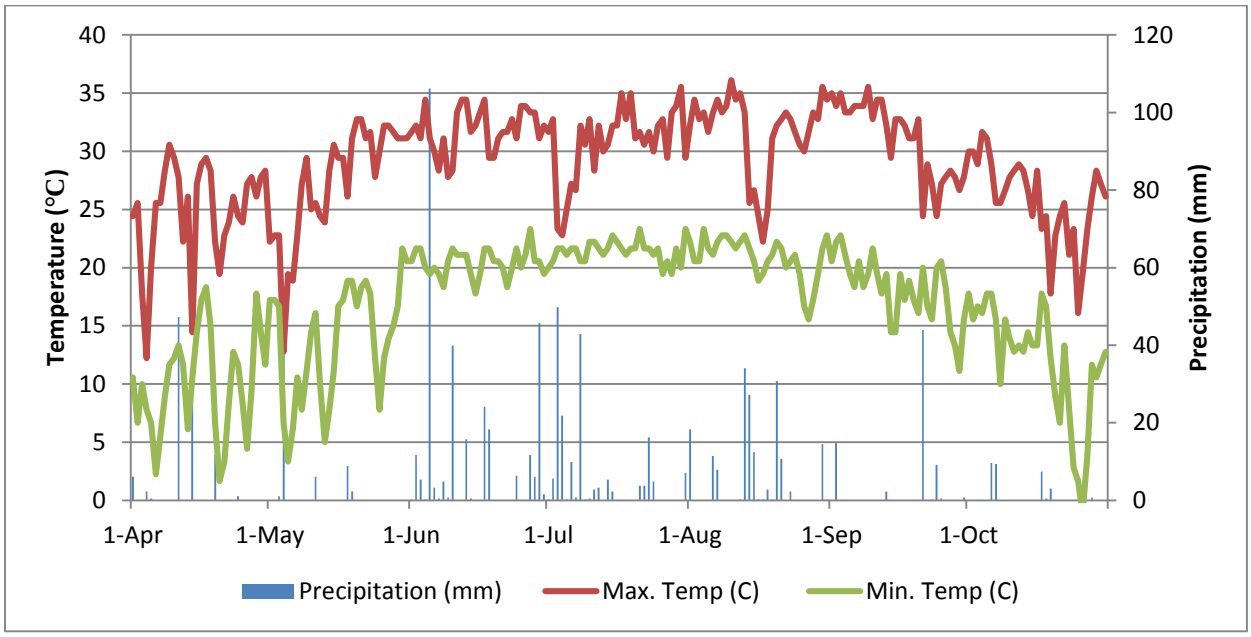


Figure 9. Minimum and maximum temperatures (C) and precipitation (cm) during the 2014 growing season. Cooler, moist conditions favored FOV development.

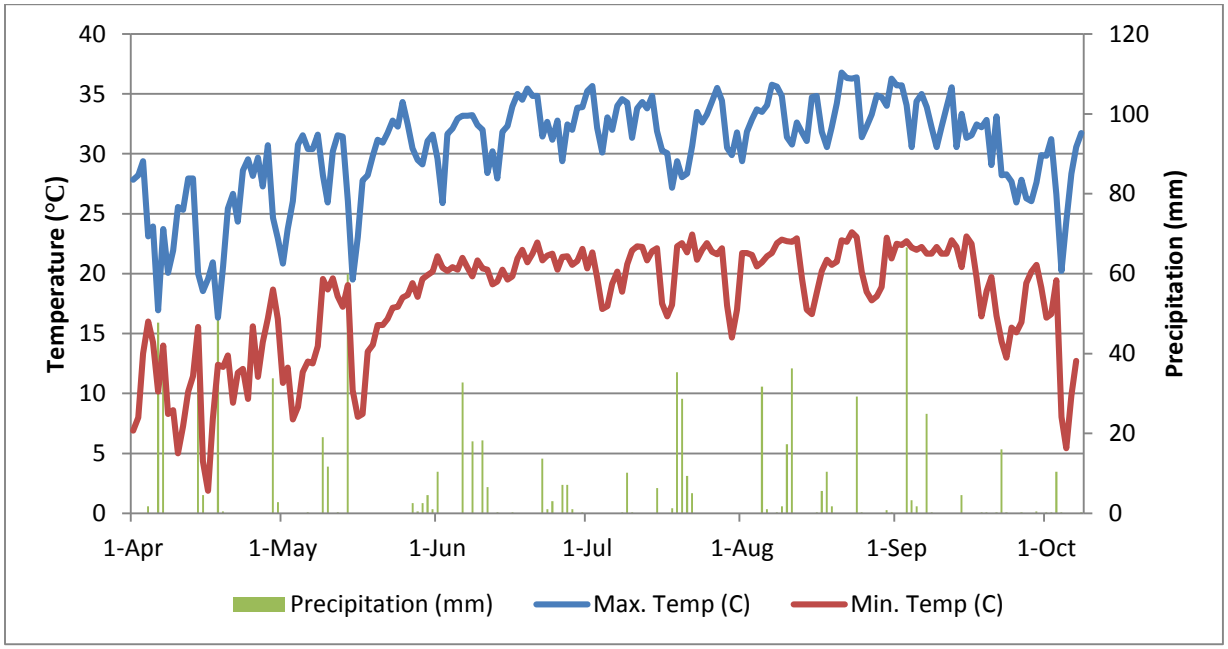
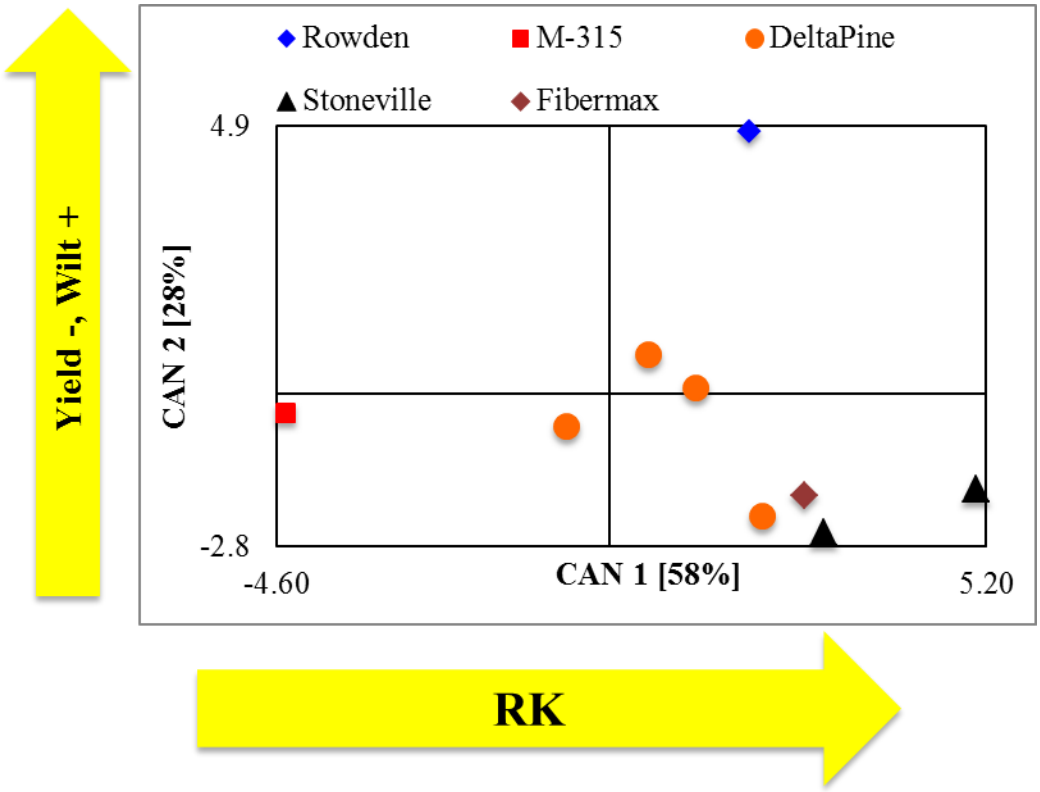
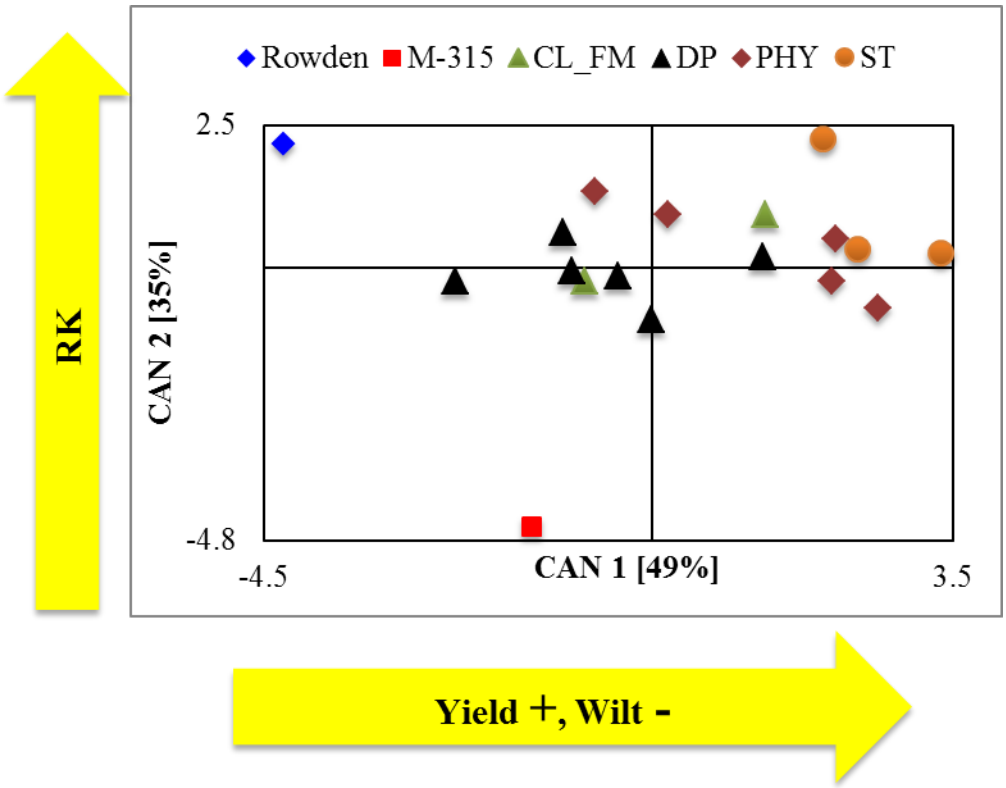


Figure 10. Canonical discriminant analysis of cotton cultivar yield, FOV incidence, *M. incognita* eggs per gram of root, and total nematode egg counts in 2013/2014. CAN 1 is driven by root-knot egg numbers (r = 0.98) thus nematodes increase moving away from the Y axis. Differences in CAN 2 are driven by seed cotton yield and FOV incidence with lower yield and higher wilt in the top half of the chart.



Variable	Can1	Can2	Abs1	Abs2	Rank1	Rank2
S14_RKgmroot	0.98	0.17	0.98	0.17	1	9
S14_RKeggs	0.98	0.17	0.98	0.17	2	10
Y13_RKeggs	0.93	0.20	0.93	0.20	3	8
N14_RKgmroot	0.90	-0.04	0.90	0.04	4	12
N14_RKeggs	0.90	-0.04	0.90	0.04	5	11
Y13_RKgmroot	0.88	0.24	0.88	0.24	6	7
N14_Wilt	0.27	0.93	0.27	0.93	11	1
S14_Wilt	0.40	0.90	0.40	0.90	8	2
Y13_Wilt	0.27	0.88	0.27	0.88	10	3
N14_kgha	0.39	-0.84	0.39	0.84	9	4
S14_kgha	-0.58	-0.76	0.58	0.76	7	5
Y13_kgha	-0.27	-0.54	0.27	0.54	12	6

Figure 11. Canonical discriminant analysis of cotton cultivar yield, FOV incidence, *M. incognita* eggs per gram of root, and total nematode egg counts for 2014. CAN 1 is driven by seed cotton yield (r = 0.94) and has a negative correlation with wilt incidence (r = .0.83) thus yield increases and FOV incidence decreases from left to right. Differences in CAN 2 are driven by nematode variables (r = 0.98) with nematode numbers increasing from bottom to top.



Variable	Can1	Can2	Abs1	Abs2	Rank1	Rank2
N_kgha	0.94	0.04	0.94	0.04	1	8
N_Wilt	-0.83	0.36	0.83	0.36	2	7
S_Wilt	-0.81	0.53	0.81	0.53	3	6
S_kgha	0.67	-0.59	0.67	0.59	4	5
S_RKeggs	0.07	0.98	0.07	0.98	7	1
S_RKgmroot	0.07	0.98	0.07	0.98	8	2
N_RKgmroot	0.41	0.73	0.41	0.73	5	3
N_RKeggs	0.41	0.73	0.41	0.73	6	4

Figure 12. Maximum likelihood evolutionary tree for *Fusarium oxysporum f. sp. vasinfectum* isolated in 2013. Races 1, 8, LA 108, LA 110, and LA 127/140 were detected. Races 3, 4, and LA 112 were not detected.

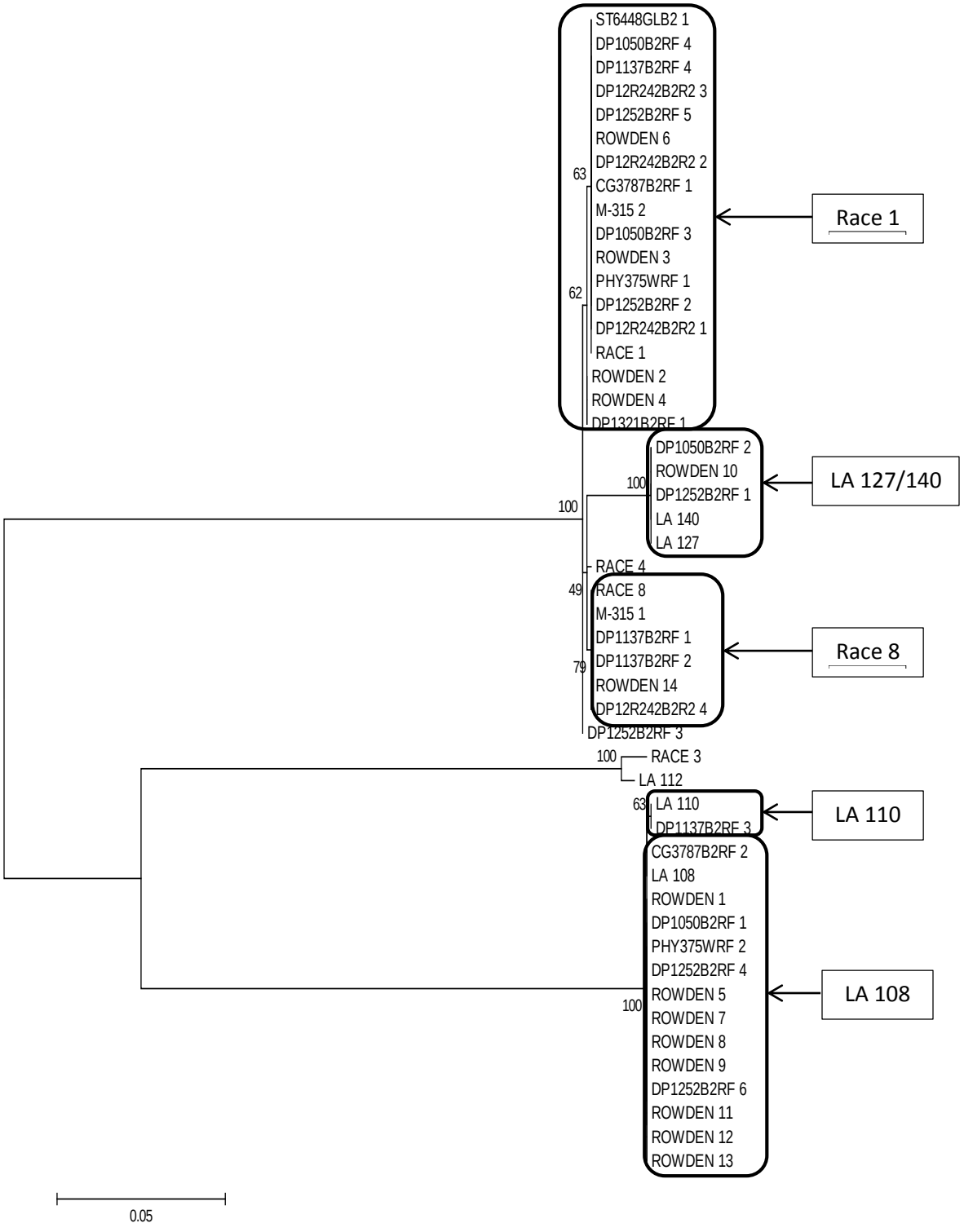


Figure 13. Disease evaluation at 33 DAP in 2014. Race 1 was the prominent race detected. Races 8, LA 108, and LA 127/140 were also detected.

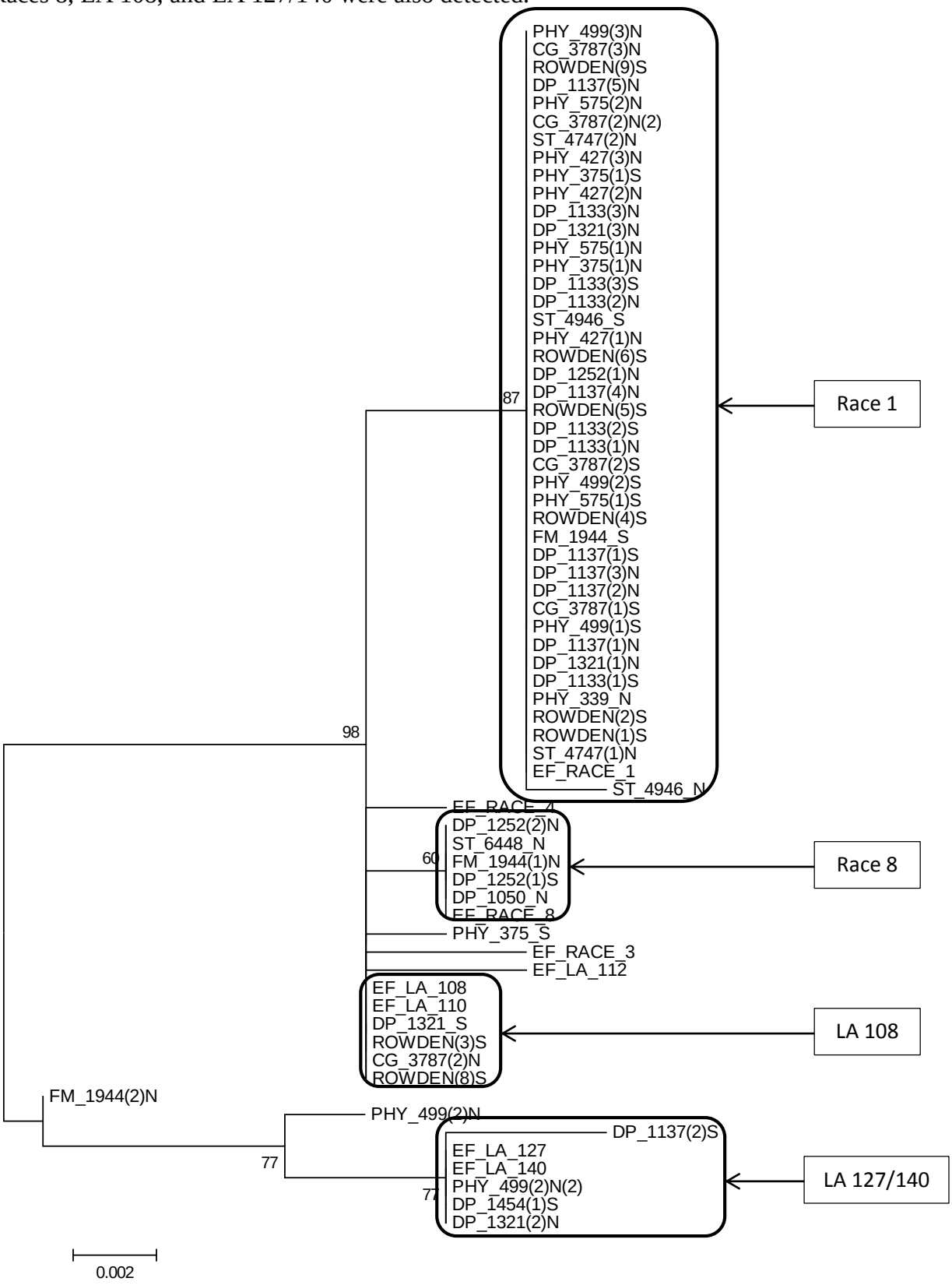


Figure 14. Disease evaluation at 93 DAP in 2014. Race 1 was the prominent race detected. Races LA 108 and LA 127/140 were also detected.