Upland Cotton Cultivar Evaluation for Reaction to *Corynespora cassiicola*

by

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Abstract

Target spot of cotton (*Gossypium hirsutum* L.*)* is caused by the fungus *Corynespora cassiicola* (Berk. & Curt. C. T. Wei.) and has become a recent problem in cotton in the humid, southeastern states. Primarily occurring in the tropics and subtropics, the disease is characterized by leaf lesions, starting small then enlarging with a concentric ring appearance, thus the name target spot. High levels of defoliation can occur and yield loss estimates vary. The causal organism has been found to be extremely variable and can infect a wide range of crops, including soybean (*Glycine max* L. Merr.), sesame (*Sesamum indicum* L.), cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.) and cotton. With the recent appearance in cotton in local areas of the southeast, it is important that resistant lines are identified. Some limited data has been reported on cotton genotypic response to *C. cassiicola* based on field observations. In 2012, CC made a natural appearance in the Regional Breeders Testing Network (RBTN) at the Tallassee, Alabama location. Lines were rated and the results indicate a differential response among lines with regard to symptom development. A greenhouse protocol was implemented to evaluate genotypes under controlled conditions, and to compare the results to that of their field performance. While we were able to induce high levels of disease in the greenhouse, we were not able to significantly differentiate disease levels among genotypes. In field evaluations of the Alabama Cotton Variety test locations, we were able to differentiate among genotypes in only a very limited number of cases. In addition, the greenhouse ratings for disease severity did not correlate with our field ratings. Thus it appears that there is a minimal amount of genetic variation for resistance to target spot in upland cotton.

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List of Abbreviations

CC	*Corynespora cassiicola*
GCREC	Gulf Coast Research and Extension Center
cm	centimeter
ft	feet
g	gram
kg/ha	kilogram per hectare
L	liter
mL	milliliter
mm	millimeter
PARU	Prattville Agricultural Research Unit
PBU	Plant Breeding Unit
PDA	Potato Dextrose Agar
RBTN	Regional Breeders Testing Network
TVREC	Tennessee Valley Research and Extension Center
WREC	Wiregrass Research and Extension Center

Literature Review

Corynespora cassiicola

Corynespora cassiicola (Berk. & Curt. C. T. Wei. abbreviated as CC) is a genetically diverse species of fungi in the phylum Ascomycota. This fungus can infect a wide number of plant species and has been found on nematode cysts and rare occasions on the human skin (Dixon, 2009). C.T. Wei first classified CC in 1950. Wei studied the history of the genus, and compared conidia production on conidiophores' to that of similar genera. In 1896, Cooke published a note about a disease of melon (*Cucumis melo* L.), later collecting CC on cucumber, that he characterized belonging to the genus *Cercospora* (Wei, 1950). In 1906, Güssow discovered the same fungus on cucumber and proposed the new genus *Corynespora*, as he noticed the conidia are formed in chains and a hyaline isthmus is present between the conidiophore and conidium (Wei, 1950). Wei reported the conidia of CC to be variable in size, reaching 520 µ in length and 9 to 13 µ in diameter (1950).

Wei observed that the specimen of *Helminthosporium cassiicola* Berk. & Curt. showed similar characteristics with *Cercospora melonis.* Wei compared conidia of *Cercospora, Corynespora,* and *Helminthosporium* and found the latter two to be most related (1950). The two genera of fungi can be distinguished by the structure of their conidia. Conidia of CC are more slender and widest near the base, whereas conidia of *Helminthosporium gossypii* are widest near the middle (Jones, 1961). In the *Helminthosporium cassiicola* folder in Herbarium I.M.I., Wei identified sixteen collections from eleven different hosts as *Corynespora* (1950). There is speculation that CC may have been present for many years as it was misidentified in 1935 as

Helminthosporium leaf spot in the Philippines (Clara, 1935). Wei chose the scientific name of CC because the species *cassiicola* predated others given to this fungus.

CC is a universal pathogen reported to cause major economic losses in over 70 countries (Dixon, 2009). Host range of this pathogen includes more than 530 plant species from 380 genera (Dixon, 2009). It has been found to be extremely variable and has been reported to cause foliar disease on soybean (Koenning, 2006), sesame (Stone and Jones, 1960), cucumber (Blazquez, 1967), tomato (Blazquez, 1972) and many other economically important species. CC has played a significant role as a devastating leaf disease of plantation rubber (*Hevea brasiliensis*) trees in Sri Lanka and other rubber growing countries (Silva, 2003). Reports of the disease were frequent in the United States between 1950 and 1970 on agricultural crops includes cotton, soybean, sesame and cowpea (*Vigna unguiculata* (L.) Walp). The fungus became uncommon for several decades in the southeastern United States, until about 15 years ago. North Carolina reported CC in 2004 on soybean, after being dormant since 1981 (Koenning, *et al.*). First observation of CC in cotton was in Georgia in 2005 (Fulmer, *et al.*, 2012), which was followed by Alabama's in 2011 (Hagan, *et al.*), Tennessee in 2013 (Butler, *et al.*, 2016) and Louisiana in 2014 (Price, 2015). The reappearance of CC across the lower and mid-South has become a major concern for cotton and soybean producers.

The primary symptom of CC in cotton is concentric lesions on the leaves, hence the common name of target spot. The target-like lesions on leaves range in frequency and size. Wei described the species as "parasitic on stems, leaves and fruits, but mostly on leaves, causing lesions of various sizes, from less than 1 mm to 1.5 or even 2 cm in diameter" (Wei, 1950).

Lesions are yellowish-brown and enlarge with disease progression. Target spot is primarily found in the tropics and subtropics, with the ideal conditions for disease onset and development being a hot, humid environment. Target spot is most severe during periods of extended leaf wetness when conidia increase in number (Kingsland, 1986). CC survives as an endophyte (Déon, 2012), saprophyte (Kingsland, 1986) or a pathogen (Dixon, 2009). As a pathogen, CC can be found in the roots, stems and leaves with defoliation progressing from the bottom of the plant outward to the top. The disease seems most aggressive when lesions are primarily present on foliage, causing premature defoliation.

Pathogenicity of CC varies greatly by host. There is no clear relationship between phylogenetic lineage and pathogenicity. Isolates have shown host specificity, while others have a wide range of hosts (Stone and Jones, 1960; Jones, 1961; Onesirosan *et al.*, 1974; Dixon, 2009). With CC isolates from sesame and soybean, Stone and Jones (1960) u observed infection on both hosts, yet the two isolates only caused small lesions on cowpea. Onesirosan (1974) studied the virulence of isolates from twenty plant species, including some cotton species, and was able to aggressively infect cotton plants with cotton isolates. Some of his other isolates included those collected on cucumber and soybean in the southern United States. Another group from two common Nigerian weeds, *Aspilia africana* and a *Lepistemon* sp., were both highly virulent on cotton but not on soybean, sesame, or eggplant (*Solanum melongena* L.) while none of the were highly virulent on cowpea (Onesirosan, 1974). Blazquez showed cross-pathogenicity with isolates from tomato being pathogenic on cucumber (1972). Furukawa infected scarlet sage (*Salvia splendens* Sellow ex Roem. & Schult.) leaves with isolates from cucumber, green pepper

(*Capsicum annuum* L.) and hydrangea (*Hydrangea* L.), but the scarlet sage isolate was not pathogenic to cucumber, green pepper, hydrangea, eggplant, tomato or soybean (2008). Kingsland noted that isolates recovered from papaya (*Carica papaya* L.) leaf debris caused target spot on tomato, cucumber and watermelon (*Citrullus lanatus* var. lanatus Thunb. Matsum. & Nakai) but not on papaya in the Republic of Seychelles (1986). Onesirosan used an isolate of papaya and could not initiate disease on seven other cultivated plants, while only infecting papaya with an isolate from papaya leaf debris (1974). As seen above, isolates tend to group by pathogenicity and appear to be distinct strains or races, only affecting certain hosts (Onesirosan, 1974).

Upland Cotton and *Corynespora cassiicola*

Cotton (*Gossypium spp.*) serves as a major textile crop in the United States, with a majority of acreage grown in southern states. The United States produced 20.2 million bales of cotton in 2017 (National Cotton Council, 2018). There are four major species of cotton used in commercial production. Upland cotton (*Gossypium hirsutum L.*) is the most widely grown species of the four, accounting for more than 90% of world lint yield. Upland cotton is an allotetraploid that derived from an Old World diploid cotton. It originated in Mexico and adapted the A genome, native to Africa, that hybridized with the Mexican D genome giving rise to the diverse species of *G. hirsutum* (Wendel, 1989).

Upland cotton (*Gossypium hirsutum L.*) is susceptible to a number of fungal pathogens, including CC, which has made its recent reappearance in the United States. CC was first identified as a pathogen of cotton in 1959 in commercial fields of the Mississippi Delta (Jones,

1961). There is speculation that the fungus could have been present for many years prior because it had been commonly misidentified as the genus *Helminthosporium*. Reports of the disease have been uncommon in the Southeastern United States until 2005 when it was identified on irrigated cotton in southwestern Georgia (Fulmer *et al.*, 2012). The reappearance of the disease is now distributed across the southeastern United States and has impacted cotton quality and yield. The primary symptoms of CC shown in cotton are concentric rings on foliage and premature defoliation. In 2011, the disease was noted statewide in Alabama cotton with heaviest defoliation seen in irrigated as opposed to dryland cotton (Hagan, 2013). Campbell *et al.* did however observe heavy defoliation in Alabama dryland cotton that received frequent late summer rains (2012). In 2013, CC made its first appearance in Tennessee (Raper, 2016). During the summer of 2014, target spot had spread into Louisiana along the Mississippi and Red Rivers (Price, 2015).

CC can have a devastating effect on cotton yield. Yield losses of several hundred kg/ha of lint have been reported (Fulmer, 2012). Recent studies show a disconnect between yield and disease intensity (cite). The relationship between yield and target spot intensity needs further examination as several cultivars show heavy defoliation yet do not suffersignificant yield loss. Phytogen 499 WRF is a cultivar that has heavy defoliation, yet its yield is normally among the highest among commercial cotton cultivars (Hagan, 2013). Bowen *et al.* found PhytoGen 499 WRF to have consistently greater defoliation than Deltapine 1050 B2RF, Deltapine 1252 B2RF or Deltapine 1137 B2RF (2018). Hagan *et al.* has reported high yielding cultivars showing highest target spot severity (2013). Yield losses are still possible; with examples of susceptible cotton cultivars having an estimated 448 kg/ha lint yield loss (Bowen *et al.*, 2018). The effect of

CC on yield may be impacted by disease onset, specifically the plant's developmental stage upon infection, the rate of disease progression, and weather conditions.

While the exact mechanism of pathogen dispersal is not known, seed transmission is a possibility since it has been reported that CC is able to survive in seeds of both soybean and sesame (Stone and Jones, 1960). Spending part of its life cycle as a saprophyte, CC can overwinter on plant debris. Jones (1961) collected cotton stem tips randomly from overwintered stalks in two different fields and found CC on 7 of 37 and 15 of 41 stems, respectively. He then prepared four colonies with this inoculum and sprayed on four plants each of soybean and cotton. Both hosts were infected. Such results demonstrate the ability of the fungus to overwinter on plant debris, specifically cotton, without a loss of pathogenicity (Jones, 1961). The closed canopies of mature cotton plants provide the suitable conditions for the fungus to thrive near the soil surface. No-till or strip-tilled fields planted with continuous cotton are reported as most affected (Raper, 2016; Campbell *et al.*, 2012). Crop rotation, sanitation and planting away from other infected fields as well as establishing disease resistant cultivars are potential management options. Weed management is also important, as the fungus can infect a wide number of weed hosts (Dixon, 2009; Onesirosan, 1974).

Reaction of Upland cotton cultivars to *Corynespora cassiicola*

Inoculation methods have been used since 1945 to induce infection onto plants to determine their reactions to a certain disease (Price, 2015; Dixon, 2009; Onesirosan, 1974; Jones, 1961). Previous research showed reactions of certain hosts, now plant pathologists are studying the reaction of specific cotton cultivars to CC in major areas of the southeastern United States

(Hagan *et al.* 2013; Fulmer *et al.* 2012). Disease reactions have ranged in intensity across different cultivars. Hagan *et al.* (2013) has reported Phytogen lines to be among the more susceptible cultivars, specifically Phytogen 499 WRF suffered premature defoliation ranging from 65% to 85%. Campbell *et al.* used the Florida 1 to 10 leaf spot scoring scale at the Wiregrass Research and Extension Center (WREC) in Headland, AL and the Plant Breeding Unit (PBU) in Tallassee, AL (2012). In both trials, the Phytogen cultivars received the highest ratings and the lowest rating was with Stoneville 5288 B2F at WREC and Delta Pineland 1050 B2RF at PBU, respectively (2012).

Management of *Corynespora cassiicola* in Upland Cotton

Management options for CC-incited diseases include scouting for the disease, weed management, sanitation (Kingsland, 1986), and the use of fungicides (Hagan, 2015). Some recent studies have reported varying levels of disease symptoms and control of CC with the use of fungicides (Hagan, 2012). It is best to plant cotton crops adjacent to non-susceptible hosts. Crop rotation is a form of management and planting after a susceptible crop is not suggested.

The objectives of my research are:

1. To develop a protocol for screening large numbers of cotton genotypes for resistance to CC in the greenhouse
2. Apply that protocol to evaluate cotton genotypes represented in the 2013 Alabama Cotton Variety Test for resistance under natural field conditions
3. Develop an inoculation system for plants in the field, and assess disease symptom development

Materials & Methods

Inoculum Preparation

An isolate of CC from cotton was obtained from Auburn University's Plant Diagnostic Lab for inoculating cotton plants. Using a sterile loop technique, the isolate was transferred from its original plates to approximately thirty tubes consisting of a half-strength potato dextrose agar (PDA) medium. After obtaining an isolate, it is critical to keep a vial and original culture on PDA plates between 0-4°C. The inoculated PDA tubes for our experiment were stored in the refrigerator to ensure a pathogenic isolate throughout the experiment. Subcultures were made from the inoculated PDA tubes approximately every six to eight weeks to maintain the original, pathogenic isolate. To initiate sporulation, the subculture was transferred to V8 agar plates (Dixon *et al.*, 2009) and stored at 28°C for optimal isolate growth (Onesirosan, 1974). The subculture was transferred onto the V8 agar plates approximately two to three weeks prior to inoculating live plants. Using a sterile loop technique, two small portions of the culture were applied to two separate areas of the V8 medium (Figure 2). This application is to ensure maximum growth on the plate. Forty plates were inoculated on average per greenhouse experimental trial. Kanamycin was the antibiotic chosen for our experiments. Two different light settings were compared to determine the best sporulation of the fungus on V8 agar media. The first light setting was a black light on a timer of every twelve hours at room temperature. The second lighting method incorporated an incubator with no light. Optimal growth required seven to ten days within an incubator with no light at a temperature of 31°C. Inoculum preparation followed the sporulation process. The standard for CC inoculum preparation has consisted of

washing conidia from V8 agar plates then filtering the conidia through two layers of sterilized cheesecloth (Onesirosan, 1974). After the V8 agar media plates had coverage of the culture, they were removed from the incubator and the mycelium was scraped from each plate. The mycelium was combined in a blender, creating a suspension that was filtered through two layers of sterilized cheesecloth. A drop of the spore suspension was placed onto a glass slide and conidia and colony forming units were quantified with a hemocytometer. The conidia visible inside the grid of the hemocytometer were counted and standardized to an average of 62,600 spores and colony forming units per mL in each inoculum mixture. Within four to six hours, the mixture was applied to the plants in the greenhouse via a foliar spray technique (Dixon, 2009).

Greenhouse Inoculation

Thirty-nine cultivars tested in the 2013 Alabama Cotton Variety Tests were evaluated in the greenhouse to assess their reaction to target spot under controlled conditions. Cotton plants were grown in a soil medium in six-inch pots with two seeds per pot. The cultivars were evaluated starting spring of 2013 and continuing until October 2014 with fifteen total experimental sets. A randomized complete block design for each set with a single plant as the experimental unit was used. Each set contained 48 plants and eight blocks. In each block one of the cultivars was left untreated as a non-inoculated control. CC conidia suspensions increased on V8 agar plates were used to inoculate each individual plant. Individual plants were placed in a white, 32 L Up & Up plastic trash bag (Figure 2). Inoculum was applied with a plastic spray bottle, each leaf was thoroughly wetted, and each bag was tied, enclosing the entire plant. Distilled water was applied to the controls, which were bagged in individual trash bags. Disease

symptoms began to appear after 48 hours (Figure 4). The symptoms were not observed on non-inoculated control plants. After two days, each bag was removed and plants were rated for disease symptoms. The plants were inoculated when five to six leaves were present, making a rating scale of 0 to 5 most practical. The visual estimate of lesions was based on the number of leaves with characteristic target spot lesions. Disease ratings were based on lesion coverage on the foliage as well as defoliation according to the following scale: 0 = no lesions present on leaves and no defoliation; 1 = 1 leaf showing a few to many small lesions and/or 1 leaf defoliated; 2 = expanding lesions covering 2 true leaves and/or 2 leaves defoliated; 3 = expanding lesions covering 3 true leaves and/or 3 leaves defoliated; 4 = lesions covering 4 of true leaves and/or 4 leaves defoliated; and 5 = lesions covering all true leaves and/or plant fully defoliated.

Field Experiment at Plant Breeding Unit

The field experiment for assessing cultivar resistance to CC was implemented in summer 2013 at PBU. The intent of this experiment was to induce disease in a field environment. Six cultivars were chosen from the 2013 Alabama Cotton Variety Trials to be tested; Phytogen 499 WRF, Phytogen 575 WRF, Fibermax 1944 GLB2, Deltapine 1252 B2RF, Deltapine 1137 B2RF, and Deltapine 1050 B2RF. Phytogen 499 WRF and Deltapine 1050 B2RF had shown consistent higher and lower disease ratings from research and the greenhouse, respectively. FiberMax 1944 GLB2 had shown severe disease symptoms in the greenhouse experiment, while Phytogen 575 WRF showed lesser disease symptoms. The remaining cultivars chosen were a fair representation of widely produced mid- and full-season cultivars. The experiment was designed

as a randomized complete block with two row plots, with four replications split into treated and untreated blocks. Each plot was 20 ft in length and each cultivar was represented in a total of eight plots. The test was randomized by Agrobase SQL to determine the placement of each cultivar within the replication. Each replication treatment was randomly allocated with the CC inoculum mixture or was left untreated, therefore acting as a control. Corn (*Zea mays* L.*)* was chosen as the three-foot border for all treated and untreated plots within the field test as seen in Figure 5. Increased distance between plots may interfere with disease spread. Four rows of corn were planted to separate each plot failed to prevent the spread of CC into the non-inoculated controls. The failure of the corn border suggests that there was a resident CC population.

The seeds for the field experiment were planted on May 11, 2013. Two inoculation treatments occurred during week 12 and 14 of growth. Plants were inoculated in the field using a three-gallon backpack sprayer. The inoculum was quantified in the laboratory beforehand and totaled on average 4,000 mL of inoculum to be used in the field. Eight thousand mL of water was added totaling 12,000 mL used in one inoculation to cover all plants. This was sufficient to ensure the canopy of each plot got full coverage of the inoculum spray. Four blocks received no CC inoculum and served as controls.

The plants were rated four times between weeks 15 and 17 of growth: August 6, August 14, August 19, and September 6, 2013. A rating system of 0 to 100 was used based on visual estimates of lesions and defoliation percentage, with 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20

to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation.

Alabama Variety Tests

The 39 cultivars of the 2013 Alabama Cotton Variety Tests were evaluated at four locations in Alabama: Belle Mina, Prattville, Tallassee, and Fairhope for target spot severity. Symptomatic plants were observed in our studies in 2012 and 2013 at all four locations. Each location × test combination comprised 25 entries, i.e., not all 39 entries evaluated in the greenhouse were present at all location x test combinations. The rating was based on a visual estimate of the entire plot of the percentage of coverage of lesions on plant and/or defoliation present using the scale described previously. Locations and rating dates were as follows, the Tennessee Valley Research and Extension Center in Belle Mina (July 30 and August 15), the Gulf Coast Research and Extension Center in Fairhope (August 8), the Prattville Agricultural Research Unit (August 13 and 20), and the Plant Breeding Unit at the E. V. Smith Research Center (August 2 and August 14).

Statistical Analysis

All data was analyzed statistically utilizing the Agrobase SQL software package. Experiment factors were location (fixed), genotypes (random), sets and blocks (random). The field tests were analyzed for correlation among location, test and rating date in comparison to the ratings produced in the greenhouse. The F value and p value were obtained for each location and

test. Correlation was also analyzed in the field tests based on cultivar and test type (Early or Full season). A mean separation test was performed on all thirty-nine cultivars represented in the greenhouse studies as well as the Alabama Variety Tests. In the first step of the analysis cultivar means were calculated for each set. These means were then analyzed using mixed models methodology as implemented in SAS® PROC MIXED, where set was the random blocking factor in the greenhouse and blocks were such in the field. Pairwise comparisons among cultivars were calculated without any adjustment for multiple comparisons based on the suggestion by Milliken and Johnson (2009) for experiments of a preliminary nature.

Results and Discussion

Inoculum Growth & Inoculation Procedures

The CC grew very well on the V8 agar and covered the majority, if not all, of each agar plate as seen in Figure 1. Inoculum that was cultured and prepared in the laboratory produced significant disease symptoms in both our greenhouse and field experimental trials. The spray and bag method in the greenhouse was a suitable method for inoculation. The bagging method provided the perfect environment for the fungus to thrive; allowing a smaller individual environment, higher humidity, and rapid disease onset.

Greenhouse Inoculation

The 39 cultivars in the 2013 Alabama Cotton Variety Trials found in Table 1 all received a mean rating based upon their performance in greenhouse trials (Table 1). Every cultivar showed symptoms of CC following inoculation. An effective inoculum was able to produce extreme disease symptoms on all plants in the humid environment of the closed trash bag. The plants foliage area of five to six leaves and the visual evidence of defoliation and target spots aided the ability to distinctly choose a rating. When looking at the differences of the means on an average of the entire genotype's population in a sample, the numbers appear similar and the differences are not as obvious. The means in the greenhouse ranged from 1.5 to 4.0 (Table 1). Deltapine 1321 B2RF received the highest mean rating of 4.0. Phytogen PX 5540-10 WRF received the second highest rating of 3.9, as well as Deltapine 1034 B2RF. These top three ranking cultivars had significantly greater disease ratings compared with the lowest five ranking cultivars. Deltapine 1212 B2RF had the lowest mean rating of 1.5 and Deltapine 1219 B2RF

received the second lowest mean of 1.7. The lowest two entries could be separated statistically ($P = 0.05$) only from nine entries with the highest mean disease ratings. The letters following the mean ratings in Tables 1-22 represent this statistical difference. Cultivars followed by any of the same letters are not statistically different with respect to symptom severity.

Field Experiment at PBU

The 2013 season experienced a frequent, heavy rainfall which led to a possible exchange of conidia between all treated and untreated fields. The highly infectious CC in that year prevented a reliable control for comparison. The combination of high moisture and severe disease decimated the plots, leaving no boll samples in two of the eight plots. We did not have sufficient sample sizes to determine if the disease treatment had any effect on total yield.

Alabama Variety Tests

Defoliation levels as high as 75% in the state of Alabama in 2013 in both Prattville and Tallassee, Alabama. A mean rating was calculated for each cultivar at each location and is displayed by rating dates and locations in Tables 2-19. All four locations were to be rated twice; however, the Fairhope location was rated only once due to weather related issues (Table 20). Within the first rating group, a significant difference in disease ratings were noted in four of the 10 test × date combinations. Significant ($P < 0.05$) cultivar effects were observed for the early season tests at PBU and TVREC as well as the TVREC IRR early season test at the latter location. Among the full season tests, only the test at TVREC had significant ($P < 0.05$) overall cultivar differences. Within Rating 2, a significant difference in disease level ratings was recorded in only one of eight test × date combinations, viz. the full season test at the PBU. Due

to the decrease in significance from Rating 1 to Rating 2, we assume it is best to use a rating scale at an earlier stage of plant development to better distinguish among genotypes for resistance to CC. It appears that it may be easier to differentiate among genotypes at the early rather than later rating dates. This may indicate that genotypes may react to the disease mainly by showing differential rates of disease development.

Correlations

Phenotypic correlations between greenhouse ratings and field ratings revealed a very poor relationship between greenhouse observations of disease following inoculation and disease development under natural conditions. Of 18 location × test × rating date combinations, only one (Prattville Early Season) was significantly ($P < 0.05$) correlated with greenhouse ratings (Table 3). This could be due to two reasons, viz; our inoculation procedure in the greenhouse, while capable of causing disease, was not able to simulate disease development and may have overcome disease defense mechanisms that would naturally occur in the field; and there also appears to be limited variability in upland cotton adapted germplasm for reaction to target spot. The disease induced in the greenhouse could have been too suddenly onset by the virulent CC isolate in comparison with production fields. Evidence for this is supported by the failure to differentiate among genotypes in a majority of the location × test × rating date combinations. With the later rating tests, the disease seemed to have covered most of the leaves and defoliation has occurred to the point where the genotype reaction to the disease appeared similar across all genotypes.

Phenotypic correlations between field disease ratings and lint yield based on 100 (or 75 for rating 2) cultivar × test type (Early or Full season) means indicated a significant negative correlation (Table 4). Larger negative correlations were observed for the second rating. There was only one significant case of correlation with the natural infection in the field and the greenhouse inoculation procedure. This could be due either to the virulence of the greenhouse inoculum or the natural susceptibility of Upland cotton to CC.

The performance of these cultivars, following exposure to CC, was difficult to determine with only the use of a visual rating scale. High defoliation levels increased the difficulty of utilizing a visual rating scale. The visual rating scale in the field needs to be performed at an early stage of plant development to be efficient. A measure could be taken on height of disease progression in plant so that each cultivar can receive a rating based on a numerical calculation taken and not only the naked eye visually rating the progression. Because of the apparent limited genetic variability for reaction to CC among adapted upland cotton germplasm, it may be necessary to explore the broader cotton germplasm collection for possible sources of resistance to CC.

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Table 1. Mean greenhouse target leafspot disease severity rating for the 39 cotton cultivars in the 2013 Alabama Cotton Variety Trials

Cultivar	Mean		Std Err
Deltapine 1321 B2RF	4.0	a	0.65
PhytoGen PX 5540-10 WRF	3.9	ab	0.59
Deltapine 1034 B2RF	3.9	ab	0.52
All-Tex DG CR108788 B2RF	3.8	abc	0.65
DynaGro CT 13414	3.6	abc	0.54
DynaGro 2285 B2RF	3.5	abc	1.13
PhytoGen PX 5403-01 WRF	3.5	abc	0.62
Deltapine 1048 B2RF	3.4	abc	0.74
PhytoGen 339 WRF	3.4	abc	0.65
Bayer ST 4946 GLB2	3.4	abcd	0.59
Americot 1550 B2RF	3.3	abcd	0.74
Croplan Genetics 3428 B2RF	3.3	abcde	0.74
Americot 1511 B2RF	3.3	abcde	0.52
Deltapine MON 12R242 B2R2	3.3	abcde	0.69
PhytoGen PX 4433-25 WRF	3.3	abcde	0.74
All-Tex DG CT12353 B2RF	3.2	abcde	0.56
PhytoGen PX 4433-27 WRF	3.2	abcde	0.74
PhytoGen 499 WRF	3.2	abcde	0.59
Croplan Genetics 3787 B2RF	3.1	abcde	0.52
Deltapine 0912 B2RF	3.0	abcde	0.42
PhytoGen 367 WRF	3.0	abcde	0.65
Deltapine 1137 B2RF	2.8	abcde	0.47
Deltapine 1050 B2RF	2.7	abcde	0.46
Deltapine 1252 B2RF	2.6	abcde	0.87
PhytoGen 375 WRF	2.6	abcde	0.46
PhytoGen PX 4444-13 WRF	2.6	abcde	0.49
PhytoGen PX 3122-40 WRF	2.5	abcde	0.65
All-Tex NITRO 44B2RF	2.3	abcde	0.62
Bayer ST 6448 GLB2	2.3	abcde	0.52
PhytoGen PX 4444-14 WRF	2.3	bcde	0.62
FiberMax 1944 GLB2	2.3	bcde	0.52
PhytoGen 575 WRF	2.2	bcde	0.50
Deltapine MON 12R224 B2R2	2.2	bcde	0.59
DynaGro 2610 B2RF	2.1	bcde	0.42
PhytoGen PX 5538-40 WRF	2.1	cde	0.62
PhytoGen PX 3003-10 WRF	2.0	cde	0.62
Americot NG 5315 B2RF	1.9	cde	0.42
Deltapine 1219 B2RF	1.7	de	0.62
Deltapine 1212 B2RF	1.5	e	0.74

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 = 1 leaf showing a few to many small lesions and/or 1 leaf defoliated; 2 = expanding lesions covering 2 true leaves and/or 2 leaves defoliated; 3 = expanding lesions covering 3 true leaves and/or 3 leaves defoliated; 4 = lesions covering 4 of true leaves and/or 4 leaves defoliated; and 5 = lesions covering all true leaves and/or plant fully defoliated

Table 2. Mean target leafspot disease severity ratings for 1st rating date of early season dry land cultivars at the Tennessee Valley Substation, 2013.

	TVREC Early Dry land	
Cultivar	Mean	
Deltapine 0912 B2RF	11.8	a
Americot 1550 B2RF	11.0	ab
PhytoGen PX 3003-10 WRF	9.0	abc
Deltapine 1034 B2RF	8.8	abc
PhytoGen PX 4444-13 WRF	8.3	abc
Deltapine 1212 B2RF	7.0	bcd
Croplan Genetics 3428 B2RF	6.8	bcd
PhytoGen PX 4444-14 WRF	6.8	bcd
Deltapine 1321 B2RF	6.5	bcd
DynaGro 2285 B2RF	6.3	cd
PhytoGen 367 WRF	6.0	cd
PhytoGen 499 WRF	5.8	cd
PhytoGen PX 4433-25 WRF	5.8	cd
FiberMax 1944 GLB2	5.5	cde
All-Tex DG CT 12353 B2RF	5.3	cde
PhytoGen 375 WRF	5.0	cde
Americot 1511 B2RF	5.0	cde
PhytoGen PX 3122-40 WRF	4.8	cde
Bayer ST 4946 GLB2	4.8	cde
Deltapine 1048 B2RF	3.5	de
PhytoGen 339 WRF	3.5	de
Deltapine MON 12R224 B2R2	3.5	de
All-Tex DGX CR108788 B2RF	3.5	de
PhytoGen PX 4433-27 WRF	3.0	de
DynaGro CT 13414	1.0	e
	Std Err: 1.63	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 3. Mean target leafspot disease severity ratings for 2nd rating date of early season dry land cultivars at the Tennessee Valley Substation, 2013.

	TVREC Early Dry land	
Cultivar	Mean	
Deltapine 1212 B2RF	10.8	a
PhytoGen PX 4433-27 WRF	10.8	a
Deltapine 1034 B2RF	10.3	a
PhytoGen PX 4444-13 WRF	10.0	ab
PhytoGen PX 4444-14 WRF	9.8	ab
Americot 1550 B2RF	9.5	ab
Deltapine 0912 B2RF	9.3	ab
Americot 1511 B2RF	8.5	ab
Deltapine 1321 B2RF	7.3	ab
All-Tex DGX CR108788 B2RF	7.0	ab
PhytoGen PX 3003-10 WRF	7.0	ab
Croplan Genetics 3428 B2RF	6.8	ab
DynaGro 2285 B2RF	6.5	ab
All-Tex DG CT 12353 B2RF	6.3	ab
PhytoGen 367 WRF	6.2	ab
PhytoGen PX 4433-25 WRF	6.0	ab
PhytoGen 375 WRF	6.0	ab
PhytoGen 339 WRF	5.7	ab
FiberMax 1944 GLB2	5.7	ab
PhytoGen 499 WRF	5.5	ab
PhytoGen PX 3122-40 WRF	5.0	ab
Deltapine 1048 B2RF	4.7	ab
Deltapine MON 12R224 B2R2	4.7	ab
DynaGro CT 13414	4.5	ab
Bayer ST 4946 GLB2	3.5	b
	Std Err: 2.42	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 4. Mean target leafspot disease severity ratings for 1st rating date of early season irrigated cultivars at the Tennessee Valley Substation, 2013.

	TVREC Early Irrigated	
Cultivar	Mean	
Americot 1511 B2RF	6.8	
PhytoGen 339 WRF	5.3	b
Americot 1550 B2RF	5.3	b
All-Tex DGX CR108788 B2RF	5.3	b
PhytoGen PX 4433-27 WRF	5.0	b
Deltapine 1034 B2RF	4.4	bd
Deltapine MON 12R224 B2R2	4.3	bd
Bayer ST 4946 GLB2	4.3	bd
PhytoGen 367 WRF	4.0	bde
DynaGro CT 13414	4.0	bde
PhytoGen PX 4444-14 WRF	4.0	bde
PhytoGen PX 3003-10 WRF	3.8	bde
All-Tex DG CT 12353 B2RF	3.8	bde
Deltapine 0912 B2RF	3.5	bde
PhytoGen PX 3122-40 WRF	3.5	bde
PhytoGen PX 4444-13 WRF	3.5	bde
Croplan Genetics 3428 B2RF	3.0	bde
Deltapine 1212 B2RF	3.0	bde
PhytoGen 375 WRF	2.5	bde
Deltapine 1321 B2RF	2.3	de
PhytoGen 499 WRF	2.0	de
Deltapine 1048 B2RF	2.0	de
FiberMax 1944 GLB2	2.0	de
PhytoGen PX 4433-25 WRF	1.5	de
DynaGro 2285 B2RF	1.3	e
	Std Err: 1.09	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 5. Mean target leafspot disease severity ratings for 2nd rating date of early season irrigated cultivars at the Tennessee Valley Substation, 2013.

	TVREC Early Irrigated	
Cultivar	Mean	
Americot 1550 B2RF	9.8	a
PhytoGen 367 WRF	8.3	ab
DynaGro 2285 B2RF	7.0	ab
PhytoGen PX 4444-14 WRF	7.0	ab
PhytoGen 339 WRF	6.5	abd
All-Tex DG CT 12353 B2RF	6.5	abd
Deltapine 1212 B2RF	6.3	abd
FiberMax 1944 GLB2	5.8	abd
Americot 1511 B2RF	5.5	abd
Croplan Genetics 3428 B2RF	5.0	abd
All-Tex DGX CR108788 B2RF	5.0	abd
PhytoGen 375 WRF	4.8	abd
PhytoGen PX 4433-27 WRF	4.8	abd
Bayer ST 4946 GLB2	4.5	bd
DynaGro CT 13414	4.5	bd
PhytoGen PX 3003-10 WRF	4.3	bd
Deltapine 1321 B2RF	4.0	bd
Deltapine 1048 B2RF	3.8	bd
Deltapine 1034 B2RF	3.5	bd
PhytoGen PX 4433-25 WRF	3.3	bd
PhytoGen PX 3122-40 WRF	3.0	d
Deltapine MON 12R224 B2R2	3.0	d
PhytoGen PX 4444-13 WRF	3.0	d
PhytoGen 499 WRF	2.8	d
Deltapine 0912 B2RF	1.8	d
	Std Err: 1.77	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 6. Mean target leafspot disease severity ratings for 1st rating date of early season cultivars at the Prattville Agricultural Research Unit, 2013.

	PF Early	
Cultivar	Mean	
DynaGro CT 13414	45.0	a
All-Tex DGX CR108788 B2RF	43.8	ab
PhytoGen PX 3003-10 WRF	42.5	ab
PhytoGen 367 WRF	42.5	ab
Deltapine MON 12R224 B2R2	42.5	ab
Deltapine 1034 B2RF	41.3	ab
Americot 1511 B2RF	41.3	ab
Croplan Genetics 3428 B2RF	40.0	abc
Bayer ST 4946 GLB2	40.0	abc
PhytoGen 339 WRF	39.3	abc
Deltapine 1048 B2RF	37.5	abc
PhytoGen PX 3122-40 WRF	37.5	abc
PhytoGen PX 4433-27 WRF	37.5	abc
Deltapine 1321 B2RF	36.3	abc
DynaGro 2285 B2RF	36.3	abc
PhytoGen PX 4433-25 WRF	35.0	abc
PhytoGen 375 WRF	33.8	abc
PhytoGen PX 4444-13 WRF	33.8	abc
PhytoGen 499 WRF	32.5	abc
All-Tex DG CT 12353 B2RF	32.5	abc
Americot 1550 B2RF	31.8	abc
Deltapine 0912 B2RF	31.3	abc
Deltapine 1212 B2RF	27.5	abc
FiberMax 1944 GLB2	25.0	bc
PhytoGen PX 4444-14 WRF	21.8	c
	Std Err: 7.46	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 7. Mean target leafspot disease severity ratings for 2nd rating date of early season cultivars at the Prattville Agricultural Research Unit, 2013.

	PF Early	
Cultivar	Mean	
PhytoGen PX 4444-13 WRF	48.5	a
Croplan Genetics 3428 B2RF	47.5	a
PhytoGen PX 3003-10 WRF	46.3	a
PhytoGen PX 3122-40 WRF	46.3	a
PhytoGen PX 4433-27 WRF	45.0	a
Deltapine 1034 B2RF	43.0	a
PhytoGen 375 WRF	42.5	a
PhytoGen 499 WRF	42.5	a
PhytoGen 339 WRF	42.5	a
Deltapine 0912 B2RF	41.3	a
Bayer ST 4946 GLB2	40.0	a
DynaGro 2285 B2RF	39.5	a
Deltapine 1321 B2RF	39.3	a
FiberMax 1944 GLB2	37.5	a
Deltapine 1212 B2RF	37.0	a
Deltapine 1048 B2RF	36.3	a
All-Tex DGX CR108788 B2RF	36.3	a
Americot 1511 B2RF	35.5	a
Deltapine MON 12R224 B2R2	35.5	a
DynaGro CT 13414	35.0	a
PhytoGen PX 4433-25 WRF	34.5	a
PhytoGen 367 WRF	34.0	a
PhytoGen PX 4444-14 WRF	33.8	a
All-Tex DG CT 12353 B2RF	33.3	a
Americot 1550 B2RF	30.5	a
	Std Err: 6.97	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 8. Mean target leafspot disease severity ratings for 1st rating date of early season cultivars at the Plant Breeding Unit, 2013.

	PBU Early	
Cultivar	Mean	
FiberMax FM 1944 GLB2	45.0	
PhytoGen 339 WRF	41.3	b
PhytoGen 367 WRF	38.8	b
PhytoGen PX 4433-25 WRF	36.3	bd
PhytoGen PX 4444-13 WRF	36.3	bd
Americot 1511 B2RF	33.8	bde
Croplan Genetics 3428 B2RF	32.5	bde
Deltapine 1212 B2RF	32.5	bde
Deltapine 1034 B2RF	31.3	bde
PhytoGen PX 3122-40 WRF	31.3	bde
Americot 1550 B2RF	30.0	bde
PhytoGen 499 WRF	30.0	bde
PhytoGen PX 4433-27 WRF	30.0	bde
PhytoGen PX 3003-10 WRF	30.0	bde
Deltapine 0912 B2RF	28.8	bde
All-Tex DG CT 12353 B2RF	28.8	bde
Deltapine MON 12R224 B2R2	27.5	bde
DynaGro 2285 B2RF	27.5	bde
Bayer ST 4946 GLB2	26.3	de
PhytoGen 375 WRF	25.0	de
Deltapine 1321 B2RF	25.0	de
PhytoGen PX 4444-14 WRF	22.8	de
Deltapine 1048 B2RF	21.3	e
All-Tex DGX CR108788 B2RF	17.5	
DynaGro CT 13414	13.8	
	Std Err: 5.18	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 9. Mean target leafspot disease severity ratings for 2nd rating date of early season cultivars at the Plant Breeding Unit, 2013.

	PBU Early	
Cultivar	Mean	
PhytoGen PX 4433-27 WRF	53.8	a
Croplan Genetics 3428 B2RF	47.5	ab
Deltapine 1212 B2RF	46.3	ab
PhytoGen PX 4444-14 WRF	46.3	ab
Deltapine MON 12R224 B2R2	45.0	abc
PhytoGen 339 WRF	41.3	abc
PhytoGen PX 3122-40 WRF	40.0	abc
DynaGro CT 13414	40.0	abc
PhytoGen 375 WRF	38.8	abc
PhytoGen 367 WRF	38.8	abc
PhytoGen 499 WRF	38.8	abc
Deltapine 1321 B2RF	38.8	abc
PhytoGen PX 4444-13 WRF	38.8	abc
Americot 1511 B2RF	37.5	abc
Deltapine 1048 B2RF	37.5	abc
FiberMax FM 1944 GLB2	37.5	abc
Americot 1550 B2RF	36.0	abc
Deltapine 1034 B2RF	33.8	abc
All-Tex DG CT 12353 B2RF	33.8	abc
DynaGro 2285 B2RF	33.3	bc
All-Tex DGX CR108788 B2RF	32.5	bc
PhytoGen PX 3003-10 WRF	30.0	bc
Bayer ST 4946 GLB2	28.8	bc
Deltapine 0912 B2RF	28.8	bc
PhytoGen PX 4433-25 WRF	25.0	c
	Std Err: 7.10	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 10. Mean target leafspot disease severity ratings for 1st rating date of early season cultivars at the Gulf Coast Research and Extension Center, 2013.

Cultivar	GCREC Early	
	Mean	
PhytoGen PX 3003-10 WRF	3.3	a
Croplan Genetics 3428 B2RF	3.1	ab
PhytoGen PX 4444-14 WRF	2.3	abc
PhytoGen 367 WRF	2.0	abc
PhytoGen 375 WRF	1.9	abc
Deltapine 0912 B2RF	1.8	abc
Deltapine 1048 B2RF	1.8	abc
Deltapine 1321 B2RF	1.8	abc
PhytoGen PX 4444-13 WRF	1.8	abc
DynaGro 2285 B2RF	1.6	abc
PhytoGen 339 WRF	1.6	abc
Bayer ST 4946 GLB2	1.5	abc
Americot 1511 B2RF	1.3	abc
Deltapine 1212 B2RF	1.3	abc
DynaGro CT 13414	1.3	abc
Deltapine MON 12R224 B2R2	1.1	abc
Deltapine 1034 B2RF	1.0	bc
PhytoGen PX 4433-27 WRF	0.9	bc
PhytoGen PX 3122-40 WRF	0.8	c
All-Tex DGX CR108788 B2RF	0.7	c
All-Tex DG CT 12353 B2RF	0.7	c
PhytoGen PX 4433-25 WRF	0.6	c
PhytoGen 499 WRF	0.4	c
Americot 1550 B2RF	0.2	c
FiberMax 1944 GLB2	0.2	c
	SE: 0.83	

No 2nd rating was taken for this location

Means followed by the same letter are not significantly different at α=0.05

Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 11. Mean target leafspot disease severity ratings for 1st rating date of full season cultivars at the TVREC dry land, 2013.

Cultivar	TVREC Full Dry land	
	Mean	
PhytoGen PX 5540-10 WRF	13.3	
PhytoGen PX 5538-40 WRF	11.0	b
Deltapine 1219 B2RF	9.7	b
Deltapine 1048 B2RF	9.5	b
PhytoGen PX 4444-13 WRF	8.5	be
All-Tex Nitro 44B2RF	8.2	bef
Deltapine MON 12R242 B2R2	7.7	bef
PhytoGen 499 WRF	7.2	ef
PhytoGen 339 WRF	6.5	ef
Deltapine 1252 B2RF	6.5	ef
Croplan Genetics 3787 B2RF	6.5	ef
PhytoGen PX 3003-10 WRF	6.5	ef
Americot NG 5315 B2RF	6.2	efh
PhytoGen PX 5403-01 WRF	6.0	efh
Deltapine 1137 B2RF	5.5	efh
DynaGro 2610 B2RF	5.2	efh
Phytogen 575 WRF	5.0	efh
PhytoGen 375 WRF	5.0	efh
Deltapine 1050 B2RF	4.7	fh
PhytoGen PX 3122-40 WRF	4.7	fh
Bayer ST 6448 GLB2	4.7	fh
PhytoGen PX 4444-14 WRF	4.5	h
Americot AM 1511 B2RF	4.5	h
PhytoGen PX 4433-27 WRF	2.7	h
PhytoGen PX 4433-25 WRF	2.0	
	SE: 1.31	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 12. Mean target leafspot disease severity ratings for 2nd rating date of full season cultivars at the TVREC dry land, 2013.

Cultivar	TVREC Full Dry land	
	Mean	
PhytoGen PX 5540-10 WRF	21.3	a
Americot 1511 B2RF	11.5	b
PhytoGen PX 4444-13 WRF	11.3	b
Deltapine 1137 B2RF	11.0	b
Deltapine 1048 B2RF	11.0	b
PhytoGen 499 WRF	10.8	b
Deltapine 1219 B2RF	10.8	b
PhytoGen PX 3003-10 WRF	10.1	b
All-Tex Nitro 44B2RF	10.0	b
DynaGro 2610 B2RF	10.0	b
PhytoGen 339 WRF	9.8	b
PhytoGen PX 5538-40 WRF	9.3	b
Americot NG 5315 B2RF	9.0	b
PhytoGen PX 4433-27 WRF	8.5	b
Deltapine 1050 B2RF	8.2	b
Croplan Genetics 3787 B2RF	8.2	b
PhytoGen PX 5403-01 WRF	8.0	b
PhytoGen 375 WRF	8.0	b
Deltapine 1252 B2RF	7.5	b
Deltapine MON 12R242 B2R2	7.0	b
PhytoGen PX 3122-40 WRF	7.0	b
PhytoGen PX 4444-14 WRF	6.7	b
Phytogen 575 WRF	6.0	b
Bayer ST 6448 GLB2	5.7	b
PhytoGen PX 4433-25 WRF	4.8	b
	SE: 2.72	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 13. Mean target leafspot disease severity ratings for 1st rating date of full season cultivars at the Tennessee Valley Substation, 2013.

Cultivar	TVREC Full Irrigated	
	Mean	
PhytoGen PX 4433-27 WRF	4.8	a
PhytoGen PX 3003-10 WRF	4.3	ab
Deltapine 1050 B2RF	4.0	ab
All-Tex Nitro 44B2RF	4.0	ab
PhytoGen 339 WRF	3.8	ab
Bayer ST 6448 GLB2	3.5	ab
PhytoGen PX 5540-10 WRF	3.3	ab
Deltapine 1252 B2RF	3.3	ab
PhytoGen PX 4444-13 WRF	3.3	ab
PhytoGen PX 5403-01 WRF	3.3	ab
Deltapine MON 12R242 B2R2	3.0	abd
Deltapine 1048 B2RF	2.8	abd
Americot 1511 B2RF	2.8	abd
Phytogen 575 WRF	2.8	abd
DynaGro 2610 B2RF	2.5	abd
PhytoGen PX 5538-40 WRF	2.3	abd
PhytoGen 375 WRF	2.0	bd
PhytoGen 499 WRF	2.0	bd
PhytoGen PX 3122-40 WRF	2.0	bd
Deltapine 1219 B2RF	2.0	bd
Americot NG 5315 B2RF	2.0	bd
Croplan Genetics 3787 B2RF	1.8	bd
Deltapine 1137 B2RF	1.5	d
PhytoGen PX 4444-14 WRF	0.5	d
PhytoGen PX 4433-27 WRF	0.5	d
	SE: 0.95	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 14. Mean target leafspot disease severity ratings for 2nd rating date of full season cultivars at the Tennessee Valley Substation, 2013.

Cultivar	TVREC Full Irrigated	
	Mean	
Deltapine 1050 B2RF	6.0	a
PhytoGen 575 WRF	5.5	a
PhytoGen PX 4433-27 WRF	5.5	a
PhytoGen PX 3003-10 WRF	5.5	a
PhytoGen 339 WRF	5.0	a
All-Tex Nitro 44B2RF	5.0	a
PhytoGen PX 5540-10 WRF	4.8	a
PhytoGen PX 5538-40 WRF	4.8	a
PhytoGen PX 4444-13 WRF	4.5	a
PhytoGen PX 4444-14 WRF	4.5	a
PhytoGen PX 4433-25 WRF	4.3	a
PhytoGen PX 5403-01 WRF	4.3	a
PhytoGen 499 WRF	4.0	a
Deltapine 1252 B2RF	4.0	a
DynaGro 2610 B2RF	4.0	a
Americot 1511 B2RF	4.0	a
Americot NG 5315 B2RF	4.0	a
Deltapine 1219 B2RF	3.8	a
PhytoGen 375 WRF	3.3	a
PhytoGen PX 3122-40 WRF	3.3	a
Bayer ST 6448 GLB2	3.3	a
Deltapine 1048 B2RF	3.2	a
Deltapine MON 12R242 B2R2	2.5	a
Deltapine 1137 B2RF	2.0	a
Croplan Genetics 3787 B2RF	2.0	a
	SE: 1.68	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 15. Mean target leafspot disease severity ratings for 1st rating date of full season cultivars at the Prattville Agricultural Research Unit, 2013

Cultivar	PF Full	
	Mean	
Deltapine 1050 B2RF	33.0	a
PhytoGen 375 WRF	32.5	a
PhytoGen PX 4444-13 WRF	32.5	a
PhytoGen PX 5538-40 WRF	32.5	a
PhytoGen PX 5540-10 WRF	31.3	a
PhytoGen PX 5403-01 WRF	31.3	a
DynaGro 2610 B2RF	30.0	ab
PhytoGen PX 4433-25 WRF	28.8	ab
Croplan Genetics 3787 B2RF	28.0	ab
Deltapine 1219 B2RF	27.5	ab
Deltapine 1137 B2RF	26.3	ab
PhytoGen PX 3003-10 WRF	26.3	ab
Deltapine 1048 B2RF	25.5	ab
PhytoGen 339 WRF	25.0	ab
Deltapine MON 12R242 B2R2	25.0	ab
Americot NG 5315 B2RF	24.3	ab
Bayer ST 6448 GLB2	23.8	ab
Americot 1511 B2RF	22.8	ab
PhytoGen PX 3122-40 WRF	21.8	ab
PhytoGen 499 WRF	20.0	ab
PhytoGen PX 4444-14 WRF	19.3	ab
Deltapine 1252 B2RF	17.5	ab
PhytoGen PX 4433-27 WRF	15.8	ab
Phytogen 575 WRF	15.0	ab
All-Tex Nitro 44B2RF	10.8	b
	SE: 7.64	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 16. Mean target leafspot disease severity ratings for 2nd rating date of full season cultivars at the Prattville Agricultural Research Unit, 2013

Cultivar	PF Full	
	Mean	
PhytoGen 339 WRF	48.0	a
DynaGro 2610 B2RF	45.5	ab
PhytoGen PX 5538-40 WRF	45.0	ab
All-Tex Nitro 44B2RF	44.5	ab
Deltapine 1050 B2RF	42.5	abc
PhytoGen PX 4433-25 WRF	42.5	abc
PhytoGen PX 3122-40 WRF	41.5	abc
Croplan Genetics 3787 B2RF	41.3	abc
Deltapine 1137 B2RF	40.5	abc
Deltapine MON 12R242 B2R2	40.5	abc
Americot NG 5315 B2RF	40.0	abc
PhytoGen PX 4433-27 WRF	40.0	abc
PhytoGen 375 WRF	38.3	abc
PhytoGen PX 5403-01 WRF	38.0	abc
Americot 1511 B2RF	37.5	abc
PhytoGen 499 WRF	36.8	abc
PhytoGen PX 4444-14 WRF	35.0	abc
PhytoGen PX 3003-10 WRF	35.0	abc
PhytoGen PX 5540-10 WRF	34.0	abc
Deltapine 1252 B2RF	30.8	bc
PhytoGen PX 4444-13 WRF	29.5	bc
Deltapine 1048 B2RF	28.8	bc
Deltapine 1219 B2RF	28.8	bc
Phytogen 575 WRF	26.3	c
Bayer ST 6448 GLB2	26.3	c
	SE: 9.13	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 17. Mean target leafspot disease severity ratings for 1st rating date of full season cultivars at the Plant Breeding Unit, 2013.

Cultivar	PBU Full	
	Mean	
PhytoGen PX 5538-40 WRF	45.0	a
PhytoGen 499 WRF	43.8	ab
PhytoGen PX 4433-27 WRF	35.8	ab
PhytoGen PX 3122-40 WRF	35.0	ab
Deltapine 1252 B2RF	30.8	abd
All-Tex Nitro 44B2RF	29.5	abd
Deltapine 1137 B2RF	28.8	abd
Deltapine 1219 B2RF	28.8	abd
Americot NG 5315 B2RF	28.8	abd
PhytoGen PX 4433-25 WRF	28.8	abd
PhytoGen PX 3003-10 WRF	28.8	abd
Americot 1511 B2RF	28.0	abd
Bayer ST 6448 GLB2	28.0	abd
Deltapine 1048 B2RF	26.3	bd
DynaGro 2610 B2RF	26.3	bd
PhytoGen PX 4444-13 WRF	25.8	bd
PhytoGen 375 WRF	23.8	d
Phytogen 575 WRF	23.8	d
Deltapine MON 12R242 B2R2	22.5	d
Croplan Genetics 3787 B2RF	20.0	d
PhytoGen PX 4444-14 WRF	20.0	d
Deltapine 1050 B2RF	18.3	d
PhytoGen PX 5540-10 WRF	17.5	d
PhytoGen PX 5403-01 WRF	15.3	d
PhytoGen 339 WRF	15.0	d
	SE: 6.52	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 18. Mean target leafspot disease severity ratings for 2nd rating date of full season cultivars at the Plant Breeding Unit Full, 2013

Cultivar	PBU Full	
	Mean	
PhytoGen PX 3122-40 WRF	53.8	
PhytoGen 499 WRF	52.5	
PhytoGen PX 5538-40 WRF	52.5	
PhytoGen PX 4433-27 WRF	45.0	b
PhytoGen PX 4433-25 WRF	43.8	b
PhytoGen PX 4444-14 WRF	43.8	b
Americot 1511 B2RF	42.5	bd
Deltapine 1252 B2RF	40.0	bd
Deltapine 1137 B2RF	38.8	bd
Phytogen 575 WRF	38.8	bd
Deltapine 1219 B2RF	38.8	bd
PhytoGen PX 5403-01 WRF	37.5	bdf
All-Tex Nitro 44B2RF	36.5	bdf
PhytoGen PX 4444-13 WRF	36.3	bdf
Americot NG 5315 B2RF	35.0	bdf
Deltapine MON 12R242 B2R2	35.0	bdf
Deltapine 1048 B2RF	34.5	bdf
PhytoGen PX 3003-10 WRF	33.0	df
PhytoGen 375 WRF	31.5	df
Bayer ST 6448 GLB2	31.3	df
DynaGro 2610 B2RF	31.0	df
Deltapine 1050 B2RF	30.0	f
Croplan Genetics 3787 B2RF	26.0	f
PhytoGen PX 5540-10 WRF	25.0	
PhytoGen 339 WRF	23.8	
	SE: 4.16	

Means followed by the same letter are not significantly different at α=0.05
Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 19. Mean target leafspot disease severity ratings for full season cultivars at the Gulf Coast Research and Extension Center, 2013.

Cultivar	GCREC Full	
	Rating 1	
PhytoGen PX 4444-14 WRF	3.0	a
Deltapine 1219 B2RF	2.9	ab
PhytoGen 339 WRF	2.8	ab
PhytoGen PX 5538-40 WRF	2.8	ab
Americot 1511 B2RF	2.6	ab
Phytogen 575 WRF	2.5	ab
Americot NG 5315 B2RF	2.3	ab
PhytoGen PX 5540-10 WRF	2.3	ab
PhytoGen PX 3003-10 WRF	2.1	ab
PhytoGen PX 5403-01 WRF	2.1	ab
Deltapine 1137 B2RF	2.0	ab
Croplan Genetics 3787 B2RF	2.0	ab
All-Tex Nitro 44B2RF	2.0	ab
Deltapine MON 12R242 B2R2	2.0	ab
PhytoGen 499 WRF	1.9	ab
PhytoGen PX 4433-25 WRF	1.9	ab
PhytoGen 375 WRF	1.8	ab
Deltapine 1050 B2RF	1.6	ab
Bayer ST 6448 GLB2	1.6	ab
PhytoGen PX 4433-27 WRF	1.5	ab
Deltapine 1252 B2RF	0.9	ab
DynaGro 2610 B2RF	0.7	ab
PhytoGen PX 3122-40 WRF	0.7	ab
Deltapine 1048 B2RF	0.6	b
PhytoGen PX 4444-13 WRF	0.6	b
	SE: 0.80	

No 2nd rating was taken for this date

Means followed by the same letter are not significantly different at α=0.05

Rating scale 0 = no lesions present on leaves and no defoliation; 1 to 20 = lesions noticeable in lower canopy, ≤ 20% of plant covered in lesions and/or ≤ 20% defoliation; 21 to 40 = lesions numerous in lower canopy reaching coverage of 20 to 40% of plant and/or 20 to 40% defoliation; 41 to 60 = lesions numerous in both upper and lower canopy and at least 41 to 60% defoliation; 61 to 80 = lesions very numerous in upper and lower canopy and/or 61 to 80% defoliation; 81 to 100= lesions present on majority of plant and/or 81 to 100% defoliation

Table 20. F-values and probability for significant difference in disease severity ratings for all 18 location x test combinations

		Rating 1			Rating 2	
Location and test	Date	F-value	*P*-value	Date	F-value	*P*-value
GCREC Early Season	8/8	0.98	0.51	†		
GCREC Full Season	8/8	0.76	0.77	†		
PBU Early Season	8/2	1.83	0.03	8/14	0.86	0.65
PBU Full Season	8/2	1.31	0.19	8/14	3.7	0.01
PARU Early Season	8/13	0.78	0.75	8/20	0.55	0.95
PARU Full Season	8/13	0.73	0.81	8/20	1.06	0.41
TVREC Early Season	7/30	2.29	0.01	8/15	0.84	0.68
TVREC Full Season	7/30	3.83	0.01	8/15	1.43	0.12
TVREC Early Season Irrigated	7/30	1.68	0.05	8/15	1.12	0.35
TVREC Full Season Irrigated	7/30	1.33	0.18	8/15	0.42	0.99

† No 2nd rating was done for these trials.

Table 21. Phenotypic correlation coefficients between greenhouse ratings and field ratings for 10 location x test combinations.

	Rating 1		Rating 2	
Location and test	r	*P*-value	r	*P*-value
GCREC Early Season	-0.16	0.44	†	†
GCREC Full Season	-0.08	0.71	†	†
PBU Early Season	-0.29	0.15	-0.28	0.18
PBU Full Season	-0.32	0.12	-0.18	0.40
PARU Early Season	0.46	0.02	-0.10	0.64
PARU Full Season	0.11	0.61	0.14	0.52
TVREC Early Season	-0.16	0.44	-0.16	0.46
TVREC Full Season	0.06	0.79	0.33	0.11
TVREC Early Season Irrigated	0.12	0.57	0.00	0.98
TVREC Full Season Irrigated	0.12	0.57	-0.14	0.50

† No 2nd rating was done for these trials.

Table 22. Phenotypic correlation between field target spot disease ratings and lint yield for early and full season trials across four locations

	Lint yield			
	Rating 1		Rating 2	
Test	r	*P*-value	r	*P*-value
Early Season	-0.28	0.0055	-0.68	<0.0001
Full Season	-0.24	0.0144	-0.59	<0.0001

Figure 1. Inoculum growth comparison of non-incubated vs. incubated plates

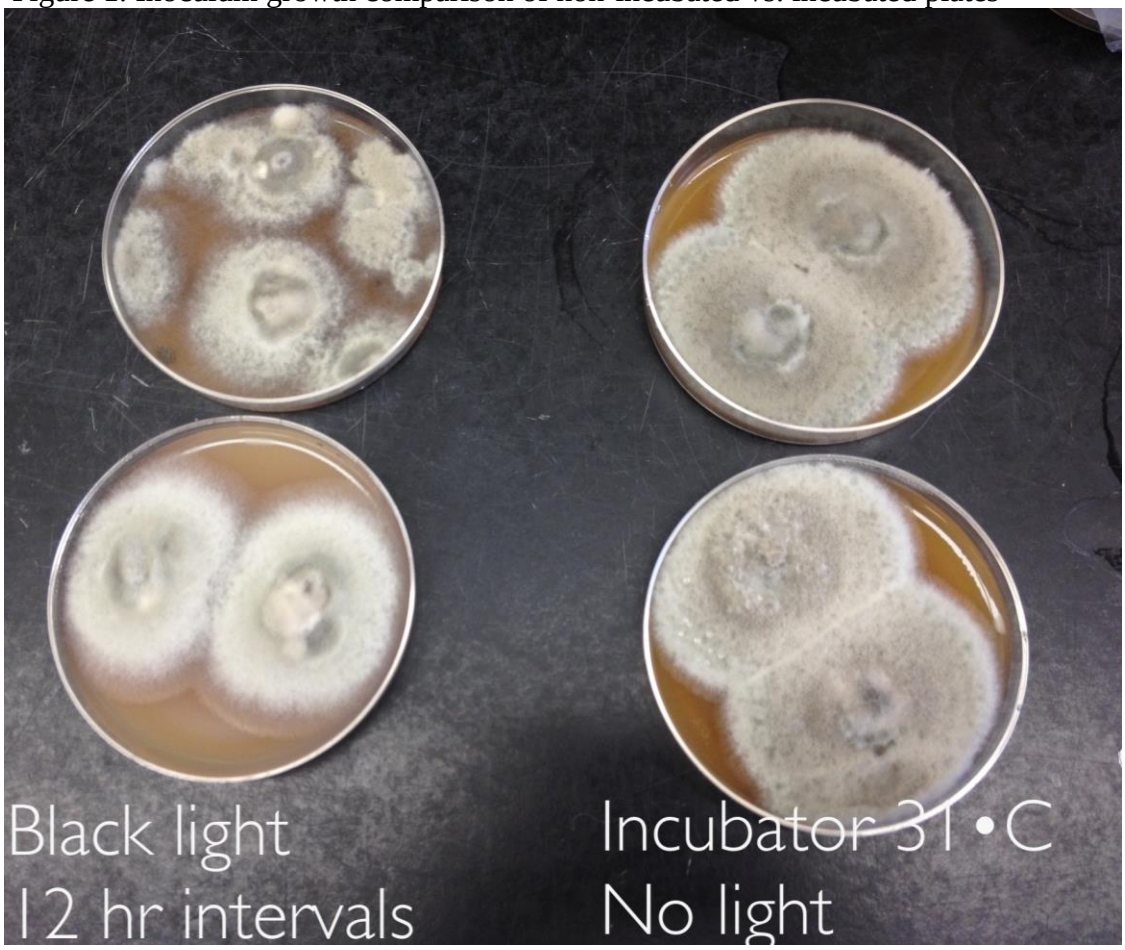

Figure 2. Spray and bagging method for inoculation in greenhouse

Figure 3. Target spot symptoms on cotton

Figure 4. Inoculated plants vs. a control in greenhouse

Figure 5. Field experiment design with corn as border at the Plant Breeding Unit in Tallassee