The study of applied management options to enhance crop protection against Verticillium wilt in *Gossypium hirsutum*.

by

Caroline Josephine Land

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
August 1, 2015

Keywords: cotton, agriculture, fungi, plant disease

Approved by

Kathy S. Lawrence, Chair, Professor of Plant Pathology
Dale Monks, Professor of Crop, Soil, and Environmental Sciences
Austin Hagan, Professor of Plant Pathology

Abstract

Verticillium wilt of cotton is incited by *Verticillium dahliae*, which colonizes the vascular cylinder of the plant and resulting in defoliation, stunting, and yield loss. The long-term goal of this project was to look closely at Verticillium wilt in Alabama and find multiple methods for management of this disease to enhance cotton yields. Specifically our objectives were: 1) assess the state cotton fields with wilt symptoms to determine Verticillium presence; 2) evaluate cotton cultivars for resistance or tolerance to Verticillium wilt in the field; 3) determine whether different soils types are conducive to Verticillium wilt disease severity; and 4) assess potential chemical fungicides for efficacy to *Verticillium* spp. *in vitro*.

In Alabama, two different species were found in the state, *Verticillium. dahliae* and *V. albo-atrum*. Both species were found in the northern part of the state where the wilt is often a yield limiting factor in cotton production. However only *V. albo-atrum* was found in the mid to southern part of the state, where Verticillium wilt is rarely a concern in cotton. Cotton variety selection impacts Verticillium development in cotton. In 2013, plants displaying the least foliar symptoms were Stoneville (ST) 4747 GLB2, FiberMax (FM) 1944 GLB2, Phytogen (PHY) 339 WRF, and Deltapine (DP) 1044 B2RF. In terms of disease incidence for 2013, the resistant check FM 1944 GLB2 had the lowest number of plants with darkened vascular systems, while ST 4946 GLB2 and FM 1944 GLB2 showed the least symptoms in 2014. Varieties all displayed vascular staining and average percent of disease incidence ranged from 46-68%. In terms of disease

incidence, ST 4946 GLB2 had the lowest number of plants with darkened vascular systems. Vascular staining and average percent of disease incidence ranged from 50-53% for PHY 339 WRF, ST 4747 GLB2 and ST 5032 GLB2. These percentages were statistically similar and indicated less disease than was noted for the resistant check FM 1944 GLB2. Ranking the cultivars by yield indicates ST 4747 GLB2 and DPLX 14R1455 B2R2 produced the greatest yield with a yield gain of 19% when compared to the resistant check FM 1944 GLB2. The cultivars DP 1137 B2RF, ST 4946 GLB2, and DPLX 13R352 B2R2 also increased yield on average of 5% compared to the check. Using the average price of cotton for 2014 of 76 cents per pound a $650 per acre revenue increase was obtained with ST 4747 GLB2 over the check FM 1944 GLB2. Gross income was also 53% higher for ST 4747 GLB2 than the most susceptible line DPLX 14R1456. Thus cotton variety selection has the greatest impact of yield and income in the Verticillium-infested field. In addition to the cultivar test a microplot trial was conducted to determine disease severity with and without irrigation in selected soil types commonly found throughout the state of Alabama. A significant interaction was found between the heaviest soil type, Houston Clay, and irrigation. The irrigated Houston Clay soil significantly supported a greater disease incidence compared to the other soil types with or without irrigation. To give producers some potential control options, fungicide active ingredients were evaluated *in vitro* for efficacy against *V. dahliae* and *V. albo-atrum*. Thirteen chemicals were tested at 100% labeled rates. *In vitro*, *V. dahliae* growth was best inhibited by Methoxy–carbamates, and Methoxy-acrylates + Triazoles chemical groups more specifically Quilt Xcel and Domark fungicides. *V. albo-atrum* growth was best inhibited by the same chemical groups (Methoxy – carbamates, and Methoxy- acrylates + Triazoles) however, specific fungicides with superior activity were Quilt Xcel and Headline. In summary, two species of Verticillium are found in the portion of the state where disease is most prevalent. ST 4747 GLB2 was the most tolerant cultivar producers can plant to combat yield loss. Producers who irrigate and are planting in heavier soils are at higher risk for Verticillium wilt in cotton. There are some potential fungicide treatments that possibly may reduce disease severity. Understanding infection risks and implementing different management options is the best way to combat this disease.

Acknowledgments

I would like to extend my expression of gratitude to everyone that helped me complete this project. A very special thank you to my major professor, Dr. Kathy Lawrence, for her guidance, encouragement, and endless support throughout this project. Thank you to the sponsors, Cotton Incorporated, Alabama Cotton Commission, and the USDA, for their contributions to the funding of this project. Thank you to my committee members Dr. Dale Monks and Dr. Austin Hagan, for their contributions of time and guidance of this project. Thank you to the graduate student and undergraduate students, Ni Xiang, Amber Smith, David Bailey, Andrew Sikkens, Justin Luangkhot, Stephen Till, Presley Townsend, Lam Duang, and Roelof Sikkens, Cassie Spencer and for their countless hours of labor and support that helped make this project possible. Thank you to colleague and collaborator Terry Wheeler who helped with many different aspects of this project. Thank you to Agri- AFC, especially Brad Meyer, whose contributions and generous support made this project possible. Thank you to Charlie Burmester who was right there with us rating plants, cutting stems, and picking cotton. Thank you to the staff of the Plant Science Research Center whose extensive time and care was put into the experimental trials. Thanks to my boyfriend, Gavin Falcona, for supporting me through this project and helping me balance everything out. I would also like to thank my parents, John and Wendy Land, without their support I could not have made it through this process, I cannot thank you enough

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Chapter I

Introduction and Review of Literature

Introduction and Problem Statement

This project focuses on the serious problem of Verticillium wilt on cotton **(*Gossypium hirsutum*) *L.***. This disease, which was first recorded in Virginia in 1918 by Carpenter (8) who reported its presence on Upland cotton, is distributed world-wide in major cotton producing countries such as Australia, Brazil and China (29). In the U.S., Verticillium wilt is present in most states across the cotton belt. Of the approximately ten million acres of cotton grown in the U.S, 97% is planted to Upland cotton (28). Cotton production is greatly impacted by the fungal plant pathogens. Losses from Verticillium wilt for the U.S., according to disease loss estimates, between the years of 1990-2013 are approximately 480 million bales (11). Verticillium wilt occurs most often occurs in the northern regions of Alabama and causes a decline in plant health and yield. It is caused by the soil born fungus *Verticillium dahliae Kleb.,* which first colonizes the root and then moves upward in the vascular system of the plant (15). This colonization of the vascular system prevents water movement creating wilted appearance of the plant. Symptoms in Alabama are not observed until boll set when diseased plants prematurely begins to defoliate. Defoliation leads to yield decrease from the lack of photosynthetic activity. Verticillium wilt symptoms included stunting, lack of lateral growth, and decreases in yield, fiber quality, and seed quality (7, 42).

Verticillium is the anamorphic form of an ascomycete imperfect fungus and reproduces asexually through the production of conidia on whorled conidiophores (18). Two *Verticillium* species are found in Alabama, *V. albo-atrum* Reinke and Berthold and *V. dahliae.* Morphologically, the difference between these two species is microsclerotia production (14). Microsclerotia are the dark torulose globose cells designed for over-wintering and enhance pathogen survival are formed by *V. dahliae*. Often these structures are formed from hyphal budding and multilateral septation. The *V. albo-atrum* does not produce microsclerotia but instead forms dark thickened resting mycelium which are dark swollen turulose hyphal cells (13). Microsclerotia are known to germinate, when exposed to root exudates, and begin another infection cycle when environmental conditions are ideal (15). *Verticillium* sp. generally prefers air temperatures ranging from 70-85 degrees F and moist soils (13).

Limited management options makes Verticillium wilt a difficult disease to control. Disease severity increases with irrigation and enhanced fertilization, two very expensive production inputs that should result in higher lint yields (15, 39). Rotation to a non-host crop such as a corn or grain sorghum will reduce disease (15); however, this is not an economical management option for many producers. Fungicides may help reduce disease severity, but high costs and limited returns make them a poor option for producers (19). The only effective management option producers have is to select a Verticillium wilt tolerant cotton cultivar. The number of cotton cultivars available to producers, however, is limited. The market life of a cotton cultivar is often less than 5 years thus a producer must constantly look for cultivars that yield well when challenged with Verticillium wilt. There is a continuing need for research on cultivar response to *Verticillium*.

Verticillium wilt and the Disease Cycle

Two *Verticillium* spp. are pathogenic to cotton, *V. albo-atrum* and *V. dahlia* (18). *Verticillium albo-atrum* was first characterized by Reinke and Berthold in 1879 on wilted potato plants, while Klebahn isolated *V. dahliae* in 1913 from wilted dahlia noting there were slight morphological differences in from the previously discovered *Verticillium* species (22). *V. ablo-atrum* and *V. dahliae* were considered to be one species until recent genetic work proved they are two distinct species (13). In the 1980's, Stunnikova and Muromtsev used binary cross-immunoelectrophoresis to analyze different antigenic composition Verticillium species (34). Verticillium is an anamorphic Ascomycota in the family Hypocreaceae (32). Common characters for this genus are verticillate or whorled conidiophores that have more than one level of whorls on an axis as seen in Figure 1(32). Conidia lack septa, produce mucoid or watery masses, and are released by the splitting of a septa resulting in the secession scar without frill or excess tissue (32). Mostly single-celled conidia are cylindrical and hyaline in color, are elongate 50-200 x 15-50 (-100) µm in size (13).

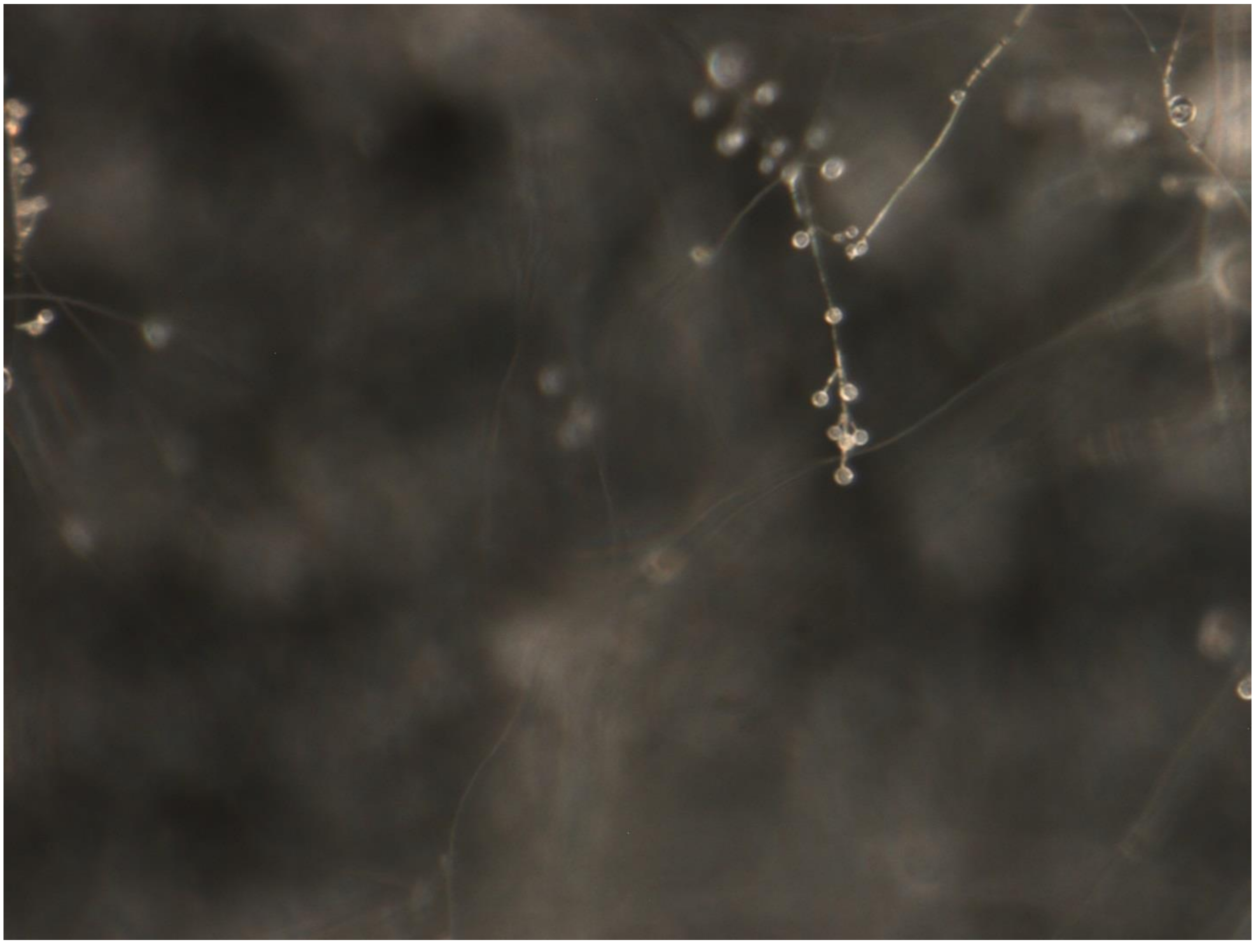

Figure 1: A characteristic verticillate conidiophore of *V. dahliae* with conidia in mucoid masses.

V. albo-atrum, which was first reported as the causal agent of potato wilt, has a host range that includes over 300 plant species including a number of vegetable and agronomic crops (4). This fungus is more commonly distributed in more northern climates of the United States however; it does exist in temperate zones, which includes Alabama. *V. albo-atrum* is a root-inhabiting fungus (13). Infections of the xylem often lead to plant death (13). Over wintering of this fungal pathogen occurs as dark resting mycelium that can be found in plant tissue or in the soil (13).

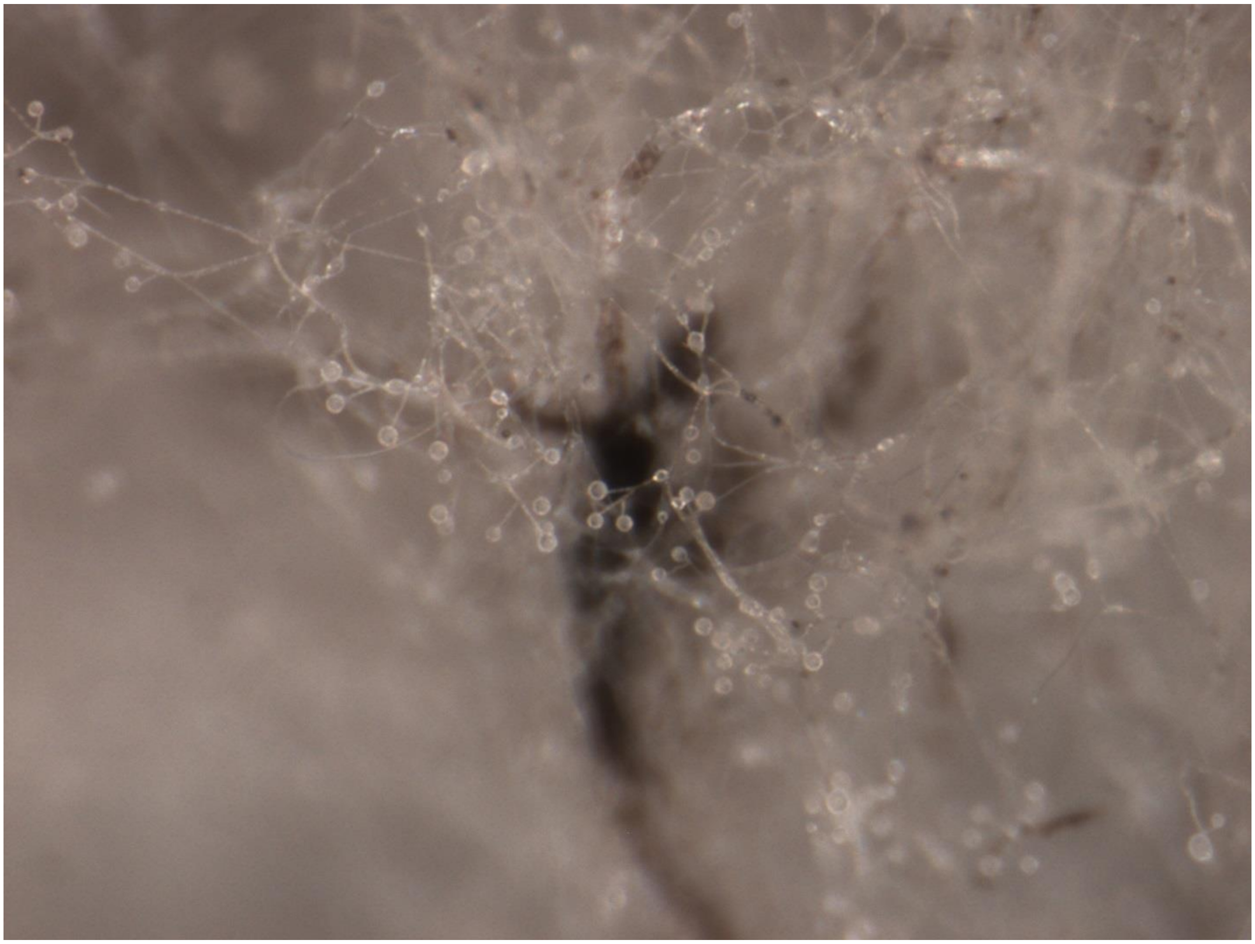

Figure 2. *Verticillium albo-atrum* culture with condiaphores and dark resting mycelium in the background.

The primary causal agent of Verticillium wilt in cotton is *Verticillium dahliae;* which is the only *Verticillium* sp. shown to cause wilt in cotton under field conditions (13). *V. dahliae* overwinters in the soil and plant debris as microsclerotia (15). Microsclerotia remain viable in extreme temperature even over common temperatures for prime growth, which range from 18-33°C (3, 20). Microsclerotia can remain dormant for at least14 years, until root exudates stimulate the germination of infectious hyphal structures (13). Areas of ruptured epidermal root cells have the highest chance of infection by germinated microsclerotia in close proximity (13). Once the fungus has entered the epidermal cells, the hyphae will grow through the cortex intercellularly and intracellularly, eventually colonize the xylem. Colonization of the xylem initiates reproduction as evidenced by the production of conidia (15). Verticillium infection progresses upward by the passive movement of conidia in the transpiration stream through the xylem or hyphal growth. Xylem vesicles become congested with gels, gums, and tylosis produced by the plant in response to the invading fungus, which results in diagnostic wilt symptoms (15). Loss of turgor pressure begins at the base of the plant and progresses upward until the leaves become chlorotic. Leaf chlorosis is followed by necrosis, premature defoliation, and eventually plant death (15, 29). The fungus overwinters in the dead plant residue collected from the past growing seasons or during the winter months. In the presence of sufficient moisture, cooler temperatures, and a suitable host, the fungi will germinate and infect plants in close proximity.

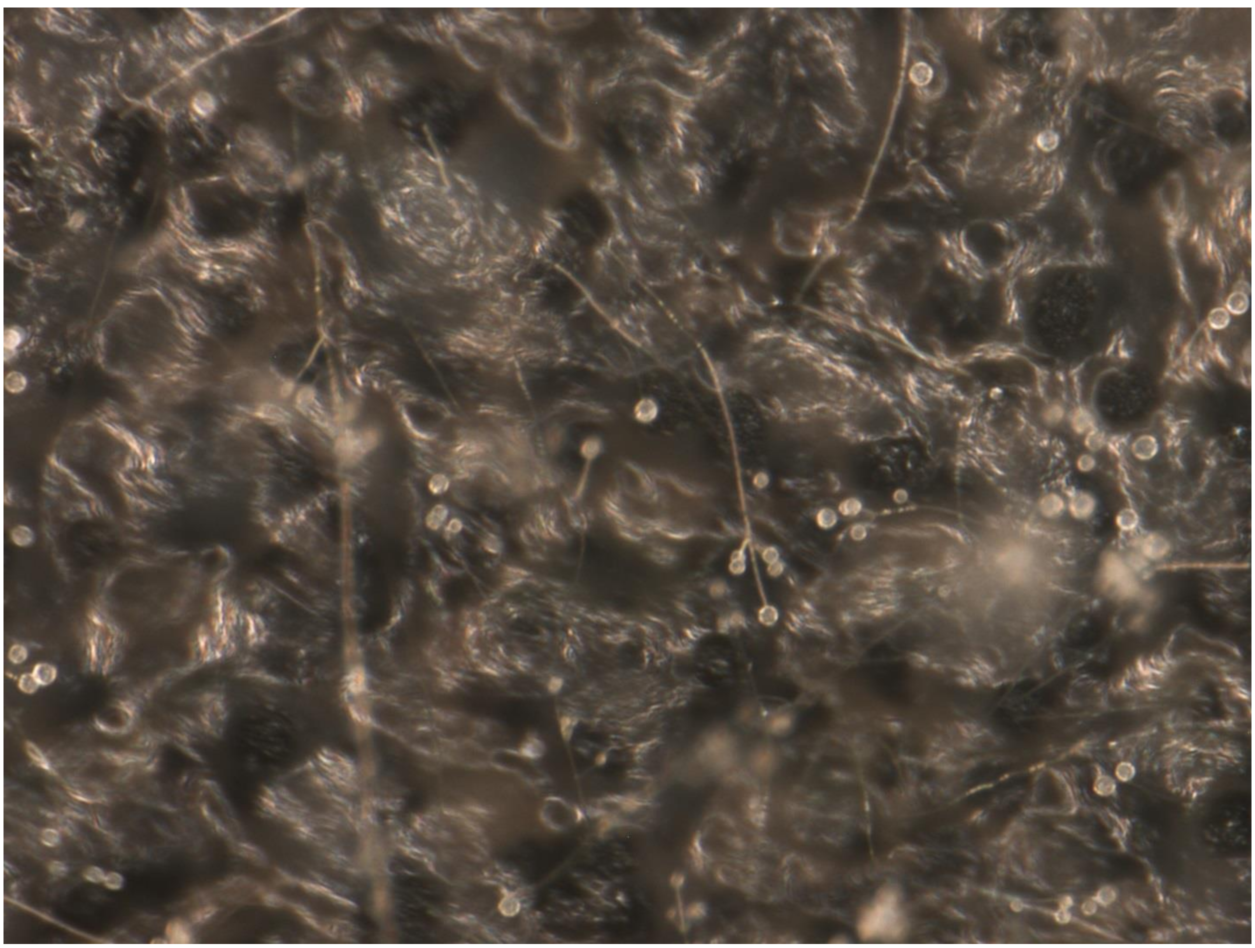

Figure 3. *Verticillium dahliae* conidiophore growing above microsclerotia formed in culture.

Verticillium wilt on Cotton

Carpenter (11) first observed Verticillium wilt in cotton in Virginia in 1918 (8). He noted that the same pathogen was isolated from okra (*Abelmoschus ecculentus*) in the area (9). Economic importance of this disease in cotton was not established until Sherbakoff in 1928 observed widespread wilt symptoms in Tennessee. Sherbakoff (33) than began an attempt to breed wilt resistant cotton (33). There are few varieties of Upland cotton that are tolerant or relatively resistant to *Verticillium dahliae* (36, 37). Tolerance to the pathogen can lead to reduced foliar symptom development and yield loss. To date, there is no known genetic source of immunity in Upland cotton (2). Other cotton genotypes, such as Pima cotton (*Gossypium barbadense*), have been tested for disease susceptibility to *V. dahliae* and results indicate various levels of Verticillium wilt resistants, however; all *Gossypium hirsutum* genotypes are susceptible pathotypes of *V. dahliae* (4).

Verticillium wilt resistance in Pima cotton is thought tio be associated with the plant's production of terpenoids which are toxic compounds produced by the plant to inhibit pathogen growth (21). When exposed to a pathogen or pest, plants begin to synthesize these compounds (21). Three terpenoids are found in cotton roots: gossypol, hemigossypol, and desoxyhemigossypol (21, 26). Harrison and Beckman (1982) saw an increase in terpenoid concentrations in Pima cotton exposed to *Verticillium sp*. Studies have shown that *Verticillium dahliae* is highly sensitive to terpenenoids (20). It is unknown what terpenoids concentrations are produced by Upland cotton following exposure to the *V. dahliae*.

Verticillium resistance is available in other crops such as tomato (*Solanum lycopersicum*). Tomato inherits a single dominant gene *Ve* that allows the plant to express resistance to certain isolates of *Verticillium dahlia;* unfortunately the genetic relationship between *V. dahliae* and cotton is not that simple (12). In cotton, Verticillium isolates have been divided into two groups, the more virulent defoliating types and non-defoliating types. Jaoquim and Rowe (23) designated all defoliating isolates to be in the group P_1 and P_2. The P_2 isolates are less virulent than the P_1 isolates (23). Unfortunately, the P_1 types are widely dispersed throughout the U.S. cotton belt (29). It has been shown by Mert et al. (2005) that a single dominant V^t gene is responsible for tolerance to defoliating isolates, while two dominant genes control the expression for resistance to non-defoliating isolates. This is indicated from a segregation of 12:3:1 ratio in F2 population for the non-defoliating isolates (27). It has been suggested that Verticillium wilt resistance in cotton is genetic; however, environment plays a major role in gene expression. Heritability of Verticillium wilt resistance, in the broad sense, has been estimated in some experiments to range from (H^2 = 0.25–0.66) (16). The higher the number the more likely the genes will be passed to the next generation through genes as opposed to environmentally induced plant responses. Variability of the H^2 score could also be contributed to the nature of the fungi and distribution of population in the soil (16).

Environmental Factors

Temperature and moisture are the two most important environmental factors that contribute to the spread and proliferation of *Verticillium dahliae* (1). Moist soils from irrigation enhance the incidence of Verticillium wilt in cotton. As the timing intervals of watering regiments increase so do the disease incidences of cotton plants, while the non-irrigated plots had very little infection present (31). Irrigation cools the soil thereby enhancing pathogen survival and increasing infection rates (25). Microsclerotia germination increases with periodical wetting and drying of the soil (17). Moisture is also an important component for conidia dispersal, along with root infection and colonization. Infected plants are more susceptible to water stress due to the presence of *V. dahliae* hyphae in the xylem inhibiting water flow (42). Plants also experience stomatal closure causing the plant to become deficient in the accumulation of carbon dioxide, an important component for photosynthesis (5). Drought-stressed cotton typically suffers less infection by *V. dahliae* compared with irrigated cotton (24). It is unknown if this relationship is due to the moisture contributing to the level of humidity or if it is related to irrigated-related cooling of the soil.

Verticillium dahliae is a temperature sensitive soil pathogen. Verticillium wilt of cotton only becomes an economically important disease when soil temperatures cooler (2). As soil temperatures decline, disease severity increases until it plateaus at 22°C (2). In 1967 Brinkerhoff noted symptom expression in cotton exposed to 28°C during the day and 18°C at night. Consequently, when he repeated the test and had a higher day temperature of 36°C and 18°C at night, symptoms did not appear (6). Ideal growing temperatures for fungal growth in soil range from 21-29°C. Temperatures in excess of 32°C inhibit mycelial growth and reproduction (15). If early *V. dahliae* infection occurs, plants can recover if soil temperatures remain high through the remainder of the production season. The critical point of disease development late in the production season occurs when soil temperatures decline and plants begin to mature (2).

Verticillium dahliae over winters in the soil thus the soil moisture and temperatures have a large impact on how pathogen virulence. *V. dahliae* and *V. albo-atrum* are both not very dynamic inhabitants and cannot grow more than a few millimeters through the soil before encountering and infecting a host (10). Increased competition from other soil microbes can reduce infection rates. In culture, a decline Verticillium wilt with increased activity of other soil microflora and microfauna illustrates the potential for disease suppressive soils as a possible control strategy (2).

Verticillium dahliae and *V. albo-atrum* prefer neutral or alkaline soils, pH 6-9. Disease incidence is higher on heavier soils with higher clay and silt content and may be linked to the lower temperatures and higher moisture levels. However, elevated levels of Verticillium wilt have also been noted on intensively irrigated cotton on sandy soils (30).

Management Options

There are several strategies that can be implemented to help manage Verticillium wilt caused by *Verticillium dahliae*. Crop rotation to non-host crops such as grasses, legumes, and crucifers suppresses *V. dahliae* inoculum levels in the soil (15). Wheeler in 2012 indicated a 10% decrease in microsclerotia levels when cotton was rotated to a non-host crop (39). She also indicated rotation systems are more effective when utilized before Verticillium wilt became a problem, that the rotated fields had significantly less wilt than those in continuous cotton, and also higher yields (40). Rotation to any crop other than cotton will reduce inoculum levels and disease severity (2). If possible, rotation to a flooded rice cropping system, where soils are flooded for an extensive period of time, substantially reduces microsclerotia levels (10). However, fields in a cotton monoculture with high microsclerotia levels show little immediate response to rotation to a non-host crop (39) and infested fields need to be cropped for several years to a non-host crop to significantly reduce *V. dahliae* populations and reduce disease incidence (37). Wheeler (40) has shown that rotation to a non-host such as sorghum reduces microsclerotia populations however this is a long term management option. However, farm programs, commodity prices, and equipment availability limit the rotation options for many producers. Cultivar selection can greatly impact the incidence of Verticillium wilt. While, upland cotton varieties do not have high level of disease resistance, differences in cultivar sensitivity to *V. dahliae* can be utilized to reduce wilt-related yield loss in cotton. Irrigation can be an integral part of a Verticillium wilt management program. Cotton grown in soil that has moderate to high densities of microsclerotia will not express wilt symptoms if soil moisture levels are inadequate (40). Unfortunately producers with irrigated land have the greatest yield potential yield and highest risk for wilt-related losses. The chemical option for Verticillium wilt control in cotton has not been explored. Previously, the now unavailable and very expensive methyl bromide was used for wilt control in cotton (38). While the soil fumigant metam sodium is widely used for Verticillium wilt control in potato in the Pacific Northwest, the $157 per acre treatment cost is far too high for a cotton production system (19). The efficacy of new released fungicides, which could be applied as an in-furrow treatment, for the control of Verticillium wilt in cotton has not been established. The most effective method to deal with Verticillium wilt is an integrated management approach since all singular methods tend to individually have marginal impact on disease incidence. The combinations of resistant cultivars, row spacing, irrigation timing, and crop rotation have all shown great results in terms of management (41). Planting cotton in narrow rows has shown to increase yields in Verticillium infested fields in California. This suggests that if growers change from the traditional 91 to 101.6 cm row spacing to 101 cm row spacing that yields will see an increase (15, 38). Reducing irrigation to a rate that induces slight drought without inhibiting yields would be difficult. Wheeler suggests in that crops with 80% of its water needs met yielded higher than crops that were fully watered (39). Perhaps an irrigation scheduling model could be developed that would provide sufficient soil moisture for wilt suppression without jeopardizing lint quality or yield.

The overall hypothesis of this study is to find multiple applied methods for management and assess risk potentials for certain environmental and cultural practices on Verticillium wilt on cotton. The objectives of this study were 1. Evaluate different cotton cultivars, available for the southeast region of cotton production, for resistance or susceptibility or tolerance to *Verticillium sp.*; 2. Assess disease severity with and without irrigation in different soil types commonly found throughout the state of Alabama; and 3. Assess different commercial chemical fungicides for efficacy to *V. dahliae*. The overall goal of this project is to provide protection from *V. dahliae* to cotton in producer's fields that have devastating losses, yet most of these fields are high input and producing fields and have potential for great yield returns.

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Chapter II.

Abstract

Verticillium wilt of cotton is incited by *Verticillium dahliae*, which colonizes the vascular cylinder of the plant resulting in defoliation, stunting, and yield loss. The long-term goal of this project was to look closely at Verticillium wilt in Alabama and find multiple methods for management of this disease to enhance cotton yields. Specifically our objectives were: 1) assess the state cotton fields with wilt symptoms to determine Verticillium presence; 2) evaluated cotton cultivars for resistance or tolerance to Verticillium wilt in the field; 3) determine if different soils types are conducive to Verticillium wilt disease severity; and 4) assess potential chemical fungicides for efficacy to *Verticillium* spp. *in vitro*. A survey of the state disclosed in the northern region, two species of Verticillium were isolated, *V. albo-atrum* and *V. dahliae* while in the central and southern part of the state only *V. albo-atrum* was found. In the 2013 field trial, two cultivars had the lowest disease severity, Bayer Experimental (BX) 1347 GLB2 and the resistant standard Fiber Max (FM) 1944GLB2. The resistant check, FM 1944 GLB2, had the fewest number of plants with discoloration in the vascular cylinder. In 2014, Stoneville (ST) 4747GLB2 and the resistant check FM 1944GLB2 had the lowest disease severity. Ranking the cultivars by yield indicates ST 4747GLB2 and Deltapine Experimental (DPLX) 14R1455B2R2 produced the greatest yield under these disease conditions. The microplot test indicated a

significant interaction between soil types and irrigation. Houston Clay irrigated had significant higher disease incidence compared to the other soil types with and without irrigation. A general screening of fungicides found the greatest control for *V. dahliae* with tetraconazole (Domark) and azoxystrobin + propiconazole (Quilt X).

Introduction

This project focuses on the serious problem of Verticillium wilt on cotton (*Gossypium hirsutum) L.*. This disease was first recorded in Virginia in 1918 by Carpenter (4) who reported its presence on Upland cotton and is distributed world-wide in major cotton producing countries such as Australia, Brazil and China (14). In the U.S., Verticillium wilt is present in most states across the cotton belt. Of the approximately ten million acres of cotton grown in the U.S, 97% is planted to Upland cotton (13). Cotton production is greatly impacted by the fungal plant pathogens. Losses from Verticillium wilt for the U.S., according to disease loss estimates, between the years of 1990-2013 are approximately 480 million bales (5). Verticillium wilt occurs most often occurs in the northern regions of Alabama and causes a decline in plant health and yield. It is caused by the soil born fungus *Verticillium dahliae Kleb.,* which first colonizes the root and then moves upward in the vascular system of the plant (8). This colonization of the vascular system prevents water movement creating wilted appearance of the plant. Symptoms in Alabama are not observed until later in the season at boll set when diseased plants prematurely begins to defoliate. Defoliation leads to yield decrease from the lack of photosynthetic activity. *Verticillium dahliae* causes stunting, lack of lateral growth, and decreases in yield, fiber quality, and seed quality. (3, 22)

Verticillium is the anamorphic form of an ascomycete imperfect fungus and reproduces asexually through the production of conidia on whorled conidiophores (7). Two *Verticillium* species are found in Alabama, *V. ablo-atrum* Reinke and Berthold and *V. dahliae.* Morphologically the difference between these two species is microsclerotia production (6). Microsclerotia are the dark torulose globose cells designed for over-wintering and enhance pathogen survival are formed by *V. dahliae*. Often these structures are formed from hyphal budding and multilateral septation. The *V. albo-atrum* does not produce microsclerotia but instead forms dark thickened resting mycelium, which are dark swollen turulose hyphal cells (8). Microsclerotia are known to germinate, when exposed to root exudates, and begin another infection cycle when environmental conditions are ideal (6). *Verticillium* sp. generally prefers air temperatures ranging from 70-85 ° F (21- 29 ° C) and moist soils (8).

There are several strategies that can be implemented to help manage the severity of Verticillium wilt caused by *Verticillium dahliae*. Crop rotation to grasses, legume, and crucifers is useful to prevent inoculum levels from building in the soil (8). Wheeler in 2012 found a ten percent decrease in soil microsclerotia levels when cotton was rotated to a crop that is not a host to *V. dahliae* (20). She also indicated rotation systems are more effective when utilized before Verticillium wilt becomes a severe problem, then the rotated areas had significantly less wilt than areas in continuous cotton, and also better yields than rotations started after economically damaging wilt was confirmed (19). Rotation to any crop other than cotton will reduce inoculum levels and disease severity (1). However, areas in cotton monoculture, which have severer Verticillium wilt infections and are planted in locations with high microsclerotia levels did not respond to rotations with a non-host crop (20). The reduction of Verticillium microsclerotia densities in the soil requires longer rotation cycles to be effective (2). Wheeler has shown that rotation to a non-host such as sorghum does reduce microsclerotia populations but reductions can be variable.

Cultivar selection is the most important choice when planting into a Verticillium infested field (Drew Schrimsher, AGRI AFC, Personal communication). Unfortunately, Upland cotton varieties are not highly resistant to Verticillium wilt. Irrigation can be an integral part of managing Verticillium wilt. Cotton grown in soil that has moderate to high densities of microsclerotia will not express wilt symptoms without adequate soil moisture (19). Unfortunately producers with irrigated land have the greatest potential yield return and wilt infection. Chemical controls are often the last option. Chemical treatments such as metam sodium are popular for Verticillium wilt control in potatoes. The average costs for metam sodium in potato fields in the Northwest United States $157/acre, a cost not reasonable for cotton production (19). The most reliable chemical control for cotton cropping systems from the 1950's to the late 1990's has been soil fumigation with methyl bromide; however, this product have been removed from the market due to EPA concerns about the earth's stratospheric ozone layer (20). The most effective method to deal with Verticillium wilt is an integrated pest management approach since all singular methods tend to have negative effects. Holistic management combinations including resistant cultivars, row spacing, irrigation timings, and crop rotation have all shown great results in terms of disease management (21). Planting cotton in narrower rows has shown to increase yields in Verticillium infested fields in California. This suggests that if growers change from the traditional 91 to 101.6 cm row spacing to 101 cm row spacing that yields will see an increase (8, 18). Reducing irrigation to a rate that induces slight drought without inhibiting yield loss is a difficult practice to do. Wheeler suggests in that cotton in an arid environment with 80% of its water needs met yielded higher than crops that were fully watered (20).

The overall hypothesis of this study is to find multiple applied methods for management and assess risk potentials for certain environmental and cultural practices on Verticillium wilt on cotton. Specifically our objectives were: 1) Identify and confirm the presence of *Verticillium sp.* in cotton fields of Alabama; 2) evaluated cotton cultivars for resistance or tolerance to Verticillium wilt in the field; 3) determine if different soils types are conducive to Verticillium wilt disease severity; and 4) assess potential chemical fungicides for efficacy to *Verticillium* spp. *in vitro*.

The overall goal of this project is to provide protection from *V. dahliae* to cotton in producer's fields that have devastating losses, yet most of these fields are high input and producing fields and have potential for great yield returns.

Materials and Methods

Identify and confirm the presence of Verticillium sp.

In 2013 a survey of Verticillium wilt incidence in cotton and isolation of the causal agents was conducted throughout the state. Petioles were collected from symptomatic cotton plants. Petioles were diced into 1 mm pieces and surface sterilized for ten seconds in 90% ETOH followed by a 1 minute wash in 6 % NaOCl solution. The surface sterilized cross sections were then aseptically placed water agar media (VWR), ten diced petioles per petri dish. Cotton petiole cultures were kept at room temperature (22.2°C – 24.4°C) for 5 to 14 days when areal whorled conidiophores with conidia appeared allowing for identification Verticillium. Isolates were identified to species by transferring vacillated conidiophores on to Verticillium minimal medium (15) and allowed to grow for 14 days. *Verticillium dahliae* was first identified by observing isolate's development and characteristics. Isolates which began growth as a white or cream color and then later developed into a darker color due to microsclerotia formation. A Nikon Ellipse T*i* was used at 15x by 10x to make measurements of conidiophores and conidia utilizing the NIS-Element BR 3.10 program (Melville, NY). Compendium of Soil Fungi (6) was used to reference measurements taken. PCR protocols using ITS primers were used to confirm the presence of *V. dahliae.*

Field Trial

In 2013 and 2014, cotton cultivars and lines were planted in grower's fields naturally infested with *Verticillium dahliae* to determine cultivar disease response to Verticillium wilt under field conditions. In the 2013 field trial, thirteen varieties were planted in a naturally infested field in Colbert county Alabama (34°49'16.8"N 87°59'37.1"W) with a soil type of Decatur silt loam (Fine, kaolinitic, thermic Rhodic Paleudults). Seed of adapted varieties and experimental lines expected to be released in the next season were provided by AGRI AFC (Decatur, AL). Cotton cultivars and lines were plated in a strip plot design with four replications with plots being 6 rows by 152 meters evenly spaced throughout the field. The field was irrigated, when needed, with a center pivot irrigation system. In 2014, the field trial was located in Madison county (34°51'10.5"N 86°31'28.9"W) with a Decatur silt loam and Dewey silt loam (Fine, kaolinitic, thermic Typic Paleudults) soil. Eighteen cultivars and lines were again planted in a strip plot design with four replications with plots being 6 rows by 152 meters. Plots were drip irrigated. In both years, parameters measured included disease incidence, disease severity, and yield were evaluated from 3 meter sections of the third row in each plot. Verticillium wilt disease severity ratings in the field and microplot trials were taken near cotton plant maturity. Foliar symptoms of

Verticillium wilt were evaluated on a scale from 1 to 5 with 1= no foliar wilting, 3= interveinal chlorosis and necrosis of the leaves with 40-60% of the plant defoliated, and 5= virtually defoliated plants having about 80-100% of the plant defoliated (Figure 1). Plants were individually rated and averaged for a total plot disease severity rating. Vascular discoloration was used to indicate disease incidence and was determined by cutting the plant stem longitudinally exposing the vascular cylinder. The number of plants with a discolored vascular cylinder indicated the percent incidence. Plant fresh weights and plant heights were also recorded. Yields were also taken at the end of the season. *Verticillium* spp. were re-isolated from all cultivars in both locations.

Verticillium sp. inoculum preparation

Verticillium cultures were isolated from symptomatic cotton plants fields with a history of Verticillium wilt in north Alabama. Petioles were taken and aseptically placed on 15% water agar (VWR, Atlanta, GA) media (20 ml per 9cm petri dish). Petioles were incubated at room temperature (22.2°C – 24.4°C) for two to three days before being assessed for growth. Growth was checked and verticillate conidiophores were transferred onto Verticillium minimal media (15) with a 5 x 5 cm piece of cellophane covering the media. Cultures were grown at 22.2–24.4°C for two weeks to allow for microsclerotia formation. Cellophane was removed from petri dishes and blended on low speed with sterile water for ten to fifteen seconds with a Waring Commercial blender (Madison, CT). The slurry was then poured into a 75 µm sieve nested in a gallon plastic bucket. The supernatant collected in the bucket is then poured over a 38 µm sieve to allow conidia to pass through. Mircosclerotia collected on the sieve were quantified using a hemocytometer. The *V. dahliae* inoculum was combined and added to field soils used in greenhouse and micro-plot trials to establish populations. A 250 cc of the microsclerotia mixture were incorporated into 24 liters of soil.

Microplot Trial

The microplot trial was conducted to determine what level of risk producers in the state had for invasive Verticillium wilt infections. The trial examined Verticillium wilt in six different soil types commonly found in cotton production throughout the state of Alabama. The second factor in the trial was the addition of daily irrigation or natural rainfall. The primary factor in the experiment were the six soil types used, soils were Houston clay (Very-fine, smectitic, thermic Oxyaquic Hapluderts), Decatur silt loam (Fine, kaolinitic, thermic Rhodic Paleudults), Lloyd loam (Fine, kaolinitic, thermic Rhodic Kanhapludults), Dothan sandy loam (Fine-loamy, kaolinitic, thermic Plinthic Kandiudults), Hartsell fine sandy loam (Fine-loamy, siliceous, subactive, thermic Typic Hapludults), and Ruston very fine sandy loam (Fine-loamy, siliceous, semiactive, thermic Typic Paleudults) (Table 1). Each microplot consisted of a 25 l outdoor plastic tree pot to simulate field conditions. Pots were arranged in a pot in pot design with a brick in between to limit root growth by air pruning. The microplot test was arranged in a split plot factorial design with five replications, totaling in 60 experimental units (Figure 10). Pots were planted with a susceptible cultivar, Croplan 3738 B2RF (B2 = Bollgard II and F = Roundup Ready Flex (Bayer CropScience, Lubbock, TX), at a rate of 5 seeds per row foot in each microplot. This design allowed the interaction of soil type and irrigation effects on Verticillium wilt incidence and severity and the subsequent effect on cotton yield. The soil types were tested under rain fed conditions (natural rainfall events) or irrigated by drip tape in addition to any rain fall it received (Irrigation-mart, Ruston LA). Irrigated microplots were watered twice daily with approximately 1 l of water. Pots were inoculated as previously described.

Fungicide Screening

Fungicide active ingredients were evaluated for efficacy to reduce *in vitro* growth for *V. dahliae* and *V. albo-atrum*. Thirteen commercially available fungicides were tested at concentrations of 100% labeled rates. *Verticillium dahliae* and *V. albo-atrum* were place onto fungicide amended potato dextrose agar (PDA) (VWR, Atlanta GA.). Fungicides were added to molten PDA after the media was allowed to cool in a water bath to 53°C. Twenty ml of amended media were poured into each 9 cm petri dishes. Non-amended plates were poured to evaluate the response of *V. dahliae* and *V. albo-atrum* to an untreated check. Three mm diameter core of actively growing fungi were aseptically inverted in the center of the plate, which were incubated for two weeks at 22-24 °C. Fungal growth was determined by right angle measurements to define growth as compared to the untreated control. The three best preforming fungicides which reduced fungal growth were selected for further testing at concentrations of 0.01, 0.1, 1.0, and 10 mg a.i. per liter. Fungal growth was compared to the unamend culture and sensitivity curves were formed in Microsoft Excel.

Statistical Analysis

Data collected in all trials were analyzed SAS 9.3 (SAS Institute, Inc.) using the PROC GLIMMIX procedure. PROC UNIVARIATE generated the graphs that were used to evaluated normality assumption of the residuals. Verticillium severity, incidence, and yield numbers required a lognormal distribution transformation to satisfy the normality assumption. Tukey-Kramer method was employed to compare means for field, microplot, and fungicide trials at a ($P > 0.10$). The LSMEANS estimates for the lognormal distribution function were transformed back to the original value using PROC MEANS. The original mean values are presented in charts with

P values to determine statistical differences. PROC CORR determined correlations between disease severity and incidence in 2013 and disease incidence and yield in the 2014 field trials. Correlations were evaluated at a 95% confidence interval and r values were considered significant at $\alpha = 0.05$.

Results

Identify and confirm the presence of Verticillium sp.

A assay results disclosed *Verticillium* spp. are present in soils statewide. Historically Verticillium wilt has been an economically important problem in the northern region of the state. Here, *V. albo-atrum* and *V. dahliae* were isolated. In the central and southern regions only *V. albo-atrum* was present. *Verticillium dahliae* and *V. albo-atrum* were characterized morphologically. *V. dahliae* isolates initially grow as a white mycelium culture and developed black microsclerotia over time. Phialides for the *V. dahliae* species ranged between 16-35 µm in length x 1.0-2.5 µm wide and conidia extended between 2.5-6 x 1.4-3.2 µm. *Verticillium albo-atrum* produced darkened resting mycelium but not microsclerotia. Phialides ranged between 20-30 x 1.4-3.2 µm in length and width while conidia were 3.5-10.5 x 2-5 µm. PCR result confirmed the presence of *Verticillium dahliae* with sequences confirmed in BLAST.

Field Trials

Verticillium wilt disease severity ratings were variable between cotton cultivars and experimental lines. In the 2013 field trial, differences in cultivars were observed in Verticillium wilt disease severity and incidence. Two cultivars had the lowest disease severity rating of the thirteen that were tested, Bayer Experimental (BX) 1347GLB2 and the negative control Fiber Max (FM) 1944GLB2 (Table 2). FM 1944GLB2 is a moderately resistant cultivar available to growers and is commonly grown in the state. Both cultivars had average visual rating of less than 2 indicating chlorosis and necrosis of the leaves with approximately 20-40% defoliation. Deltapine Experimental (DPLX) 12R224B2R2 and Phytogen (PHY) 375WRF had the highest wilt ratings. Both cultivars had average disease severity ratings above 4, implicating the majority of plants were 60-80% defoliated (Figure 2). The incidence of Verticillium wilt vascular staining differed ($P \geq 0.1$) among cultivars and experimental lines. The negative control, FM 1944 GLB2, did have the fewest number of plants with discoloration in the vascular cylinder with less than 35% symptomatic plants (Table 2). The eleven other cultivars had percentages of vascular discoloration ranging from 45 to 78%. DPLX 12R242 B2R2 appeared to be the most susceptible varieties that year (Figure 3). Pearson's correlations between Verticillium vascular staining and foliar wilting indicated a moderate correlation ($R^2 = 0.5482$; $P \leq 0.05$) between visual symptoms and the signs of the disease in the vascular system (Figure 4). Thus plants may be colonized with *V. dahliae* but only show wilt symptoms half the time. *Verticillium* species were isolated on water ager from petioles of all varieties and no significant differences between the infection rates of *Verticillium spp*. isolated from the thirteen cultivars. *Verticillium spp*. was re-isolated from all petiole tissues cultured from symptomatic plants.

In 2014, Stoneville (ST) 4747GLB2 responded similarly to resistant check FM 1944GLB2 with the lowest Verticillium wilt disease severity ratings of the eighteen cultivars tested (Table 3). Both cultivars displayed slight foliar necrosis and chlorosis of the leaves. Cultivars with the highest ratings were DPLX 14R1456B2R2, DPLX 12R224B2R2 and PHY 333WRF displayed interveinal chlorosis, wilting, and defoliation (Figure 5). Disease incidence varied between cultivars. Five cultivars, BX 1534 B2RF, ST 5032GLT, PHY 339WRF, ST 4747GLB2, and ST 4946GLB2, had less vascular discoloration ($P \geq 0.1$) than the FM 1944GLB2 standard (Figure 6). Twelve other cultivars had higher percentages vascular discoloration ranging from 67-91% than FM 1944GLB2. Yields indicated significant differences between cultivars when challenged with Verticillium wilt. Ranking the cultivars by yield indicates ST 4747GLB2 and DPLX 14R1455B2R2 produced the greatest yield under high disease pressure yielding 19 % more when compared to the negative control FM 1944B2RF. Yields for DP 1137B2RF, ST 4946GLB2, and DPLX 13R352B2R2 averaged 5 % higher than the FM 1944B2RF standard (Figure 7). Comparing the data between disease incidence and severity indicated a significant positive correlation (R^2=0.62213; $P \leq 0.0001$) between visual symptoms and the signs of the disease in the vascular system (Figure 8). A correlation between incidence and yield was significant (R^2= -0.49814; $P \leq 0.0001$) indicating a higher disease incidence contributed to a 50% yield reduction (Figure 9).

Microplot Trial

The microplot test indicated a significant interaction between the soil types and irrigation. in regards to disease incidence and severity (Figure 10). Specifically the soil type with the most severe Verticillium wilt was the irrigated Houston clay soil. This soil supported 38% more disease when irrigated than with the dryer natural rainfall. The irrigated Houston clay plots on average had significant higher disease severity ratings with an average of 3.4 ($P \geq 0.1$). The irrigated Decatur silt loam, non-irrigated Decatur silt loam, and irrigated Dothan sandy loam soils all were statistically similar and ranged in disease incidence between 23-18 percent. The Decatur silt loam soil supported similar levels of Verticillium wilt incidence regardless of irrigation. No significant differences in disease incidence or severity were found between the loam and sandy loam soil types with or without additional irrigation. (Table 4). Decatur silt loam with or without irrigation and the Houston clay irrigated soil had significantly more disease incidence than all other soil types. These soils had the highest clay and silt content and held water thus displayed less moisture stress. The soil type that proved to be the least conducive to Verticillium wilt was the Hartsells fine sandy loam which had no disease present regardless of irrigation (Table 4). There were no significant differences noted between yield measurements.

Fungicide Screening

Commercial fungicide compounds were tested for efficacy on *V. albo-atrum* and *V. dahliae in-vitro.* Thirteen fungicides affected fungal growth. Greatest control or reduction of *V. dahliae* growth was observed with tetraconazole (Domark®) (Gowan, Morrisville NC) and azoxystrobin + propiconazole (Quilt Xcel ®, Syngenta Crop Protection, Greensboro, NC). These fungicides did not allow for any radial growth beyond the *V. dahliae* inoculation core and an 87% reduction in growth over the non-amended *V. dahliae* plates. Mefenoxam gave suppression of *V. dahliae* growth (Ridomil®, Syngenta Crop Protection, Greensboro, NC) and similar colony diameters were noted for this fungicide and untreated check (Table 5). When looking at efficacy from a larger group such as chemical classes, Methoxy-acrylates + Triazoles had the significantly lowest amount of growth, however inhibition of growth within each chemical class was variable. This combination of chemical classes inhibited growth 25% compared to the Methoxy-Carbamates. Methoxy-acrylates + Triazoles were followed by Methoxy-Carbamates which gave the next best efficacy reducing fungal growth by 87 % over the untreated control. Levels of control varied within these chemical classes. Acylalanines allowed for the most fungal growth out of the chemical classes tested (Table 6). *V. albo atrum* had slight differences of effective treatments than the *V. dahliae* (Table 5). The best control was by pyraclostrobin (Headline SC ®, BASF Crop Protection USA, Research Triangle Park, NC) and azoxystrobin + propiconazole (Quilt Xcel®). Both of these fungicides were statically similar ($P \geq 0.1$) and contained the radial growth to the inoculation core. Mefenoxam, metalaxyl (Allegiance®, Bayer CropScience, Research Triangle Park, NC), and the untreated *V. albo-atrum* had statically similar growth ($P \geq 0.1$) which ranged from 25.5-28.4 mm (Table 6). Results from comparing chemical classes of these fungicides were similar to that of *V. dahliae*. Methoxy-acrylates + Triazoles and Methoxy-Carbamates had statically similar efficacy levels and allowed for the lowest level of growth. Methoxy-acrylates + Triazoles and Methoxy- Carbamates did not have radial growth larger than the inoculation core. Similarly to V. dahliae inhibition of growth within chemical classes were variable. Acylalanines allowed for the highest amount of growth out of the chemical classes tested. Acylalanines even allowed for a growth 8% higher than the untreated *V. albo-atrum* (Table 6). A lack of control by this fungicide class was expected due to its activity against Oomycetes and no other classes of fungi.

Sensitive curves were created for the three best preforming fungicides for *V. dahliae*. Measurements indicated no differences between the un-amended control fungal plates. Plates with 0.01 mg active ingredient (a.i.) / l significantly reduced *V. dahliae* growth between all three fungicides ($P \geq 0.1$). Tetraconazole exhibited the highest sensitivity to the pathogen, pyraclostrobin and azoxystrobin + propiconazole allowed for approximately 80% and 100% more growth than tetraconazole. Plates with 0.10 mg a.i./l also found significant growth difference between fungicides ($P \geq 0.1$). Tetraconazole and azoxystrobin + propiconazole on average reduced *V. dahliae* growth by 38% more than pyraclostrobin (Figure 11). No significant differences were observed between fungicides in the 1 mg a.i. /l or 10 mg a.i. / l. Fungicides collectively inhibited growth on average 47.7 mm of growth (Figure 11).

Discussion and Conclusion

The overall findings of this research show that there are options for managing Verticillium wilt and ways to assess risk of infections. Results indicate cultivar selection has a significant effect on the yield produced in Verticillium wilt infected fields. While statistical differences were noted between cultivar's disease incidence percentages and severity ratings, the pathogen was still present in all cotton cultivars tested, indicating a truly resistant cultivar is not available for Alabama growers. Over twenty different cultivars were tested over a two year period and all had some level of infection from Verticillium wilt confirming previous findings Bell , 1992 (1). New cultivars are preforming better than the standard FM 1944 GLB2, the semi-resistant check commercially available to growers in Alabama. Currently there are no known commercial cultivars that are completely resistant to Verticillium wilt and FM 1944GLB2 will not be available by 2016. Previous variety trials performed by Wheeler, 2007(17), in the high plains of Texas suggest that there are more cultivars with tolerance than resistance to Verticillium wilt. The new cultivar released, ST 4747 GLB2 has comparable disease severity ratings and incidence percentages. It also produced over 800 kg/ha of seed cotton more than to FM 1944 GLB2, thus appearing to have a higher level of tolerance to Verticillium wilt. Our Alabama variety responses also indicated tolerance rather than resistance is present in our cotton cultivars.

The microplot trial indicates a producer's level of risk for Verticillium wilt infection can be assessed based on soil type and irrigation usage. Understanding environmental and cultural influences that enhance Verticillium wilt pathogenicity are important when calculating control measures. The addition of water enhanced the pathogenicity of this disease, in most cases. Irrigation is an expensive input that could potentially enhance disease causing greater yield losses. Irrigation increased the incidence of Verticillium wilt compared to the non-irrigated plots in four out of the six soil types, confirming previous studies Kadolph and Langfold 1998 (10) and Karaca et al. 1971 (11). Heavier soils, i.e. soils with a higher silt and clay content, were also found to have greater disease infection compared to lighter sandier soils, contradicting Rudolphs (16), who indicated that cotton in sandier soil had a higher frequency of irrigation therefore had a higher infection rate than cotton in heavier soils. Here, the Houston clay and Decatur silt loam had significantly higher number of plants infected compared to the lighter sandier soils. These soil types are the heaviest in the state. This is consistent with where infection occurs in the state. Results show a significant relationship between irrigation and heavier soil types with higher silt and clay content. The Houston Clay irrigated treatment had significantly more disease than the other soil and watering combinations. Karaca in 1971 (11) theorized this relationship is probably due to the evaporative cooling effects that occur to soils after irrigation is applied.

The in-vitro fungicides tested indicate a promising potential for reduction of Verticillium fungal growth. Results from the broad spectrum in vitro screening of fungicide for both *V. dahliae* and *V. albo-atrum* showed significant differences between active ingredients. Little work has been done with this pathogen and it’s responses to fungicides (12). In vitro screenings of *V. dahliae* displayed mycelium, and microsclerotia sensitivity to tetraconazole and azoxystrobin + propiconazole. Both fungicides allowed for little growth and have active ingredients included in the chemical classes of demethylation inhibitor (DMI) suggesting that *V. dahliae* may have sensitive to this mode of action. DMIs affect the phospholipid bilayers which are needed in the formation of cell walls (12). *V. albo-atrum* revealed levels of efficacy from by Pyraclostrobin and azoxystrobin + propiconazole. The chemicals classes are both included in the Quinone Outside Inhibitor (QOI) which effect the respiration of the fungi cells (12). These finding suggest that *V. albo-atrum* may have sensitivity to some active ingredients included in this mode of actions. Unfortunately current labeling of effective fungicide that show efficacy on *V. dahliae* and *V. albo-atrum*, only pyraclostrobin is labeled on cotton (9). Sensitivity curves for *V. dahliae* were formed to measure the three best preforming fungicides in the broad spectrum test. Understanding sensitivity levels can allow producers to apply the minimum amount of chemical and still retain the highest efficacy possible. Fungicides are expensive inputs and should often be applied only when economical risk is noted for a potential crop. Tetraconazole exhibited the greatest amount of fungal sensitivity to a fungicide at the lowest amount of active ingredients tested.

Overall these studies demonstrations the importance's of an integrated management strategy for control of Verticillium wilt. If a producer has a history of wilt and is planting into a risky soil type with the input of irrigation, then cultivar selection and potential fungicide applications may be need to achieve desired yields.

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Appendix

Figure 1: Foliar symptoms of Verticillium wilt were evaluated on a ratings scale from 1 to 5 with 1= no foliar wilting, 2 = interveinal chlorosis and necrosis of the leaves, 3= interveinal chlorosis and necrosis of the leaves with 10-30% of the plant defoliated, 4= interveinal chlorosis and necrosis of the leaves with 40-60% of the plant defoliated, and 5= virtually defoliated plants having about 70-100% of the plant defoliated. Plants were individually rated and averaged for a total plot disease severity rating.

Table 1. Soil types used in the microplot trial with corresponding descriptions.	
Soil type with % sand, silt and clay	**Description**
Houston Clay 6.5, 48, 45	The Houston series consists of very deep, somewhat poorly drained, very slowly permeable soils that formed in clayey sediments overlying chalk or calcareous clays. They are on uplands and old stream terraces of the Alabama, Mississippi, and Arkansas Blackland Prairie and the Southern Coastal Plain major land resource areas. Near the type location, the average annual air temperature is about 63 degrees F. and the average annual precipitation is about 53 inches. Slopes are dominantly 0 to 5 percent but range to 17 percent.
Decatur Silt Loam 24, 49, 28	The Decatur series consists of very deep, well drained, moderately permeable soils that formed in residuum derived from limestone. These soils are on level to strongly sloping uplands in valleys. Slopes are dominantly 1 to 10 percent but range up to 25 percent. Near the type location the mean annual temperature is 62 degrees F., and the mean annual precipitation is more than 49 inches.
Lloyd Loam 52, 23, 25	The Lloyd series consists of very deep, well drained, moderately permeable soils on uplands in the Southern Piedmont. The soils formed in residuum derived from intermediate and mafic, igneous and high-grade metamorphic rocks. Slopes are commonly 2 to 10 percent but range to 50 percent. Near the type location, mean annual temperature is about 61 degrees F., and mean annual precipitation is about 45 inches.
Dothan Sandy Loam	The Dothan series consists of very deep, well drained, moderately slowly to slowly permeable soils on broad uplands of the

57, 28, 15	Southern Coastal Plain (MLRA 133A) and to a much lesser extent in the Eastern Gulf Coast Flatwoods (MLRA 152A) Major Land Resource Areas. They formed in thick beds of unconsolidated, medium to fine-textured marine sediments. Slopes range from 0 to 15 percent. Near the type location, the average annual precipitation is about 53 inches and the average annual air temperature is about 65 degrees F.
Hartsells Fine Sandy Loam 56, 33, 12	The Hartsells series consists of moderately deep, well drained, moderately permeable soils that formed in loamy residuum weathered from acid sandstone containing thin strata of shale or siltstone. These soils are on nearly level to moderately steep ridges and upper slopes of hills and mountains.
Ruston Very Fine Sandy Loam 59, 33, 8	The Ruston series consists of very deep, well drained, moderately permeable soils that formed in loamy marine or stream deposits. These soils are on uplands of the Western and Southern Coastal Plains. Slopes range from 0 to 8 percent.

Table 2. Cotton cultivar response to Verticillium wilt as measured by disease severity and disease incidence, in 2013.

Cotton cultivars		Disease severity**		Disease incidence %***	
1	BX 1347 GLB3	1.8*	d	57.0	ab
2	DP 1044 B2RF	2.3	cd	46.1	ab
3	DP 1133 B2RF	2.9	abcd	55.4	ab
4	DP 1137 B2RF	3.5	abc	67.9	ab
5	DP 1321 B2RF	3.0	abcd	51.7	ab
6	DPLX 12R224 B2R2	2.9	abcd	56.3	ab
7	DPLX 12R242 B2R2	4.3	a	78.5	a
8	FM 1944 GLB2	2.0	cd	31.3	b
9	PHY 339 WRF	2.3	cd	44.9	ab
10	PHY 375 WRF	4.0	ab	72.7	ab
11	PHY 499 WRF	3.3	abcd	48.1	ab
12	ST 4946 GLB2	3.3	abcd	55.4	ab
13	ST 6448 GLB4	2.6	bcd	52.9	ab
	Means	2.91		55.25	

* Means followed by the same letter do not differ significantly at the 0 .10 level of probability as indicated by Tukey Kramer Grouping.
** Disease severity ratings of foliar symptoms were evaluated on a scale from 1 to 5 with 1= no foliar wilting, 3= interveinal chlorosis and necrosis of the leaves with 40-60% of the plant defoliated, and 5= virtually defoliated plants having about 80-100% of the plant defoliated.
*** Disease incidence was determine by cutting each plant in a m section and counting those with vascular discoloration.

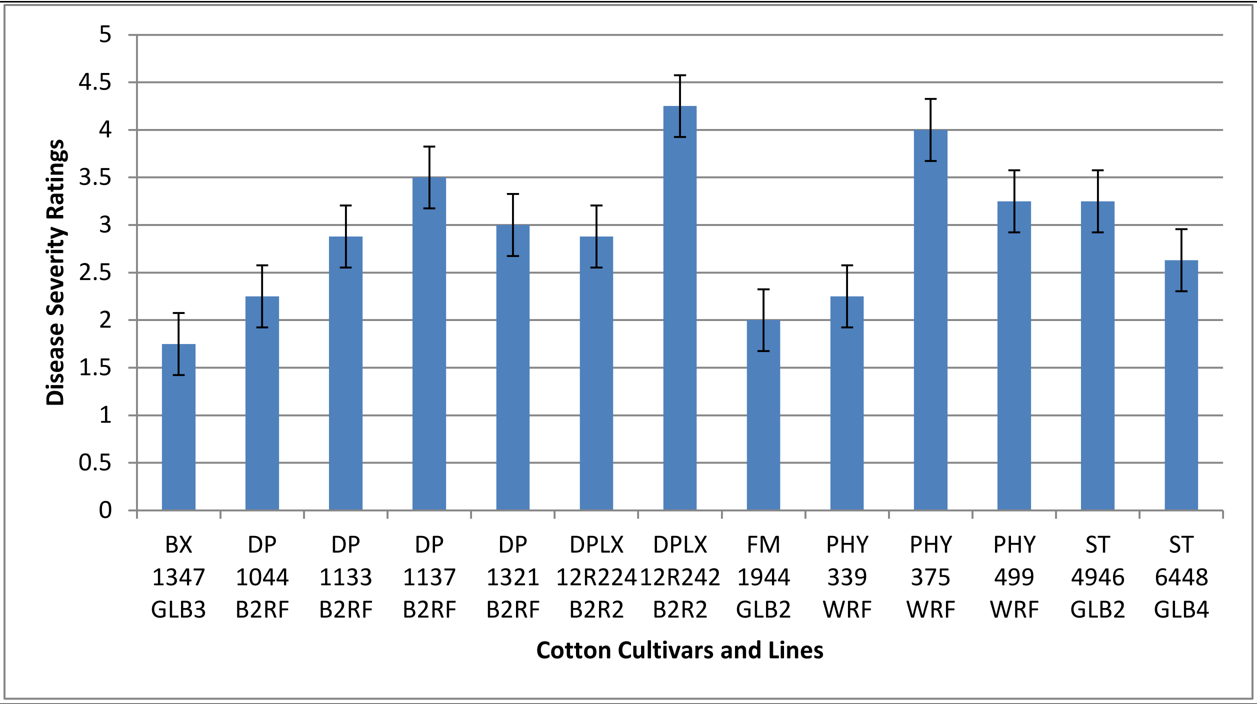


Figure 2: Verticillium wilt disease severity rating of cotton cultivars and lines from the 2013 field trial. Disease severity ratings of foliar symptoms were evaluated on a scale from 1 to 5 with 1= no foliar wilting, 3= interveinal chlorosis and necrosis of the leaves with 40-60% of the plant defoliated, and 5= virtually defoliated plants having about 80-100% of the plant defoliated.

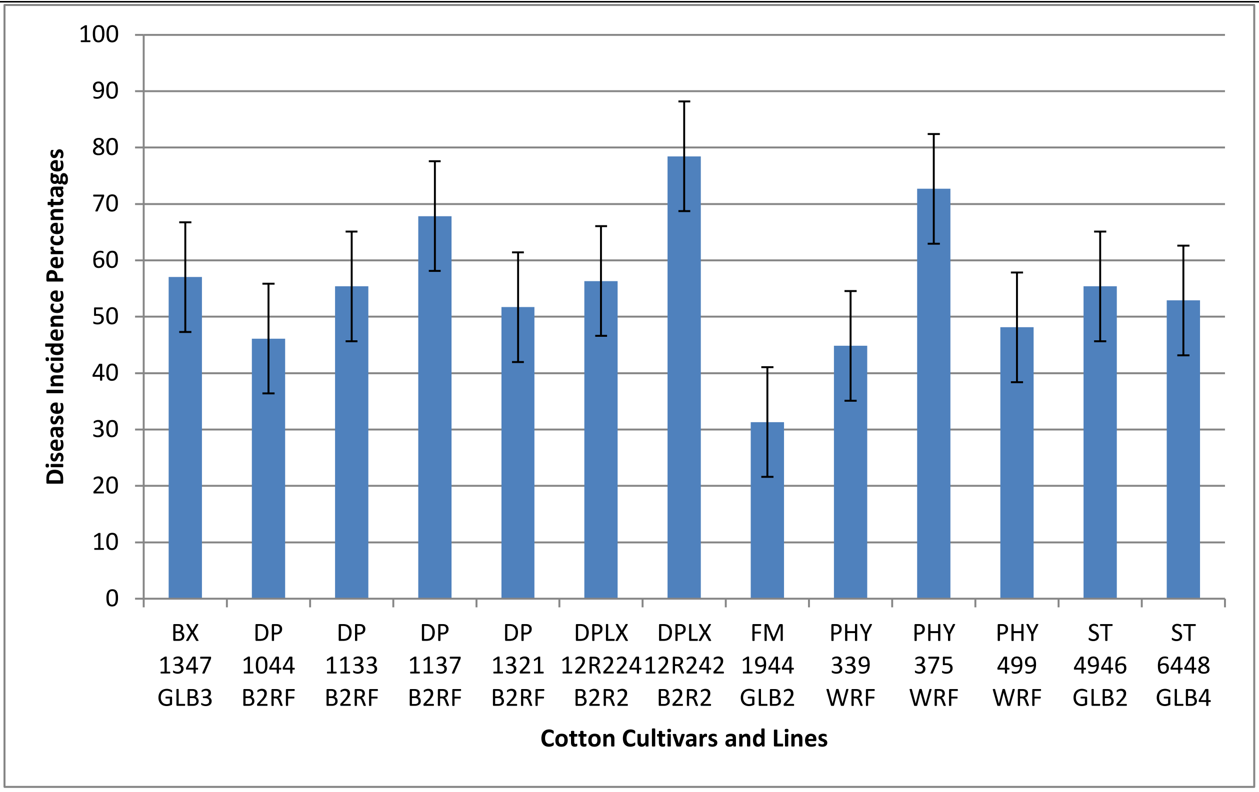


Figure 3: Verticillium wilt disease incidence percentages of cotton cultivars and lines from the 2013 field trial. Disease incidence was determined by cutting each plant in a meter section and counting those with vascular discoloration.

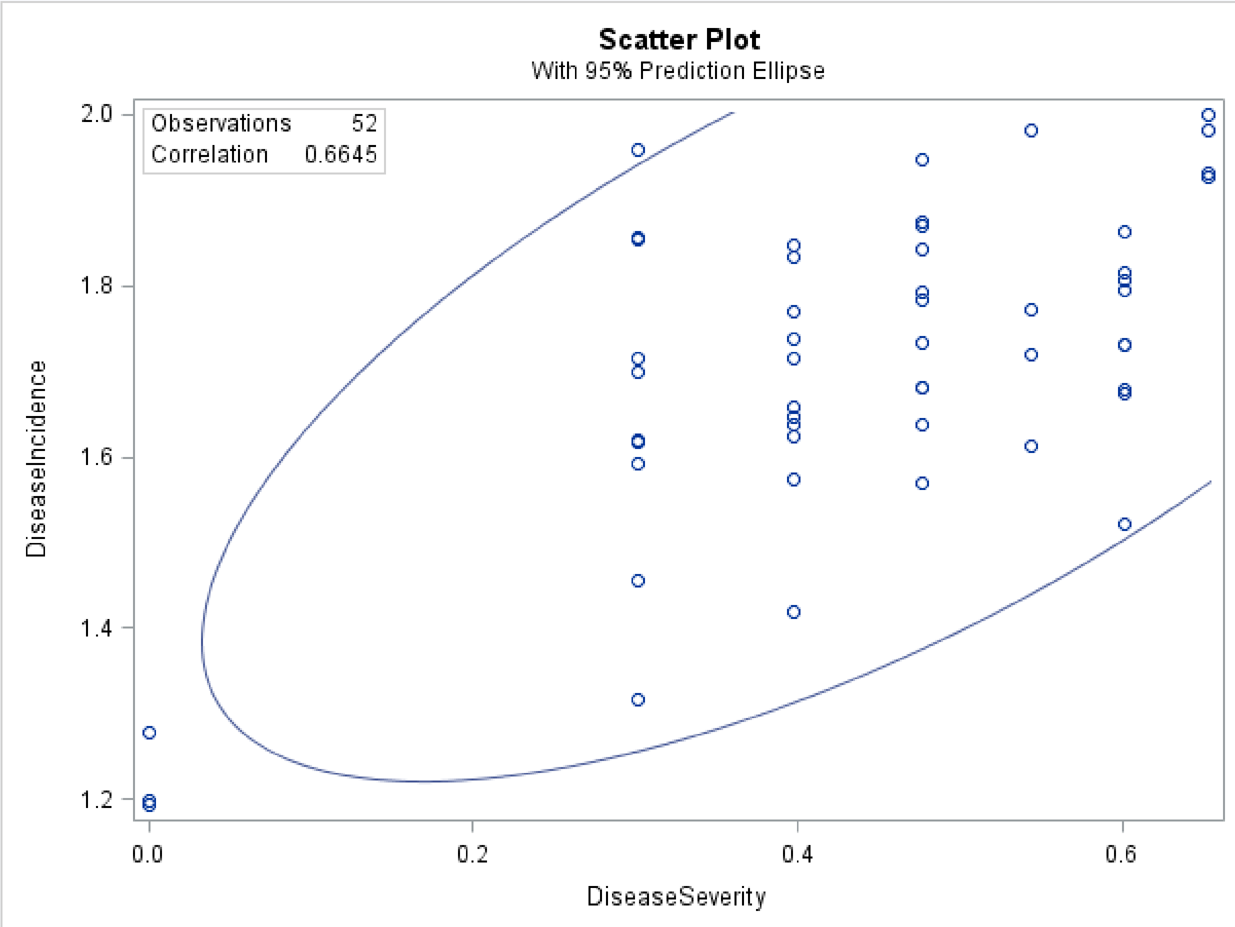


Figure 4: Verticillium disease incidence by disease severity correlation from the 2013 field trial. Correlation indicate a dependent relationship between disease severity and disease incidence ($P \leq 0.05$)

Table 3. Cotton cultivar response to Verticillium wilt as measured by disease severity, disease incidence, and yield in 2014

Cotton Cultivars		Disease Severity**		% Disease Incidence ***		Yield kg/ha	
1	CROPLAN 3787 B2RF	2.9	abc*	87.8	ab	2902.6	bcde
2	DP 1321 B2RF	2.7	abc	73.8	abc	3316.0	abcde
3	DP 1133 B2RF	2.87	abc	74.0	abc	3178.5	abcde
4	DP 1137 B2RF	3.2	abc	83.4	abc	4110.2	abcde
5	DPLX 12R224	4.1	a	89.6	a	2186.3	e
6	DPLX 13R310	3.1	abc	83.0	abc	2403.6	de
7	DPLX 13R352	2.5	abc	67.4	abcd	3899.9	abcd
8	DPLX 14R1455	2.3	abc	66.9	abc	4298.7	ab
9	DPLX 14R1456	4.2	a	90.6	a	2173.9	e
10	PHY 333 WRF	4.0	a	89.1	a	2998.3	abcde
11	PHY 339 WRF	2.7	abc	49.5	cd	3761.8	abcde
12	PHY 499 WRF	3.7	abc	72.5	abc	3501.7	abcde
13	FM 1944 GLB2	1.7	bc	66.1	abcd	3783.9	abcde
14	ST 4747 GLB2	1.6	c	51.4	cd	4630.1	a
15	ST 4946 GLB2	2.3	abc	45.7	d	3985.8	abcd
16	ST 5032 GLT	2.6	abc	52.2	bcd	3023.0	abcde
17	ST 6448 GLB2	2.8	abc	74.1	abc	3725.6	abcde
18	BX 1534	2.8	abc	58.8	abcd	2431.0	cde
	Means	2.9		70.94		3350.6	

* Means followed by the same letter do not differ significantly at the 0 .10 level of probability as indicated by Tukey Kramer Grouping.
** Disease severity ratings of foliar symptoms were evaluated on a scale from 1 to 5 with 1= no foliar wilting, 3= interveinal chlorosis and necrosis of the leaves with 40-60% of the plant defoliated, and 5= virtually defoliated plants having about 80-100% of the plant defoliated.
*** Disease incidence was determine by cutting each plant in a m section and counting those with vascular discoloration.

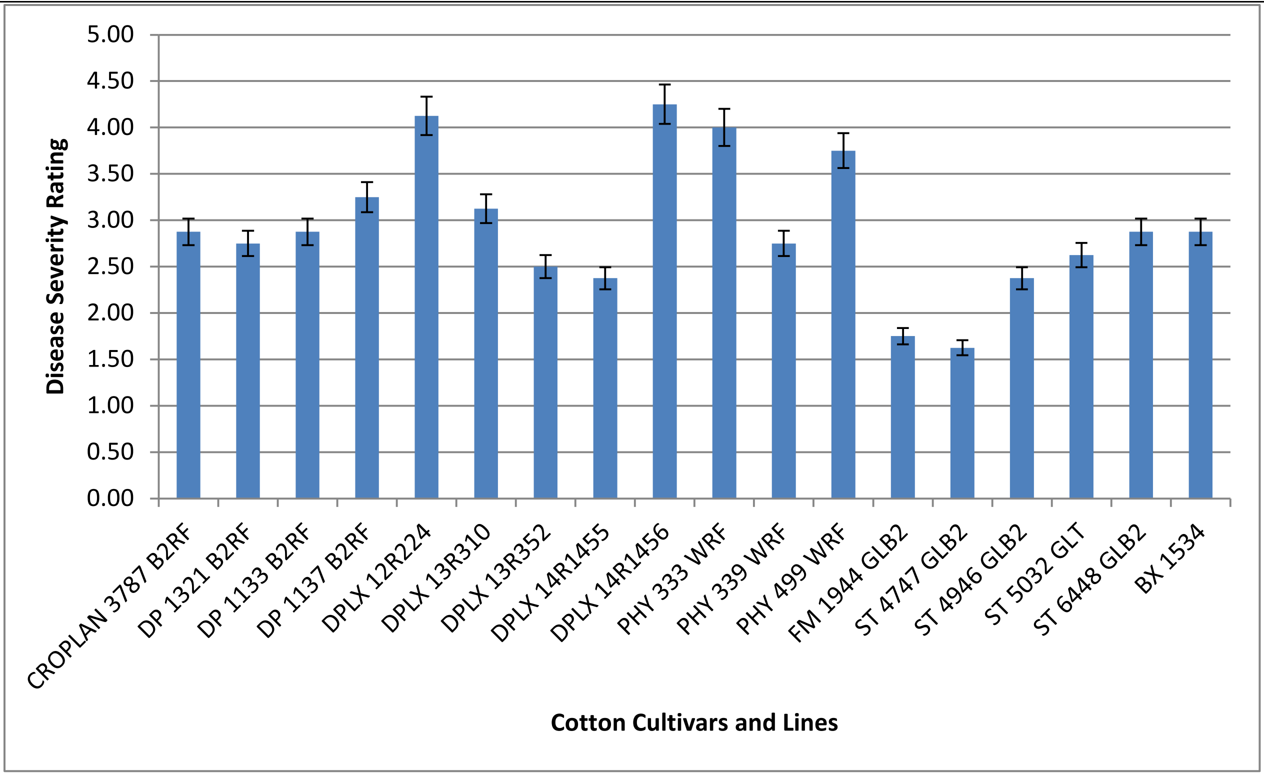


Figure 5: Verticillium wilt disease severity rating of cotton cultivars and lines from the 2014 field trial. Disease severity ratings of foliar symptoms were evaluated on a scale from 1 to 5 with 1= no foliar wilting, 3= interveinal chlorosis and necrosis of the leaves with 40-60% of the plant defoliated, and 5= virtually defoliated plants having about 80-100% of the plant defoliated.

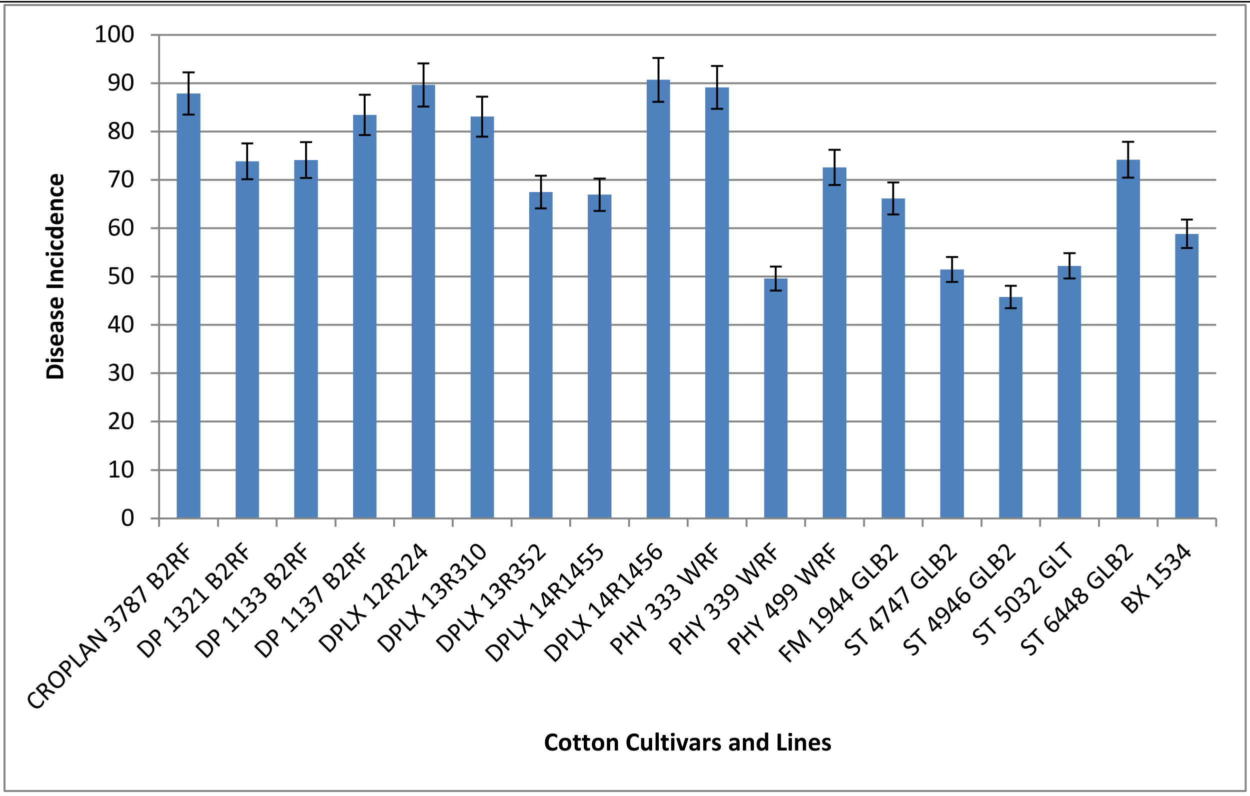


Figure 6: Verticillium wilt disease incidence percentages of cotton cultivars and lines from the 2014 field trial. Disease incidence was determined by cutting each plant in a meter section and counting those with vascular discoloration

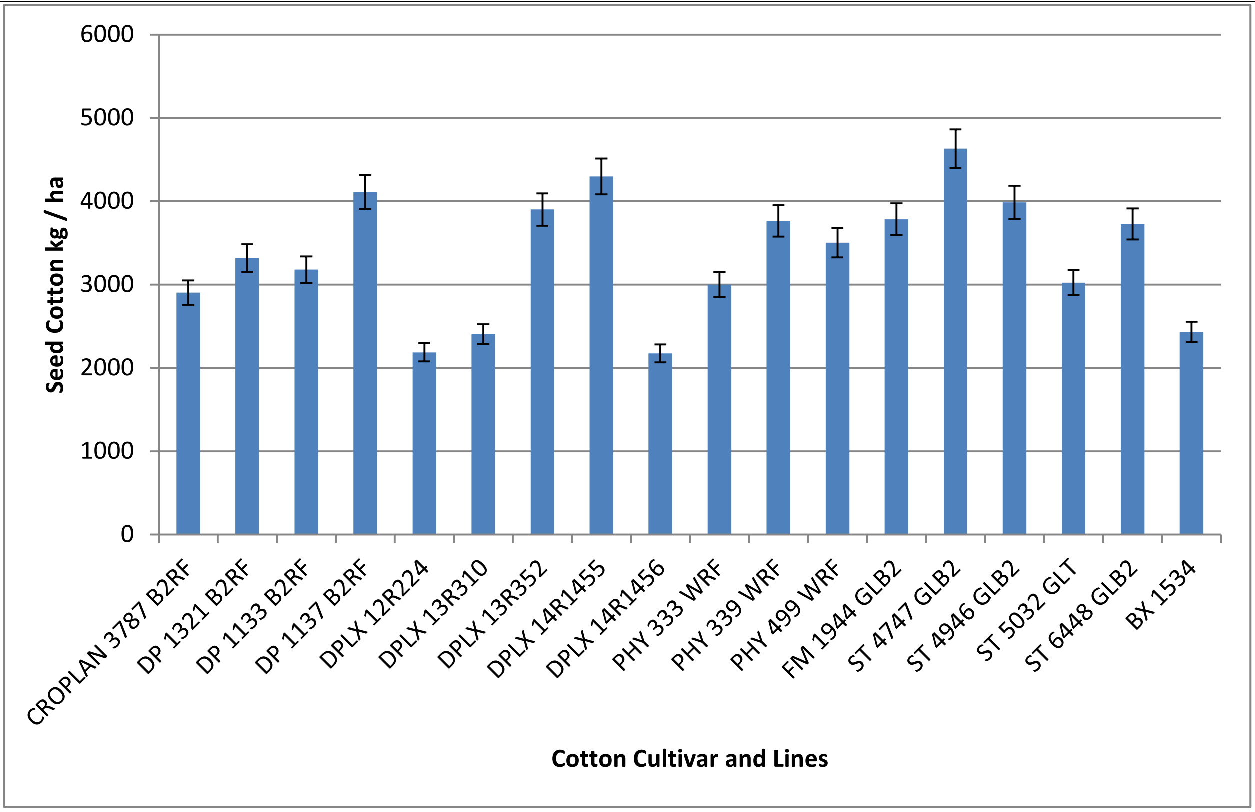


Figure 7: Cotton cultivar yields from the 2014 field trial. in kg per hectare. Yields were obtained by hand picking 3 meter sections in each row to determine kg per hectare

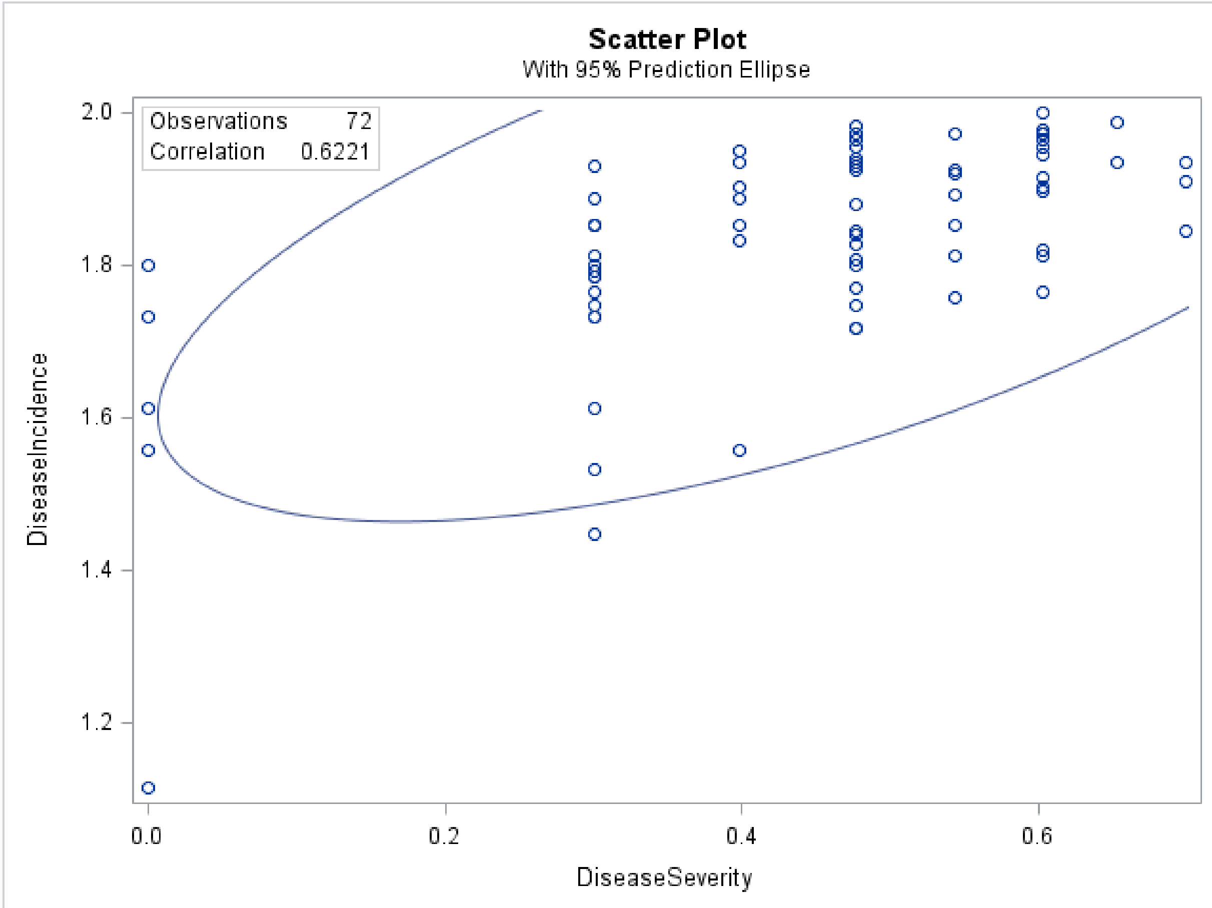


Figure 8: Disease incidence by yield correlation from the 2014 field trial. The correlation indicates a dependent relationship between disease severity and disease incidence at ($P \leq 0.05$).

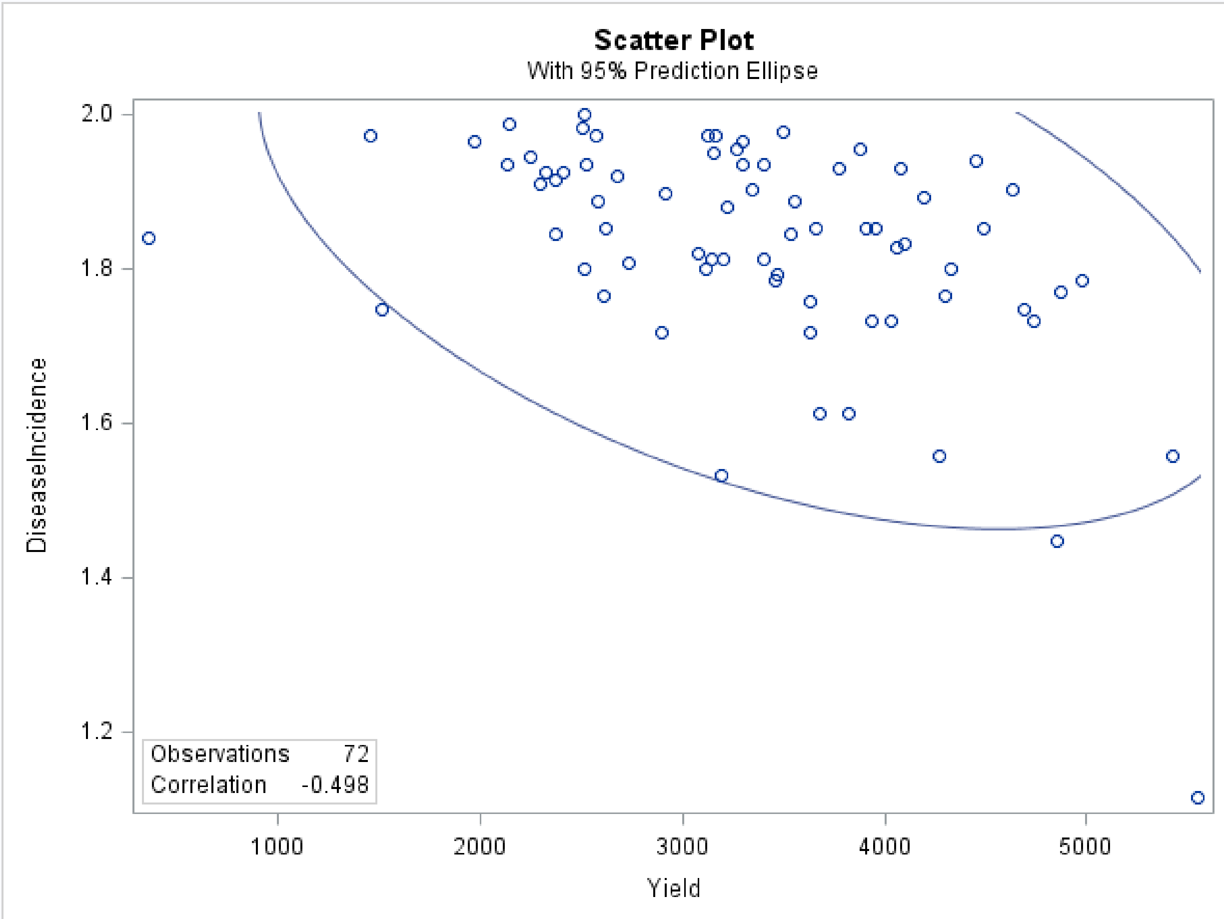


Figure 9: Disease incidence by yield correlation from the 2014 field trial Correlation shows a dependent relationship between disease severity and disease incidence (P≤ 0.05)

Table 4. Cotton microplot trial the Alabama soil types with or without irrigation and the disease incidence and severity ratings.

Soil type		Irrigation	% Disease incidence **		Disease severity ***	
1	Houston Clay	Irrigated	43	a*	3.4	a*
2	Decatur Silt Loam	Irrigated	23	ab	2.0	ab
3	Lloyd Loam	Irrigated	9	b	2.0	ab
4	Ruston Very Fine Sandy Loam	Irrigated	5	b	2.6	ab
5	Hartsells Fine Sandy Loam	Irrigated	0	b	2.0	ab
6	Dothan Sandy Loam	Irrigated	18	ab	3.2	a
7	Houston Clay	Non- Irrigated	5	b	2.0	ab
8	Decatur Silt Loam	Non- Irrigated	22	ab	1.2	b
9	Lloyd Loam	Non- Irrigated	7	b	1.4	ab
10	Ruston Very Fine Sandy Loam	Non- Irrigated	0	b	1.0	b
11	Hartsells Fine Sandy Loam	Non- Irrigated	0	b	1.4	ab
12	Dothan Sandy Loam	Non- Irrigated	7	b	1.8	ab

* Means followed by the same letter do not differ significantly at the .1 level of probability as indicated by Tukey Kramer Grouping.

** Disease incidence was determine by cutting each plant in a m section and counting those with vascular discoloration.

*** Disease severity ratings of foliar symptoms were evaluated on a scale from 1 to 5 with 1= no foliar wilting, 3= interveinal chlorosis and necrosis of the leaves with 40-60% of the plant defoliated, and 5= virtually defoliated plants having about 80-100% of the plant defoliated.

Figure 10. An aerial view of the microplot test. Plots with a red line beneath indicate natural rain feed plotsand the blue line indicates irrigated plots.

Table 5 *Verticillium dahliae* and *albo-atrum* fungal growth in response to fungicides.

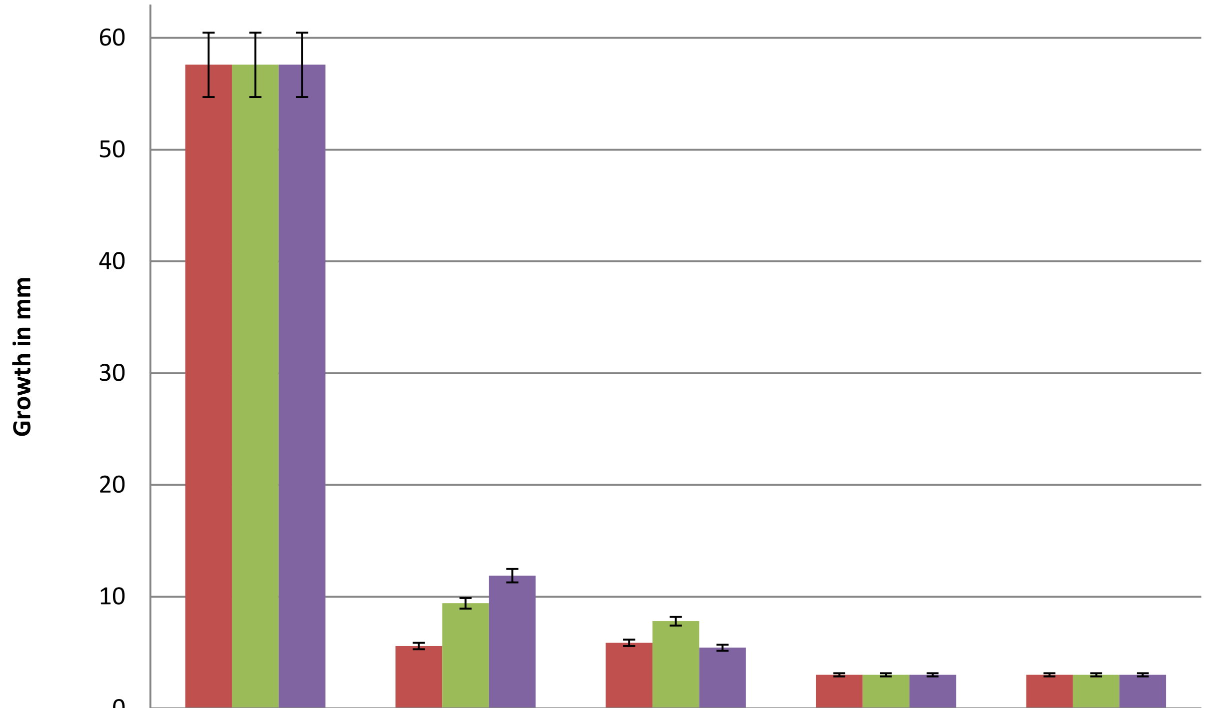
	Verticillium dahliae			*Verticillium albo-atrum*	
Fungicides, chemical name and trade name	Radial growth mm		Microsclerotia formation	Radial growth mm	
Mefenoxam (Ridomil)	24.8*	a**	Present	28.4*	a**
Fluxastrobin + Imidacloprid (Velum)	17.6	b	Present	16.2	cd
Iprodione (Rovral)	14.9	bc	Absent	13.1	de
Metalaxyl (Allegiance)	13.5	c	Present	27.5	a
Azoxystrobin (Quadris)	13.5	c	Present	15.4	cde
Fluxastrobin (Evito)	11.5	cd	Absent	17.1	c
Triadimenol (Baytan)	10.9	cd	Absent	20.6	b
Trifloxystrobin (Trilex)	10.5	cde	Absent	20.5	b
Ipconazole (Vortex)	8.2	de	Absent	6.5	gf
Prothioconazole (Proline)	8.1	de	Absent	7.5	f
Pyraclostrobin (Headline)	4.7	fe	Absent	4.0	g
Azoxystrobin + Propiconazole (Quilt X)	3.5	e	Absent	4.0	g
Tetraconazole (Domark)	3.5	e	Absent	12.5	e
(Check)	28.4	a	Present	25.5	a
Means	11.42			15.62	

*Radial growth was measured in mm 14 days after inoculation.
**Means followed by the same letter do not differ significantly at ($P \leq 0.001$) level as indicated by Tukey Kramer Grouping.

Table 6. *Verticillium dahliae* and *albo-atrum* fungal growth in response to fungicide classes

	Verticillium dahliae		*Verticillium albo-atrum*	
Chemical Classes	Radial growth mm		Radial growth mm	
Acylalanines	19.4*	b**	27.9*	a**
Triazoles	7.7	e	11.8	e
Methoxyacrylates	13.5	cd	15.4	cde
OximinoAcetates	10.5	de	20.5	bc
Methoxy-Carbamates	4.7	ef	4.0	f
Dihydro-Dioxazines	11.5	d	17.1	cd
Unknown1	14.9	cd	13.1	de
Unknown2	17.5	cb	16.2	cd
Methoxy-acrylates+Triazoles	3.5	f	4.0	f
Check	28.4	a	25.5	ab
Mean	13.15		15.55	

*Radial growth was measured in mm 14 days after inoculation.
**Means followed by the same letter do not differ significantly at the ($P \leq 0.001$) level of probability as indicated by Tukey Kramer Grouping.



	0	0.01	0.1	1	10
Domark	57.6	5.5791	5.8696	3	3
Headline	57.6	9.4048	7.8069	3	3
QuiltX	57.6	11.8772	5.4271	3	3

Figure 11. *Verticillium dahliae* fungal sensitivity curves from the three best performing fungicides which reduced fungal growth were selected for further testing at concentrations of 0, 0.01, 0.1, 1.0, and 10 milligrams of a.i. per liter.