EVALUATION OF COTTON GERMPLASM (*GOSSYPIUM* SPP.) FOR RESISTANCE TO ENDEMIC DISEASES IN THE SOUTHEAST UNITED STATES

by

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(Under the Direction of Peng W. Chee)

ABSTRACT

Cotton (*Gossypium* spp.) is a globally important crop valued for both its lint fiber and seed, with significant economic impact in the United States. The *Gossypium* genus includes over fifty species, highlighting profound genetic diversity. Upland cotton, *G. hirsutum*, is the predominant species grown in the United States. In cotton breeding, phenotypic selection has heavily focused on traits like fiber quality and lint yield, which has led to a gradual decline in genetic diversity within cultivated gene pool. The decrease in genetic diversity has hampered breeding for disease resistance, emphasizing the need to incorporate diverse germplasm into breeding programs to maintain a robust cotton gene pool. This study evaluated a large diverse selection of germplasm for resistance to endemic diseases including fusarium wilt (*Fusarium oxysporum* f. sp. *vasinfectum)*, target spot (*Corynespora cassiicolla*), and areolate mildew (*Ramulariopsis* spp.). Multi-year screening identified valuable sources of disease resistance among elite breeding lines, obsolete varieties, and wild accessions. The results from this study offer new knowledge about cotton germplasm regarding sources of disease resistance in cotton,

providing cotton breeders tools to enhance cotton resilience, and discovering genes related to pathogen resistance.

INDEX WORDS: *Fusarium oxysporum* f. sp. *vasinfectum*, *Ramulariopsis*, *Corynespora*, cotton, fusarium wilt, areolate mildew, target spot, germplasm, host resistance, disease resistance, cotton breeding

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DEDICATION

To my wife Lydia, who has been crucial in my path to finishing this degree and has been my biggest support throughout my student career. To my four amazing children who have supported their father through the good, the bad, and loving me, nonetheless. I would like to thank my late grandmother Jean Beasley. A great woman, who survived World War II in England as a child, and immigrated to the United States to eventually be one of the biggest and profound influential people in my life. She ultimately is responsible for initiating my interest in plants and leading me to develop a special interest in plant pathology and plant breeding.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Cotton background, history, and production

Cotton (*Gossypium* spp.) is an important crop around the world, grown for its lint fiber and seed. Cotton belongs to the hibiscus family *Malvaceae*, which includes other crops such as okra and cacao (Wendel et al., 1992; Wendel et al., 2009). The cotton genus (*Gossypium L*.) has long been viewed as important because of its value to the world economy. This prompted many to understand the diversity and origin of cultivated cotton (Wendel et al., 2009). The domestication process involved four species, two of which were native to the Americas, and two to Africa-Asia. American species included *G. hirsutum* and *G. barbadense*, and African-Asian species comprised of *G. arboreum* and *G. herbaceum* (Wendel and Cronn 2003). Each species was domesticated mainly because of favorable physical characteristics selected by native people in each region. Although domestication involved four main species, the *Gossypium* genus has over fifty species of profound diversity, dating back to millions of years ago (Wendel et al., 1992; Fryxell, 1992; Wendell et al., 2009).

Over the last decade, world cotton production on average was 24.8 Mt (USDA Cotton Outlook, 2023-2024). In 2024, world production is expected to rise by 3% and U.S cotton production is expected to rise to 3.48 Mt, a considerable increase from 2023 production, which was 2.7 Mt (USDA Cotton Outlook, 2023-2024). Cotton is a main staple in agriculture in the southern United States and across the Cotton Belt. Cotton is grown in over 100 countries and

exported/imported to over 150 countries (USDA/FAS, 2024). The top producing countries include China, India, Brazil, and the United States (2023-2024 USDA Cotton Outlook).

Cotton fiber is processed into home textiles and clothes while the seeds are used to supplement feed for livestock. Cotton seed oil is also used in cosmetics, soap, used to cook chips, and found in salad dressing. Many uses are not associated with the normal consumer's perception of cotton lint, ranging from medical supplies, gun powder and X-ray film.

Breeding goals and essential traits

There are many traits associated with cotton production that are valuable. Importance of traits can depend on geographical area related to disease resistance, smaller markets such as cottonseed oil, other morphological features that increase picking ability, but the majority of cotton improvement can be directly related to lint fiber, which is where the bulk of the value resides. In cotton production, fiber quality is the second most important breeding objective after lint yield (Bowman, 2000). These two output traits increase revenue for growers as well as manufacturers (Wang and Memon, 2020). The third breeding objective for cotton breeding programs is improving host plant resistance (Bowman, 2000). Plant protection is one of the most significant input costs for cotton production, therefore resistant varieties, when available, are the best defense against many aggressive diseases affecting cotton. This is especially the case given that fungicide applications or fumigation achieves little efficacy against a plethora of pathogens that attack cotton. Through decades of breeding efforts, many resistant traits have been introgressed into modern cultivars including resistance or tolerance to diseases such as bacterial blight, fusarium wilt, root-knot nematode, verticillium wilt, and several viruses (Zhang and Boopathi, 2022).

Diversity and germplasm sources of *Gossypium*

Species from the *Gossypium* genus can be found nearly worldwide including regions of extensive diversity such as Australia, Africa, and Mexico. Species are grouped by genome (A-G, and K) and can be associated to each genome group by place of origin and morphological characteristics (Beasley, 1941). Geographical origin also designates four subgenera in association with genome groups (Fryxell, 1979; Fryxell,1992). Genomes of importance are group A from Africa or Asia and group D that is analogous to the American diploid species because they contributed to the American tetraploid species that were domesticated and are grown in commercial production today (Beasley, 1941). Speculation surrounds the actual formation of AD allopolyploid cottons, but cotton researchers generally agree that their parentage is from the living descendant of the A-genome species *G. arboreum* or *G. herbaceum* and the D-genome *G. raimondii* (Wendel and Cronn 2003).

There are only two cultivated allotetraploid cotton species that are recognized, *G. hirsutum* and *G. barbadense*. Among *G. hirsutum*, the taxonomic classification of this species includes several land race designations recognized in the breeding community (Lubbers et al., 2005; Lubbers et al., 2009, Zohary and Hopf 2000). They include ‘yucatanese’, ‘punctatum’, ‘palmeri’, ‘latifolium’, and ‘marie-galante’, with ‘latifolium’ being the most important because it is considered the source of the Upland cotton germplasm. (Lubbers et al., 2009). Upland cotton is grown in major producing areas within the United States including Texas, Georgia, Mississippi, Alabama, North Carolina, California, Arkansas, Missouri, Oklahoma, and Arizona. United states production ranks third globally behind China and India (USDA/ERS, 2022).

Upland cotton (*G. hirsutum)* accounts for approximately 97% of total United States cotton production, and Pima (*G. barbadense)* cotton less than 3% (USDA/ERS, 2022). Upland

cotton improvement primarily took place within the United States, particularly in region known as the Cotton Belt (Ware et al., 1951; Lubbers et al., 2009). The genetic diversity of Upland cotton is mainly derived from material originating from Mexico, which later was introduced into Texas. (Ware et al., 1951; Lubbers et al., 2009). Although many different characterized 'types' of Upland existed (the Eastern Big Boll type, Western Big Boll type, Semi-cluster type, Cluster type, Rio Grande type, Early type, Long Limb type, Upland Long Staple type, and the Intermediate or Miscellaneous type), the Western Big Boll contributes to most of the elite cultivars in modern United States production (Lubbers et al., 2009). The Western Big Boll germplasm was further improved by cotton seed companies, Stoneville and Deltapine. Acala Upland cotton, which was derived from genetics introduced from Guatemala and Mexico, also contributes important acreage within California, and south to New Mexico. It is known to be characterized by its excellent fiber quality. (Lubbers et al., 2005; Lubbers et al., 2009).

Pima cotton, although produced on a smaller scale, is still important because of its extra-long and strong fiber properties (Kerr et al., 1960). The modern cultivated *G. barbadense* can be traced completely or partially back to 'Sea Island cotton.' Sea Island cotton is comprised of mainly Egyptian germplasm and is described as having remarkably long fiber and superb quality (Percy et al. 2009). Improvements of Sea Island cotton was made primarily for fiber quality and disease resistance. *Fusarium oxysporum* f. sp. *vasinfectum* became an overwhelming issue in the mainland United States, causing observable symptoms, mortality, and yield loss. Given the need for host resistance to fusarium wilt, breeding for resistance was focused on heavily infested fields starting in the twentieth century (Smith et al., 1960; Jones et al., 1967; Hyer et al., 1979). Although exceptional in fiber quality, Sea Island cotton was low in production compared to Pima and Egyptian cotton (Percy et al., 2009). The attempt to achieve high quality with higher

production led the endeavor to introduce Egyptian cotton into the United States (Ware, 1936; Ware, 1952). Given the fact that Egyptian cotton germplasm was not fully adaptable to the United States, breeding efforts eventually led to what is considered the gene pool for American-Egyptian cotton, Pima S-1 and Hybrid-B gene pools (Percy et al., 2009; Feaster and Turcotte, 1962). The Pima S-1 gene pool was developed by crossing three founder *G. barbadense* germplasm lines with substantial diversity followed by introgression from *G. hirsutum* to improve productivity (Feaster and Turcotte, 1962). Pima S-1 was the gateway to Pima breeding in the United States with many future improvements.

Years of selection have ultimately narrowed the genetic diversity of elite cotton varieties. (Wendel et al., 1992; Wendel et al., 2009). Since the elite cotton gene pool has a low level of genetic diversity, many breeding programs resort to using obsolete varieties, germplasm lines, and wild or exotic germplasm (Abdurakhmonov et al., 2012). This is especially true regarding disease resistance improvement such as for FOV resistance. Most traits related to yield, fiber quality, and other agronomically important characteristics are available in elite germplasm, though slight improvements are made periodically.

Breeding methods in cotton

The current approach to cotton improvement can be attributed predominantly to traditional breeding methods as well as implementation of modern technologies including marker assisted breeding and sequencing. Knowledge from classical cytogenetic and quantitative genetic research, dating back to the mid- 19th century, has provided the foundation for understanding the inheritance of both simple and complex traits (Chee and Campbell., 2009). Phenotypic selection of desired traits, mainly oriented around fiber quality and lint yields, has driven much of the improvement of cotton (Bridge et al., 1971; Hoskinson and Stewart 1977; Bridge and Meredith

1983; Bassett and Hyer 1985; Zhang et al., 2005, Chee et al., 2005a, Chee et al., 2005b; Campbell et al., 2008). This in return has significantly decreased genetic variation throughout the last century (Chee and Campbell., 2009). Advancements in molecular genetics and biotechnology have integrated modern breeding techniques, such as using DNA markers, mapping populations, and genetic linkage maps, to help locate the positions of quantitative trait loci (QTL's) and measure their effects on traits like fiber quality, disease resistance, and abiotic stress tolerance. Genetic research is also aimed at understanding the cotton genome of cultivated allotetraploid species (Sreedasyam et al. 2024). Since QTL mapping is performed routinely as a tool to identify genomic regions that are responsible for important traits, discussion of how this method is used for potential cotton improvement is important. QTL mapping is not necessarily a breeding method, but it is a critical component of what influences modern cotton breeding and can aid in traditional breeding schemes (Ulloa et al, 2011; Li et al., 2018).

Native diseases of importance

Fusarium oxysporum* f. sp. *vasinfectum

Fusarium wilt is a serious disease of cotton in all production areas around the world. It can cause severe damage to all domesticated cotton species and has the potential to create severe yield loss in commercially grown cotton. The causal agent of fusarium wilt of cotton is *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) (Atkinson, 1892; Armstrong and Armstrong 1958). This pathogen is a complex and diverse organism that varies in virulence and pathogenicity. Since the causal agent is a soilborne pathogen, fusarium wilt is typically observed in aggregated patterns. Initial foci of fusarium incidence increase consistently from year to year after infestation occurs. This can vary depending on crop rotation, alternate host weed species, cultural practices, soil nutrition, sanitation methods, and pesticide/fumigant treatments (Davis et al., 2006). Once FOV

is present, it is impossible to eliminate, but it is possible to be managed. FOV can potentially disseminate directly or indirectly through various means, including unsanitary equipment, old plant debris, irrigation, and infested seed. Management practices vary depending on the race of FOV responsible for the disease (Davis et al., 2006). Several races of FOV have been identified as causing disease in cotton, including some that are more aggressive when associated with nematodes, while others can cause significant disease in absence of nematodes (Holmes et al., 2009; Cianchetta et al., 2015). Since the predominant FOV races found in United States cotton growing areas are associated with nematodes, much of the control tactics used historically focused heavily on nematode control and not necessarily on FOV singularly (Hillocks, 1984; Shepard and Kappelman; 1986).

New research has indicated that controlling nematodes alone cannot be facilitated as a solution in the presence of aggressive FOV infection (Wheeler et al., 2022). The severity of fusarium wilt can be directly correlated to inoculum density, which in return can be correlated with genetic diversity of the strain, ideal environmental conditions, or both (DeVay et al., 1997). To control FOV, an integrated approach that combines several management practices is very important. In most integrated pest management strategies, resistant varieties are at the forefront. (Cianchetta and Davis 2015). Since pesticide applications have marginal efficacy on FOV and are generally only suppressive to the FOV/nematode complex, the need for integrating resistance to FOV in elite cotton germplasm is crucial.

Predominate Races of FOV effecting Upland Cotton Production

In plant pathology, the term race characterizes the pathogen's capacity to cause disease to a specific genotype within a species, generally related to some level of genetic resistance (Anderson et al., 2020). However, the designation of race to describe FOV in cotton does not

follow the typical and historical definition used in most pathogen systems (Halpern et al., 2020). Race designation for FOV essentially characterizes the consecutive order it was discovered and distinguishes pathogenicity to other crops such as tobacco, soybean, and okra. Fusarium wilt was first discovered and documented in 1892, with the first genotype "Race 1" documented in the United States in 1958 (Atkinson, 1892; Armstrong and Armstrong 1958).

According to sample surveys through previous research endeavors, Race 1 is the predominate race causing significant disease in Upland cotton (Holmes et al., 2009; Cianchetta et al., 2015, da Silva et al. 2019a). Races that have been identified over the years include races 1 through 8; however, recent genetic evaluations indicate redundancy in race designation. For example, races 2 and 6, 3 and 5, and 4 and 7 are indistinguishable based on DNA sequence analysis (Nirenberg et al., 1994; Hering et al., 1999; Skovgaard et al., 2001). FOV Race 4 is of great importance because of its ability to cause disease in cotton without nematode association and in 'heavier' soil types such as clay or loam. New genotypes have been discovered in the Southeast United States (Holmes et al., 2009; Cianchetta et al., 2015), but race 4 has not been identified outside of Texas, New Mexico, and California (Kim et al., 2005; Halpern et al., 2018; Zhu et al., 2020). It is still of concern for the potential possibility of race 4 moving to other cotton growing regions by infested seed or plant material. Other variables to consider include recent reports from da Silva et al. (2019a) which suggests that *Meloidogyne incognita* (Southern root-knot nematode) is not the only nematode associated with fusarium wilt incidence in Georgia. While *Meloidogyne incognita* have historically been associated with fusarium wilt in Georgia, the new survey data suggests that *Belonolaimus longicaudatus* (Sting nematode) is also associated with the disease at a higher rate than previously thought (da Silva et al. 2019a). Given the prevalence of sting nematode in sampling, earlier control methods such as nematicides and

varieties with root-knot nematode resistance may not be enough for suppression of native FOV in Georgia. Sting nematode, being an extremely aggressive and mobile ectoparasitic nematode, control methods should be more directly focused on controlling fusarium wilt. Resistant varieties to the FOV pathogen, regardless of its race or genetic characterization, are key to management in the future.

FOV Native Race Structure in Georgia

Survey work conducted by da Silva et al. (2019a) aimed to understand the distribution of FOV and the predominant race structure relevant to cotton farms in Georgia. In the study, samples were taken during 2015 and 2016 from 27 fields in a total of 10 counties throughout the cotton growing region. From those fields, 10 plant samples were taken exhibiting fusarium wilt symptoms. Soil samples were also taken during the season to quantify the relationship of plant parasitic nematodes and FOV observations. The results showed that FOV race 1 was the dominant race, but other genotypes were also present including LA 110, LA 108, MDS 12, LA 127, and LA 140. Other surveys conducted have also concluded that common races in Georgia include FOV race 1, 2, and 8 (Holmes et al., 2009; Cianchetta et al., 2015). FOV4 (Race 4) is impactful given its ability to cause disease in absence of nematode interaction, but currently has not been identified in Georgia. This is still a concern and could be a potential threat in the future.

Target Spot (*Corynespora cassiicola*)

Target spot caused by *Corynespora cassiicola* (Berk. & Curt.) was first identified in the southeastern United States in Alabama in 1959 on Upland cotton (Jones, 1961). *C. cassiicola* causes lesions on cotton foliage that typically first appear in lower canopy. Spores spread to the lower canopy by wind or rain/irrigation splash from previous crop debris. The disease favors

moderate temperatures (25-30°C), long periods of leaf wetness, and high humidity. Lesions will be light to dark brown concentric rings, which is why the common name "target spot" is used to distinguish this foliar disease. As infection increases premature defoliation will follow resulting in yield loss. Target spot was not considered a disease of concern until it appeared in commercial fields recently. First reports within the United States include Georgia (Fulmer et al., 2012), Alabama (Conner et al., 2013) Arkansas (Faske, 2013), North Carolina (Edmisten, 2012), Virginia (Mehl and Phipps, 2013), Louisiana (Price et al., 2015), Tennessee (Butler et al., 2016), Mississippi (Shultz et al., 2017), and Florida (Sumabat et al., 2018). Other occurrences of the disease were also documented in Brazil and China (Galbieri et al., 2014; Wei et al., 2014).

Control of Target Spot

Target spot yield loss and defoliation can be extremely variable because of variety differences, morphological differences in cotton canopy, and weather (Bowen et al., 2018). Because of this variability, growers have a challenging time deciding on timely fungicide applications. A study by Hagen et al. (2015) estimated yield loss as high as 448 kg lint/ha when cotton was not treated properly for target spot. Other research suggests that one, and in some situations two applications, will provide a yield response. One application at onset of target spot could potentially increase yield by 4 to 6 % (Mehl et al., 2017; Bowen et al., 2018). Fungicides are used primarily for control of target spot including active ingredients such as azoxystrobin, pyraclostrobin, pyraclostrobin + fluxapyroxad, azoxystrobin + benzobendiflupyr, azoxystrobin + difenconazole, difenconzole + pydiflumetofen, flutriafol, flutriafol + azoxystrobin, and prothioconazole. Given that *C. cassiicola* is classified as a pathogen with high risk for fungicide resistance development (FRAC, 2019), fungicides may not always be the best control method for future management of target spot. Other cropping systems such as tomato and soybean have

reported Quinone outside inhibitor (QoIs/strobilurins) resistance in *C. cassiicola* (MacKenzie et al., 2020; de Mello et al., 2021). Since QoIs are primarily used for target spot management in cotton, the future of maintaining efficacy in this class of fungicide is doubtful.

Genetic Resistance to Target Spot in *Gossypium* spp.

Definitive genetic resistance has not been identified in commercial cotton germplasm, but there have been noticeable differences in susceptibility to target spot. A study by Hagen et al. (2015) showed differences in certain commercial varieties where PhytoGen brand PHY 499 WRF (PHY 499; PhytoGen Cottonseed; Dow AgroSciences, Indianapolis, IN), had an average 448 kg of lint/ha loss and Deltapine brand DP 1252 B2RF (Deltapine Cottonseed; Bayer, St. Louis, MO), had losses of 269 kg of lint/ha compared with nontreated controls. It is imperative that more evaluations are performed to identify germplasm sources for resistance to target spot to be included in grower disease management practices.

Areolate Mildew (*Ramulariopsis gossypii and Ramulariopsis pseudoglycines*)

Areolate Mildew [*Ramulariopsis areola Atk., Ramulariopsis gossypii (Speg.)*] sometimes referred to as grey mildew or Ramularia leaf spot, has become an extremely important disease of Upland cotton. This disease was first reported in Auburn, AL, United States in 1890, and has been identified around the world affecting all four commercially grown cotton species including *G. arboreum, G. herbaceum, G. hirsutum* and *G. barbadense* (Atkinson, 1890; Bell, 1981). Areolate mildew in the United States was considered a minor issue until recently. It has increasingly become a disease managed aggressively by growers because of potential yield loss. Areolate mildew has historically and progressively been a considerable threat to cotton production in Brazil, India, and East Africa. In Brazil, the predominate pathogen species causing

areolate mildew has been *Ramulariopsis pseudoglycines*, which has also been recently discovered in Mississippi, USA (Conner et al., 2023). Areolate mildew is a polycyclic disease that can cause multiple cycles of infection during a growing season, while also surviving on dead organic matter from the host throughout the winter (Watkins et al., 1981; Johnson et al., 2013). Inoculum can be disseminated in various fashions including wind, rain, and mechanical transmission. Disease progression is favored by high relative humidity, frequent rainfall, and temperature ranging from 16-30°C (Johnson et al., 2013). In the initial phase of infection, disease symptoms include light green to yellow lesions that appear on the upper surface of the leaf in the lower canopy. These lesions eventually develop white, powdery sporulation that are confined within leaf veins and have an irregular, angular appearance like other diseases such as bacterial blight (Bell, 1981). Eventually, the pathogen defoliates the plant prematurely, which equates to yield loss and fiber quality issues (da Silva et al., 2019b).

Areolate Mildew Control

Currently, control methods within cotton growing regions in the United States consists strictly of crop rotation and fungicide use. Since areolate mildew has only become an issue recently, much of the methodology behind fungicide use and timing is still being evaluated. Current fungicide recommendations in Georgia encourage applications between the first and sixth week of bloom depending on pathogen onset (Kemerait, 2021). In contrast to the United States, Brazil currently uses six to eight applications of several fungicides including chemistries with efficacy such as pyraclostrobin +fluxapiroxade, carboxamide + copper oxychloride,trifloxystrobin + prothioconazole + bixafen, azoxystrobin +difenoconazole + chlorothalonil, fentin hydroxide,mefentrifluconazole, isofetamid, chlorothalonil, azoxystrobin+ difenoconazole, mancozeb, chlorothalonil, and piraclostrobin + metconazole (da Silva et al.,

2019b). Much of the concern around fungicide use is losing effectiveness and reducing sensitivity to certain fungicide modes of action. Mathioni et al. (2022) showed that the CYTB-G143A substitution was present in all 165 isolates evaluated. This substitution is associated with reduced sensitivity or resistance to quinone outside inhibitors (QoIs or commonly known as strobilurins, FRAC 11). A survey in Brazil has also confirmed that the predominant species in Brazil is *Ramulariopsis psuedoglycines*, which carries the CYTB-G143A substitution (da Silva et al., 2023). In the United States, azoxystrobin is a predominate chemistry used for areolate mildew control. The possibility of losing this chemistry could be detrimental economically and holistically to how growers manage this disease in United States cotton production.

Genetic Resistance to Areolate Mildew in *Gossypium* spp.

In any disease management program, a resistant variety should be the first line of defense against a diverse pathogen that can be economically devastating. *Gossypium* species (*G. hirsutum, G. barbadense and G. arboreum*) have been documented to have varying levels of resistance to areolate mildew (da Silva et al., 2019b). Observations in India and Brazil have shown variance in pathogen aggression, proving a difference of virulence in *Ramulariopsis pseudoglycines* strains (Rathaiah, 1976, Pezenti et al., 2013). This is important to note, considering that pathogen diversity could correlate with geography as well as germplasm acceptable for that region. There has been much work done in India and Brazil to understand germplasm resistance to areolate mildew (Dake and Kannan 1982; Chauhan 1983; Sharma et al. 1986; Ascari et al., 2016). Most of the resistance identified is quantitatively inherited, which results in varying levels of resistance to multiple biotypes of the pathogen. There has not been any study to investigate germplasm resistance in North America, although differences among commercial varieties are apparent.

Justification

The overall focus of this research is to better understand the disease resistance available in cotton germplasm that provides opportunities for pre-breeding resistance to native diseases that are predominant in the southeastern United States and possibly improve the knowledge of pathogen diversity in relation to host resistance. The specific objectives of this research include: (i) evaluating diverse cotton germplasm lines for resistance to fusarium wilt; (ii) evaluating diverse cotton germplasm lines for resistance to areolate mildew and target spot; and (iii) releasing germplasm for use in future breeding efforts.

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CHAPTER 2

EVALUATION OF DISEASE RESISTANCE IN COTTON GERMPLASM TO FUSARIUM WILT[1]

[1] Beasley, E. D., Lubbers, E., Suassuna, N. D., Jones, D. C., Kemerait, R., & Chee, P. W. To be submitted to a peer-reviewed journal.

Abstract

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) poses a significant threat to cotton production, particularly in regions with high pathogen pressure. This study screened 430 cotton accessions, including elite lines from University of Georgia and University of New Mexico, obsolete cultivars, and wild accessions from the USDA National Cotton Germplasm Collection for resistance to FOV across four years (2018-2021). Trials were conducted in a commercial field in Brookfield, Georgia under natural fusarium wilt infestation to assess disease incidence. Germplasm lines were classified into six resistance categories based on disease severity, ranging from highly resistant (0% incidence) to highly susceptible (60-100% incidence). Results indicated germplasm such as TX 0195-1, GA 2016090, TX 2369, GA 2016110, and TX 2322 exhibited consistent resistance, with 32-6 (a selection from GA 2016090) demonstrating the lowest average disease incidence across multiple trials. The result from this study provides valuable information related to the spectrum of fusarium wilt resistance among a very diverse group of cotton germplasm. This information has potential for breeding programs aimed at developing cotton cultivars with improved resistance to fusarium wilt. Benefits of this research also include utilizing the identification of susceptibility among germplasm to disregard from further testing in the future.

Introduction

Cotton (*Gossypium* spp.) is primarily grown for its lint fiber and is considered the most important fiber crop due to its significant value to the global economy. Cotton is grown in over 100 countries and exported/imported to over 150 countries (USDA/FAS, 2024). The top producing countries include China, India, Brazil, and the United States (USDA/FAS, 2024). In 2023, the leading cotton lint producers and exporters have been the United States, Brazil, and

Australia and their exports reached 29%, 26 %, and 13%, respectively. The top importers were China, Vietnam, and Bangladesh, which imported around 61% of world cotton trade during the same period (2023-2024 USDA Cotton Outlook). In the United States, cotton is a main staple in agriculture in the southern states from Virginia to California and from Kansas to the lower Rio Grande Valley of Texas, with production in 2023 estimated at 2.7 million Mt (12.4 million bales of 480 lbs.) (2023-2024 USDA Cotton Outlook). Cotton production is extremely important to the United States economy given that it is the largest cotton exporter globally. The United States cotton sector connects domestic farmers to international markets, fostering both agricultural and economic growth (2023-2024 USDA Cotton Outlook).

The *Gossypium* genus has over 50 known species that can be found worldwide in areas of diversity including Australia, Africa, and Mexico (Wendel et al., 1992; Fryxell, 1992; Wendel et al., 2009; Wendel and Grover, 2015). Among the known germplasm exists an extensive array of morphological diversity with varying genome sizes (Hendrix and Stewart, 2005; Wendel and Grover, 2015). Species are grouped by genome (A-G, and K) and can be associated to each genome group by place of origin and morphological characteristics. (Beasley, 1941). Geographical origin also designates 4 subgenera in association with genome groups (Fryxell, 1992). Genomes that are important to the improvement of commercial cotton are group A indigenous to Africa and Asia, and group D that is native to the Americas. These genomes contributed to the two domesticated tetraploids AD genome species currently used in commercial production: *Gossypium hirsutum* L., commonly known as Upland cotton, and *Gossypium barbadense L.*, which is known as Pima, Sea Island, and Egyptian cotton. Upland cotton (*G. hirsutum)* accounts for approximately 97% of total United States cotton production, and Pima (*G. barbadense)* cotton less than 3% (USDA/ERS, 2022).

Fusarium wilt (FW), first discovered and documented in 1892, is a major disease affecting cotton production worldwide, resulting in significant yield losses in commercially grown cotton (Atkinson, 1892). The disease is caused by *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), a complex soilborne pathogen with high variability in virulence and pathogenicity (Armstrong and Armstrong, 1958). The severity of the wilt disease is often exacerbated by the presence of parasitic nematodes such as the southern root-knot nematode (*Meloidogyne incognita*), which form a FOV-nematode complex (Ridgway et al., 1984). Due to its soilborne nature, FW typically appears in aggregated patterns, with the disease expanding progressively each year after initial infestation, often spreading through contaminated equipment, plant debris, irrigation, and infested seed. The spread of FW is influenced by various factors such as crop rotation, alternate weed hosts, cultural practices, soil nutrition, sanitation methods, and pesticide treatments (Davis et al., 2006). Once FOV is established, eradication is impossible, but it can be managed through appropriate practices, depending on the specific race or genotype of FOV causing the infection (Davis et al., 2006).

Race designation for FOV was established based on pathogenicity to other crops such as tobacco, soybean, and okra. Eight races, designated as race 1 through 8, have been identified as infecting cotton; however, recent genetic evaluations suggest redundancy in these race designations (Wagner et al., 2022). For example, races 2 and 6, 3 and 5, and 4 and 7 are indistinguishable based on DNA sequence analysis (Skovgaard et al., 2001). While race 1 is the predominate race causing significant disease in Upland cotton in the U.S., race 4 is of great concern due to its potential to spread beyond its current range in California, Texas, and New Mexico. It could be introduced to other cotton-growing regions via infested seed or plant material and cause significant damage even in the absence of nematodes (Holmes et al., 2009;

Cianchetta et al., 2015). New genotypes have also been discovered in the Southeast United States, but very little is known about their pathogenicity (da Silva et al. 2019).

Historically, control methods for fusarium wilt have focused heavily on managing nematodes (Ridgway et al., 1984), but recent research indicates that nematode control alone is insufficient when dealing with aggressive FOV infections. For example, Wheeler et al. (2022) showed that a cultivar can have excellent resistance to root-knot nematode (RKN) and yet be susceptible to FOV. Further, the severity of FW can be directly related to inoculum density, as well as shifts in genetic diversity of FOV (DeVay et al., 1997). In the survey conducted by da Silva et al. (2019), it was demonstrated that RKN is not the only prominent nematode associated with FW incidence in Georgia. The study indicates that *Belonolaimus longicaudatus* (Sting nematode) may play a similar dominant role, comparable to RKN, in the severity of FW. Since pesticide applications provide only limited suppression of the FOV-nematode complex, effective management of FOV requires an approach with resistant cotton varieties being an initial factor.

Many current elite cultivars were bred entirely for nematode resistance but not FOV resistance (Wheeler et al. 2022). To improve our understanding of FOV resistance in cotton, it is essential to evaluate new germplasm lines from diverse genetic backgrounds. The objective of this study was to evaluate a range of germplasm, including elite public breeding lines, obsolete cultivars, and wild accessions, to better characterize host plant resistance to FOV within cotton germplasm. By understanding both resistant and susceptible sources, we can advance durable resistance for future breeding endeavors in the public breeding sector.

Materials and Methods

Cotton genotypes and evaluation site

A diverse collection of 433 cotton genotypes were screened for resistance to fusarium wilt, including 264 from the unimproved or wild accessions from the USDA National Cotton Germplasm Collection in College Station, Texas, 117 from obsolete cultivars and 49 public breeding lines from New Mexico and Georgia. The trial included a susceptible check, Rowden, a resistant check, M-315, and an elite commercial cultivar, Deltapine 1646. Additional checks were added throughout the study to serve as 'running' checks to identify disease presence.

The experimental site was a commercial grower's field with a history of high fusarium wilt incidence located in Brookfield, Georgia, about 8 miles southeast of Tifton (da Silva et al. 2019a). This field was managed with normal cropping rotation practices consistent with row crop production in Georgia, having a two-year cotton, one-year peanut rotation. Approximately four acres of land was selected within the field to create a fusarium wilt nursery, which was divided into four blocks to accommodate four replications of each experimental unit (Fig. 2.1). The experimental design was a Randomized Complete Block Design (RCBD). Each plot consisted of single rows measuring 9.144 meters in length and 0.9144 meters in width, replicated once in each block.

Preliminary disease Evaluations under field conditions

In 2018, a large diverse group of germplasm lines were screened to identify sources of resistance to be further evaluated in subsequent years. The experiment was planted on July 11, and evaluation of disease symptoms began with an initial stand count 10 days after planting. The incidence of FW was recorded over time by counting infected or dead plants (Fig. 2.2). Three

subsequent disease assessments were conducted on August 8th, August 22nd, and September 5th. The percentage of incidence for each

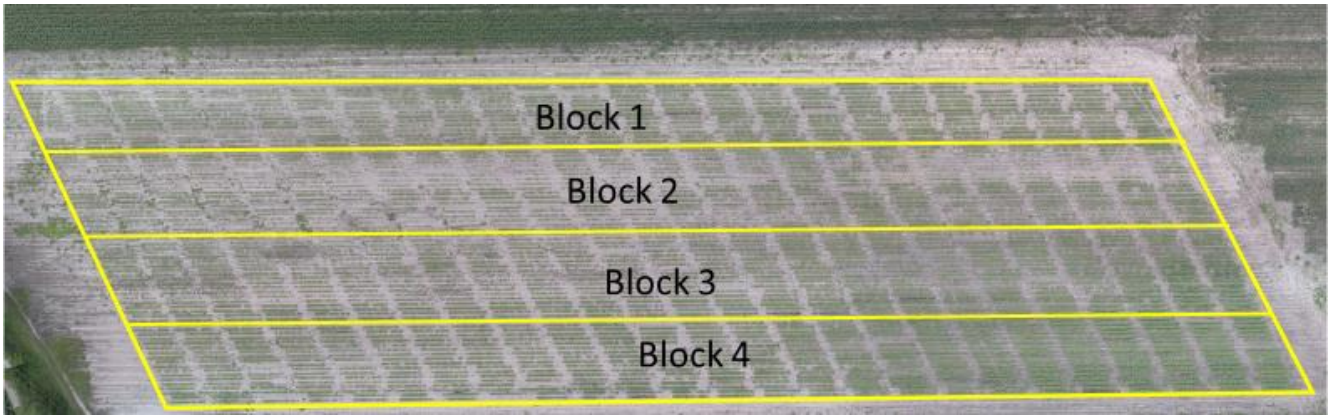

Fig. 2.1- Aerial photograph of plot design and disease incidence, 2018**.**

plot was calculated after the last assessment by dividing dead and diseased plants by total number of plants in each plot and multiplying the total by 100. Vascular discoloration, which indicates the presence of FOV and correlates with host resistance, was also assessed in genotypes that exhibited 15% incidence or less above ground symptoms of FW. For these genotypes, incidence for vascular discoloration was taken by cutting at least 10 plants at the soil level and approximately six inches above the base of the plant (Fig 2.3).

Germplasm lines were separated into six categories based on the percentage of disease incidence: highly resistant (0%), resistant (0≥2.5%), moderately resistant (2.5≥ 10%), moderately susceptible (10≥25%), susceptible (25≥60%), and highly susceptible (60≥100%). In 2018, Block 2 had significantly more disease pressure compared to other replications, providing better classification of resistance

Fig 2.2- Fusarium wilt symptoms of diseased (A) and (B) dead plants.

Figure 2.3- Vascular discoloration of cotton stem with severe discoloration (**A**) and mild discoloration (**B**).

designation. Resistance levels were determined by final disease incidence percentage per plot (Table 2.1).

Since germination was an issue among select germplasm lines, observations of each line varied from no observation to four across all four blocks. A Kruskal-Wallis Test was used to rank all genotypes using average means of disease incidence given the variability of assessments made for each line. One genotype, Wilt Wonder Wannamaker, did not germinate in any plot and no assessment was made for this genotype. Nematode samples were taken in each plot to differentiate areas of higher disease incidence, but no threshold numbers of significance were identified.

Validation of FW disease incidence

From 2019 to 2021, field trials were conducted to validate the disease incidence classifications for selected genotypes identified in 2018 and to select lines with high resistance to FW along with superior agronomic traits. The trial was conducted in the same fusarium wilt nursery in the specific area where Block 2 was located the 2018 trial. A total of 31 genotypes were selected in the test, with Rowden and M-315 serving as susceptible and resistant check, respectively. The experimental design was a Randomized Complete Block Design (RCBD), with six replications planted in 2019 and four replications in 2020 and 2021. In 2019, the planting date was June 24, and an initial stand count 10 days after planting and followed by assessments on July 17th, August 23rd, and September 23rd. Single-plant selections were made from genotypes displaying low disease incidence as well as favorable agronomic characteristics. The seeds from selected plants were used in the following planting season. In 2020, the planting date was June 17, with an initial stand count 10 days after planting followed by assessments on July 9th, July 23rd, and August 10th. Rowden was not included in the 2020 evaluation due to seed

supply shortages. In 2021, the same lines evaluated in 2020 were planted with the addition of Rowden. The planting date was May 27th, with an initial stand count 13 days after planting, followed by assessments on June 23rd, July 15th, August 5th, and August 26th.

Statistical analysis

Statistical analyses were conducted using JMP®, Version 17.0 (SAS Institute Inc., Cary, NC, 1989–2023). A one-way ANOVA with blocking was performed, and a Student's-t test was used for multiple comparison of mean disease ratings to determine significant differences between genotypes over the four years. A p-value of 0.05 was used to evaluate significance. For the 2018 data set, final severity assessments were used to rank the resistance of each genotype. In 2019-2021, the same ANOVA procedure was used using the final severity assessment. In 2018, a Kruskal-Wallis Test was also needed to rank germplasm to compare unbalanced observations.

Results

Disease ratings for germplasm classification were used from Block 2 for ranking resistance due to disease incidence being higher and significant in comparison to the other blocks. The preliminary ratings in the 2018 trial concluded that 29 genotypes were classified as highly resistant, 17 as resistant, 95 as moderately resistant, 114 as moderately susceptible, 117 as susceptible, and 50 as highly susceptible (Table 2.1). Rowden was used as the susceptible check and averaged 33.33% disease incidence, whereas M-315, the resistant check averaged 5.18%. Of the 29 genotypes classified as highly resistant, exhibiting 0% disease incidence, seven (24%) were from elite lines, 13 (45 %) were from obsolete lines, and nine (31%) were wild type lines from the Cotton Germplasm collection in College Station, TX. These genotypes were DELTAPINE 55 (652-679), TX 1409-2, ACALA 1517D, TX 2321, HYC79-6, TX 0202, DUNN

400, SA-1643XNM 12Y1004, CHACO 510 INTA, GA 2015046, TX 0616-3, LOCKETT 77, MD 15, TX 1171-1, DELCOT 277, TX 1151, NM 12Y1004, TIDEWATER #5 (G. BARB. X G. HIR.), TX 0373, TIDEWATER 29, TERRA 207, FJA, SA1643xNM12Y1005, TX 2357, GA 2016090, GA 2016110, GA 2017138, HART, and TX 0135-2. The resistant lines category, which has less than 2.5% disease incidence, contained 17 genotypes including 9 (53 %) from elite lines, three (18%) from obsolete lines, and 4 (24%) from the wild lines. These genotypes were CAHUGLBBCS-1-88, DELTAPINE 26, AUBURN 623 RNR, CD3HCHULBH-1-88, REBA 288, SEABROOK SEA ISLAND 12B2, AUBURN BR-1, DELCOT 311, TX-2359, NM 12Y1004XSA1177, NM 13P1121, DP 1646 (CHECK), DELTAPINE 1646, TX-0043, DUNN HS 120, ACALA 1517 WILT, and TX-2369. The moderately resistant category, with disease incidence between 2.5% $\geq$10%, consisted of 95 genotypes with 15 (16%) elite lines, 27 (28%) obsolete lines, and 53 (56%) wild lines. The remaining 280 (67% of total lines) genotypes screened in 2018 fell in the moderately susceptible, susceptible, and highly susceptible categories (Table 2.1). Eleven lines were not evaluated due to limited observations with no observations located in Block 2.

In the 2019 growing season, 13 highly resistant, 5 resistant, 3 moderately resistant, 5 highly susceptible, and 5 susceptible representative genotypes were selected for further evaluation (Table 2.2). Table 2.7 provides germplasm tested for 2018-2021 which varies dependent on seed supply. Table 2.3 shows the mean disease incidence for the 2019 trial. Rowden, the susceptible check had an unexpected disease incidence of 4.95%, whereas the resistant check M- 315 had incidence of 5.88% (Table 2.3). TX 2359 exhibited the highest disease incidence, with disease incidence of 55.83%, while TX 0616-3 showed similar incidence at 42.08%. TX 2383 had observed incidence at 37.49% and TX 0064 with 26.73%.

Table 2.1. 2018 block 2 classification of germplasm by diseases incidence (%)

Fusarium Wilt Incidence (0-100%)						
Genotypes	***(0%) HR****	***(0>2.5%) R***	***(2.5≥10%) MR***	***(10≥25%) MS***	***(25≥60%) S***	***(60≥100%) HS***
Wild/Unimproved (TX)	9	3	53	73	83	43
Elite	7	4	15	15	8	2
Obsolete	13	10	27	26	26	5

* Highly resistant=severity =0%, Resistant= (Severity 0>2.5%), Moderately Resistant= (2.5≥ 10%), Moderately Susceptible= (10≥25%), Susceptible= (25≥60%), and Highly Susceptible= (60≥100%).

Several lines, such as TX 1094, TX 0202-1, TX 2320, TX 1308, TX 1233, TX 2420-1, TX 0122-3, and GA 2017138 had disease incidence that ranged from 16.86% to 13.53%, respectively. Germplasm lines TX 2322, TX 1210-2, TX 2316, TIDEWATER #5, DELTAPINE 55, TX 0135-2, GA 2016 110, TX 1171-1, TX 0043, TX 0141-2, TX 2369, TX 1409-2, DELCOT 277, FJA, GA 2016 090, TX 0762-2, TX 0195-1, GA 2015046, and TX 2375 had disease incidence between 12.73% and 0%. Given the lack of disease uniformity it was decided to screen germplasm again in 2020 and 2021. In 2019, single plant selections were made from several favorable lines that had lower disease incidence consistently from 2018 and 2019 data (Table 2.4). Single plant selections were evaluated in 2020 and 2021.

Table 2.5 shows the mean disease incidence for the 2020 trial. M-315, the resistant check, had an incidence rating of 2.9%. TX-2359 had mean disease incidence of 11.40%, GA 2017 138 with 9.97%, and 3-1 having incidence of 8.73%.

Table 2.2. Germplasm selected for evaluation in 2019-2021**.**

Germplasm	***Resistance*** [1]
DELCOT 277	HR
DELTAPINE 55	HR
FJA	HR
GA 2015046	HR
GA 2016090	HR
GA 2016110	HR
GA 2017138	HR
TIDEWATER #5	HR
TX 0135-2	HR
TX 0202-1	HR
TX 0616-3	HR
TX 1171-1	HR
TX 1409-2	HR
TX 0043	R
TX 1308	R
TX 2322	R
TX 2359	R
TX 2369	R
TX 0141-2	MR
TX 0195-1	MR
TX 2420-1	MR
TX 2316	S
TX 0064	S
TX 2375	S
TX 1233	S
TX 1094	S
TX 1210-2	HS
TX 0762-2	HS
TX 2383	HS
TX 0122-3	HS
TX 2320	HS
M-315	R-CHECK
ROWDEN	S-CHECK

[1]-Resistance: HR- highly resistant, R-resistant, MR-moderate resistance, MS-moderate susceptibility, S-susceptible, and HS-highly susceptible.

Table 2.3. 2019 germplasm mean disease incidence results.

GERMPLASM	****LSD=13.7532***	***Mean FOV DI (%)***
TX 2359	A	55.83
TX 0616-3	B	42.08
TX 2383	BC	37.49
TX 0064	CD	26.73
TX 1094	DE	16.86
TX 0202-1	DE	16.83
TX 2320	DE	16.76
TX 1308	DE	14.79
TX 1233	DE	14.68
TX 2420-1	DE	14.05
TX 0122-3	DEF	13.61
GA 2017138	DEF	13.53
TX 2322	EF	12.73
TX 1210-2	EF	12.40
TX 2316	EF	12.33
TIDEWATER #5	EF	12.03
DELTAPINE 55	EF	11.25
TX 0135-2	EF	11.15
GA 2016110	EF	10.88
TX 1171-1	EF	10.60
TX 0043	EF	10.33
TX 0141-2	EF	9.75
TX-2369	EF	9.23
TX 1409-2	EF	9.03
DELCOT 277	EF	8.79
FJA	EF	8.58
GA 2016090	EF	6.56
M-315	EF	5.88
TX 0762-2	EF	5.74
ROWDEN	EF	4.95
TX 0195-1	EF	4.88
GA 2015046	EF	4.67
TX 2375	F	0

**Levels not connected by same letter are significantly different.*

Table 2.4. 2019 single plant selections collected.

Line number	Germplasm	Single Plant Selections
3	TX 2322	2
7	TX 0043	2
16	TX 1171-1	2
24	FJA	10
25	DELTAPINE 55	10
28	GA 2015046	10
29	GA 2016110	10
30	GA 2017138	10
32	GA 2016090	10
Selections were planted in the greenhouse for seed increase on January 2, 2020, by planting 4 seed per line.		

Several other lines, including TX-0064, 28-1, 32-4, 30-6, TX-1409-2, TX-0768-2, TX-0122-3, TX-0202-1, 29-4, 7-1, TX-0135-2, TX-1210-2, TX-2320, 32-5, 16-1, 30-2, TX-1171-2, 29-9, TX-2383, TX-2322, DELTAPINE 55, TX-0043, and TX-1233 had disease incidences ranging from 8.31% to 3.15%. M-315, DELCOT 277, TX-1094, GA 2016110, TX-2375, FJA, TX-2369, TX-0616-3, GA 2015046, GA 2016090, TX-0141-2 ranged from 2.91% to 1.98%. Germplasm including TX-0195-1, TX-2420-1, 28-4, 28-9, TX-1308 and 30-4 had disease incidence ranging from 1.54- 0.27% (Table 2.5). Extremely low disease pressure resulted in very little separation among lines to discern higher levels of resistance.

In 2021, disease pressure was not as high as 2018, but uniformity was very consistent throughout the trial giving adequate rating of resistance. Results of disease incidence were favorable as the susceptible check, ROWDEN, showed the highest disease incidence at 43.85% (Table 2.6). Germplasm lines 28-4**,** TX-1409-2**,** 30-6**,** 30-2**,** TX-0616-3 showed similar disease

incidence levels as Rowden, ranging from 26.43- 23.52%. All other germplasm showed significantly lower disease including 32-5, TX-1171-2, 7-1, TX-0202-1, 28-9, DELCOT 27, TX-0064, TX-2320, 3-1, M-315, DP 399, TX-1308, 29-4, TX-0122-3, 30-4, GA 2016110, DELTAPINE 55, TX-0768-2, 32-4, and FJA with disease incidence percentages between 20.65% and 10.02%.

Table 2.5. 2020 germplasm mean disease incidence results.

GERMPLASM	****LSD=8.435***	***Mean FOV DI (%)***
TX 2359	A	11.4
GA 2017138	AB	9.97
3-1	ABC	8.74
TX 0064	ABCD	8.31
28-1	ABCD	7.84
32-4	ABCD	7.65
30-6	ABCD	7.54
TX 1409-2	ABCD	6.57
TX 0768-2	ABCD	6.55
TX 0122-3	ABCD	5.88
TX 0202-1	ABCD	5.84
29-4	ABCD	5.69
7-1	ABCD	5.58
TX 0135-2	ABCD	5.47
TX 1210-2	ABCD	5.22
TX 2320	ABCD	5.07
32-5	ABCD	5.06
16-1	ABCD	4.74
30-2	ABCD	4.71
TX 1171-2	ABCD	4.59
29-9	ABCD	4.15
TX 2383	ABCD	3.71
TX 2322	ABCD	3.37
DELTAPINE 55	ABCD	3.32
TX 0043	ABCD	3.21
TX 1233	ABCD	3.15
M-315	BCD	2.91
DELCOT 277	BCD	2.83
29-1	BCD	2.76

32-6	BCD	2.66
TX 1094	BCD	2.5
GA 2016110	BCD	2.47
TX 2375	BCD	2.39
FJA	BCD	2.23
TX 2369	BCD	2.19
TX 0616-3	BCD	2.18
GA 2015046	BCD	2.13
GA 2016090	BCD	2.03
TX 0141-2	BCD	1.98
TX 0195-1	CD	1.54
TX 2420-1	CD	1.49
28-4	CD	1.34
28-9	CD	0.89
TX 1308	CD	0.53
30-4	D	0.27

**Levels not connected by same letter are significantly different.*

The remaining germplasm including GA 2015046, TX 0141-2, TX 0043, 29-9, TX 2383, TX 1210-2, TX 2369, TX 2375, TX 0135-2, TX 1094, TX 0195-1, 16-1, TX 1233, 29-1, TX 2420-1, GA 2016090, GA 2017138, TX 2359, 28-1, and TX 2322 had incidence ranging from 9.31% to 1.49%, with 32-6 (a selection made from GA 2016090) having 0% disease incidence (Table 2.6).

Summary across years

Over the four years of testing FW resistance, germplasm lines showed varying levels of susceptibility and resistance. Much of the germplasm evaluated were observed in all four years of screening apart from single plant selections and a few germplasm lines only evaluated after 2019. This includes TX 1171-1, TIDEWATER #5, TX 2316, TX 0762-2 for only the 2018-2019 evaluations, TX 1171-2, TX 0768-2 for all years except 2019, ROWDEN for years except 2020,

and TX 202-1 during the 2019-2021 experiments. The remaining germplasm included all four years of evaluations.

For germplasm with observations in all four years, TX 0195-1, GA 2016090, TX 2369, GA 2016110, GA 2017138, TX 2322, FJA, TX 0135-2, TX 2420-1 GA 2015046, TX 0141-2, TX 0043, DELTAPINE 55, DELCOT 277, and TX 1409-2 showed incidence ratings from 3.47 to 13.24%. The mean disease incidence of these lines is consistent considering variability in disease intensity during all four years (Table 2.7).

Table 2.6. 2021 germplasm mean disease incidence results.

GERMPLASM	****LSD=22.01765***	***Mean FOV DI (%)***
ROWDEN	A	43.85
28-4	AB	26.43
TX-1409-2	ABC	26.02
30-6	ABCD	23.83
30-2	ABCD	23.65
TX-0616-3	ABCD	23.52
32-5	BCDE	20.65
TX-1171-2	BCDE	19.50
7-1	BCDE	19.38
TX-0202-1	BCDE	17.04
28-9	BCDE	16.49
DELCOT 277	BCDE	16.46
TX-0064	BCDE	16.10
TX-2320	BCDE	15.84
3-1	BCDE	14.50
M-315	BCDE	14.04
DP 399	BCDE	13.81
TX-1308	BCDE	13.07
29-4	BCDE	12.95
TX-0122-3	BCDE	12.91
30-4	BCDE	12.30
GA 2016110	BCDE	12.13
DELTAPINE 55	BCDE	11.54
TX-0768-2	BCDE	10.83

32-4	BCDE	10.52
FJA	BCDE	10.02
GA 2015046	BCDE	9.31
TX-0141-2	BCDE	8.97
TX-0043	BCDE	8.14
29-9	BCDE	7.93
TX-2383	BCDE	7.40
TX-1210-2	BCDE	7.07
TX-2369	BCDE	5.42
TX-2375	BCDE	5.18
TX-0135-2	BCDE	5.01
TX-1094	BCDE	4.76
TX-0195-1	BCDE	4.75
16-1	CDE	4.36
TX-1233	CDE	4.27
29-1	DE	3.91
TX-2420-1	DE	3.61
GA 2016090	DE	3.25
GA 2017138	DE	3.24
TX-2359	DE	3.20
28-1	DE	2.86
TX-2322	E	1.50
32-6	E	0.00

**Levels not connected by same letter are significantly different.*

Germplasm that appeared to be inconsistent and with higher susceptibility include lines TX 2375, 30-6, TX 0616-3, TX 2359, TX 1233, TX 1094, TX 0064, TX 1210-2, TX 0122-3, TX 2320, and TX 2383 with severity percentages ranging from 16.94 to 33.86% for all four observations which indicate less value in providing adequate resistance to fusarium wilt. M-315, the resistant check, averaged 7.13%, whereas the susceptible check Rowden averaged 27.37%. Single plant selections had levels of disease incidence that were numerically lower to their original parent line on a few occasions which include 16-1 (TX 1171-1 selection), 28-1 (GA 2015046 selection), 29-1 (GA 2016110 selection), and 32-6 (GA 2016090 selection).

Table 2.7. Summary of disease incidence 2018-2021

Germplasm	*2018 Block 2 DI (%)**	*2019 Mean DI (%)*	*2020 Mean DI (%)*	*2021 Mean DI (%)*	*Standard Dev.*	*Mean DI (%)***
32-6			2.66	0	1.88	1.33
29-1			2.76	3.91	0.82	3.34
TX 0195-1	2.71	4.88	1.54	4.75	1.63	3.47
16-1			4.74	4.36	0.28	4.55
GA2016090	0	13.54	2.03	3.25	6.04	4.71
TX 2369	2.44	9.23	2.19	5.42	3.29	4.82
GA2016110	0	4.68	2.47	12.13	5.24	4.82
GA2017138	0	6.57	9.97	3.24	4.3	4.95
TX 2322	2.5	12.73	3.37	1.5	5.2	5.03
FJA	0	8.58	2.23	10.02	4.85	5.21
TX 1171-1	0	10.6			7.5	5.3
28-1			7.84	2.86	3.53	5.35
TX 0135-2	0	11.15	5.47	5.01	4.56	5.41
TX 2420-1	2.64	14.05	1.49	3.61	5.81	5.45
GA2015046	0	10.89	2.13	9.31	5.33	5.58
TX 0141-2	2.57	9.75	1.98	8.97	4.12	5.82
TX 0043	2.39	10.33	3.21	8.14	3.84	6.02
TIDEWATER #5	0	12.04			8.51	6.02
29-9			4.15	7.93	2.68	6.04
30-4			0.27	12.3	8.51	6.28
DELTAPINE 55	0	11.25	3.32	11.54	5.79	6.53
DELCOT 277	0	8.8	2.83	16.46	7.28	7.02
M-315	5.72	5.88	2.91	14.04	4.8	7.14
TX 1308	2.5	14.79	0.53	13.07	7.25	7.72
28-9			0.89	16.49	11.03	8.69
32-4			7.65	10.52	2.03	9.08
29-4			5.69	12.95	5.14	9.32
TX 1409-2	0	9.03	6.57	26.02	11.09	10.41
3-1			8.74	14.5	4.08	11.62
7-1			5.58	19.38	9.77	12.48
32-5			5.06	20.65	11.02	12.86
TX 0202-1		16.84	5.84	17.04	6.41	13.24
28-4			1.34	26.43	17.75	13.89
30-2			4.71	23.65	13.4	14.18
TX 2375	54.06	0	2.39	5.18	25.86	15.41

30-6			7.54	23.83	11.53	15.69
TX 0616-3	0	42.08	2.18	23.52	19.84	16.95
TX 2359	2.28	55.84	11.4	3.2	25.44	18.18
TX 1233	54.06	14.69	3.15	4.27	23.92	19.04
TX 1094	54.06	16.86	2.5	4.76	23.86	19.54
TX 1171-2	48.72		4.59	19.5	22.46	24.27
TX 0064	53.49	26.73	8.31	16.1	19.73	26.16
TX 1210-2	81.09	12.41	5.22	7.07	36.56	26.45
ROWDEN	33.34	4.95		43.85	20.13	27.38
TX 0768-2	73.08		6.55	10.83	37.24	30.15
TX 0122-3	90.63	13.61	5.88	12.91	40.07	30.76
TX 2316	52.78	12.34			28.6	32.56
TX 2320	96.78	16.76	5.07	15.84	42.45	33.61
TX 2383	86.85	37.49	3.71	7.4	38.43	33.86
TX 0762-2	81.58	5.75			53.63	43.67

*Disease incidence calculated by year germplasm line was evaluated 2018-2021.
**Mean DI (%) averaged across years regardless of number of observations.

The remaining selections were rated higher in disease incidence percentage compared to the parent germplasm (Fig. 2.4).

Discussion

Continuous monoculture has historically intensified the need for pest management practices to mitigate the inevitable effect of pathogen survival, reproduction, and dissemination. Soilborne pathogens, including FOV pose a unique challenge as they persist in soil by survival structures called chlamydospores, which can endure fumigation applications (Davis et al., 2006). Crop rotation, a typical management approach, requires seven years for FOV, making it impractical for most growers. While nematicides, such as aldicarb (AgLogic©), fluopyram (Velum Prime©), and fumigants including Telone II©, offer control options, they are costly in relation to monetary returns for the grower (Starr et al., 2007). Concern in recent years have driven innovation toward more effective strategies for controlling FW. In integrated disease

management, host-resistance is widely recognized as the most effective method for reducing fungicide use, offering advantages in economic input, sustainability, and environmental stewardship (Egan and Stiller, 2022). Unfortunately, with the exception of FOV race-4, recent breeding efforts have largely prioritized developing resistance to nematodes, such as root-knot and reniform nematodes, rather than directly addressing FOV resistance. While nematode-resistant cultivars provide some benefit by reducing FOV severity, they do not fully address the need for FOV-resistant varieties (Wheeler et al., 2022).

Recent studies in Georgia indicate that FOV exhibits greater genetic diversity than previously recognized, likely contributing to the variability in severity observed in recent years (Holmes et al., 2009; Cianchetta et al., 2015; Da Silva et al., 2019a; Halpern et al., 2020). Additionally, nematode diversity impacting FW incidence now extends beyond root-knot nematode, with sting nematode (*Belonolaimus longicaudatus*) posing a notable risk (da Silva et al., 2019a). This mobile, aggressive ectoparasitic nematode may circumvent traditional control methods, such as nematicides and RKN-resistant cultivars, reducing the effectiveness of FOV control where sting nematode are prevalent (da Silva et al., 2019a). These findings bring transparency to the limitations of relying solely on nematode-resistant varieties, as many cultivars previously deemed FOV resistant address nematode resistance rather than directly FOV resistance. Historical studies have also noted that fusarium wilt could occur without nematode pressure (Nelson et al., 1981). Given the prevalence of sting nematode in Georgia, identifying FOV resistance in germplasm is critical. Hence, long-term FW control options must include both the incorporation of robust FOV resistance into breeding programs alongside nematode resistance.

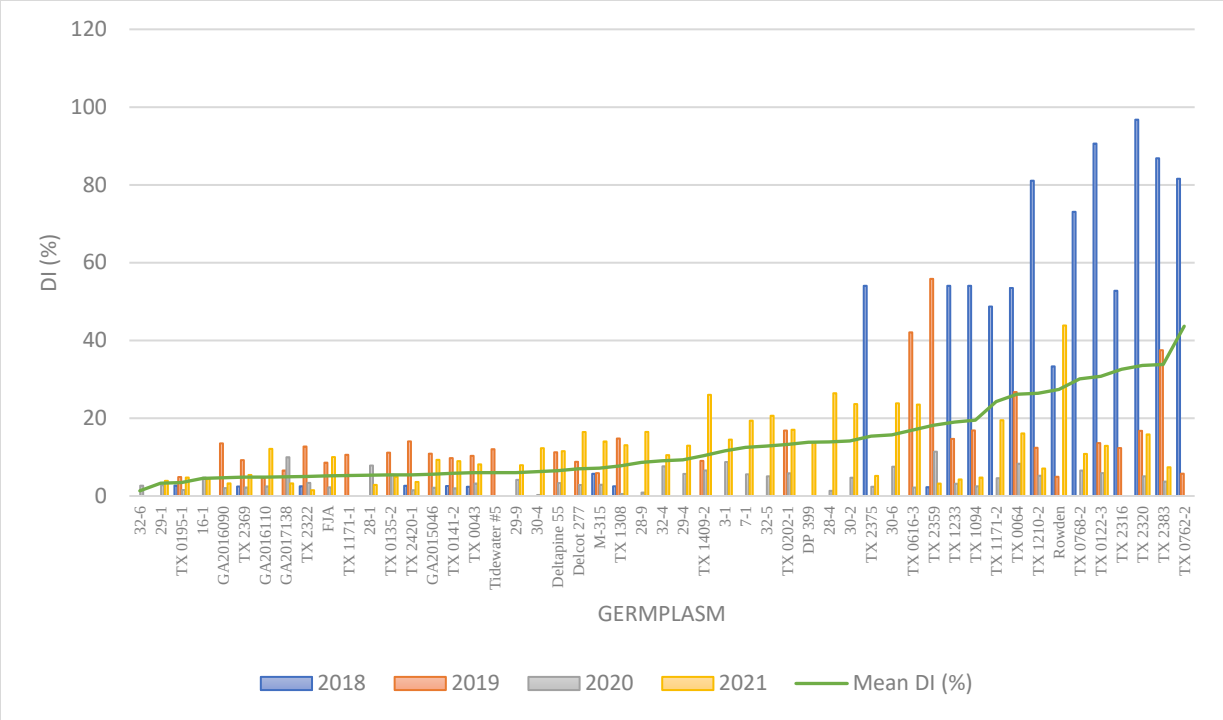

Fig 2.4. Mean fusarium wilt incidence (%) by germplasm 2018-2021.

This study emphasizes the importance of evaluating new germplasm lines from diverse genetic backgrounds, including wild accessions, obsolete varieties, and elite breeding lines, to find effective solutions for managing FW. Such an approach is necessary to gain a comprehensive understanding on the full spectrum of host resistance in cotton germplasm, especially as interactions between FOV and multiple nematode species complicate effective control. Focusing on breeding for dual resistance to both FOV and nematodes, such as sting nematode, could offer a more robust and sustainable solution for FW management in cotton (Huo et al, 2023).

In this study, several obsolete varieties, including DELTAPINE 55, DELCOT 277, TIDEWATER #5, and FJA appear to provide consistent levels of tolerance to FOV, along with elite breeding lines from the University of Georgia breeding program. The TX wild accessions

showed considerable variability in resistance, indicating a truly diverse germplasm pool (Table 2.1, Table 2.7, Figure 2.1, and Table 2.12). For example, the line TX 0195-1 showed low incidence percentages (ranging from 3.33% to 4.70%) over multiple years of testing, alluding to strong resistance whereas TX 0762-2 had mean disease incidence of 43.67%. Other lines in the study, including 16-1, GA 2016090, TX 2369, GA 2016110, GA 2017138 and TX 2322 exhibited mean incidence percentages ranging from 4.55% to 5.03%, indicating a good level of FOV resistance. FJA, TX 1171-1, and 28-1 also have consistent resistance throughout testing periods. Other germplasm like TX 0135-2, TX 2420-1, GA 2015046, TX 0141-2, TX 0043, TIDEWATER #5, 29-9, 30-4, DELTAPINE 55, and DELCOT 277 showed slightly higher mean disease incidence comparable to M-315 which averaged 7.14%. The stable resistance observed in most of the obsolete germplasm is expected, as FW resistance has been documented in these lines. However, identifying high resistance levels in certain elite lines is intriguing, given that breeding efforts did not specifically target fusarium wilt resistance. Notably germplasm like 29-1, a selection of GA 2016110 and 32-6, which is a selection of GA 2016090, exhibited exceptional performance during the 2020-2021 trials, consistently showing lower mean disease incidence of 3.34% and 1.33% respectively. 32-6 having the lowest mean disease incidence was the most resistant line. (Table 2.7).

More susceptible germplasm was identified in the wild accessions and single plant selections, which included TX 1308, 28-9, 32-4, 29-4, TX 1409-2, 3-1, 7-1, 32-5, TX 0202-1, 28-4, 30-2, TX 2375, 30-6, TX 0616-3, TX 2359, TX 1233 and TX 1094 that showed mean disease incidence values between 7.72% and 19.54%, indicating higher susceptibility to fusarium wilt over the entire testing period. Among the lines similar to the susceptible check, Rowden, were TX 1171-2, TX 0064 and TX 1210-2, which had mean disease incidence percentages exceeding

24% and Rowden having 27.38% disease incidence. Other highly susceptible lines across the four years were TX 0768-2, TX 0122-3, TX 2316, and TX 2320, showing mean disease incidence percentages between 30.15% and 33.86%. Finally, TX 0762-2 exhibited the highest average disease severity at 43.67%, making it one of the most susceptible lines in the experiment.

Even so, consistency among GA public breeding lines, obsolete varieties, wild germplasm, and 2019 single plant selections (Table 2.2) are promising for cotton breeders given the performance through observation of various levels of fusarium wilt incidence throughout 2018-2021. Completion of four years of field experiments provide results that identify certain breeding lines providing resistance to a native population of FOV in South Georgia. There appears to be variability of resistance from year to year with specific germplasm and more consistency with others.

In summary, given that FOV is a soilborne pathogen that typically causes disease in uneven, aggregated patterns influenced by environmental factors, nematode interaction, and inoculum density, year-to-year variability in field results is expected (DeVay et al., 1997; Davis et al., 2006). Even though variance is expected, this research still offers valuable insight into the levels of disease resistance among this group of germplasm against an aggressive population of native FOV specific to Georgia. The findings from this study provide previously unknown knowledge that can aid in understanding genetic resistance, potentially uncovering novel genes or quantitative factors, and offering improvement opportunities for cotton breeding programs aiming to enhance FW resistance in elite cotton cultivars.

Table 2.8. 2018 germplasm evaluation ANOVA

Summary of fit	
Rsquare	0.548351
Rsquare adj	0.394371
Root mean square error	12.31868
Mean of response	8.971707
Observations (or sum wgts)	1708

Analysis of variance

Source	***Df***	***Sum of squares***	***Mean square***	***F ratio***	
Model	434	234538.2	540.411	3.5612	
Error	1273	193177.44	151.75		***Prob > f***
C. Total	1707	427715.64			<.0001

Effect tests

Source	***Nparm***	***Df***	***Sum of squares***	***F ratio***	***Prob > f***
Germplasm	431	431	93629.52	1.4316	<.0001
Block (block)	3	3	139388.79	306.1812	<.0001

Table 2.9. 2018 germplasm evaluation block 2

Summary of fit	
Rsquare	0.995561
Rsquare adjusted	0.885626
Root mean square error	7.838735
Mean of response	24.36879
Observations (or sum wgts)	439

Analysis of variance

Source	***Df***	***Sum of squares***	***Mean square***	***F ratio***	***Prob > f***
Germplasm	421	234264.2	556.447	9.0559	<.0001
Error	17	1044.58	61.446		
C. Total	438	235308.8			

Table 2.10. 2018 Ranking of germplasm by score mean based on number of observations

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)					
GERMPLASM	Count	Score Sum	Expected Score	Score Mean	(Mean - Mean0)/Std0
DELCOT 277	4	1288	3418	322	-2.221
DELTAPINE 55 (652-679)	4	1288	3418	322	-2.221
GA 2015046	4	1288	3418	322	-2.221
GA 2017138	4	1288	3418	322	-2.221
NM 12Y1004	4	1288	3418	322	-2.221
TERRA 207	4	1288	3418	322	-2.221
TIDEWATER 29	4	1288	3418	322	-2.221
TIDEWATER #5 (G. BARB. X G. HIR.)	4	1288	3418	322	-2.221
GA 2016090	3	966	2563.5	322	-1.923

HART	3	966	2563.5	322	-1.923
HYC79-6	3	966	2563.5	322	-1.923
MD 15	3	966	2563.5	322	-1.923
TAM 2561 RKNR	2	644	1709	322	-1.57
ACALA 44WR	1	322	854.5	322	-1.109
AHA X C 100W X C 100W	1	322	854.5	322	-1.109
EARLY WILT WANNAMAKER'S	1	322	854.5	322	-1.109
REBA W 296	1	322	854.5	322	-1.109
SA-0848	1	322	854.5	322	-1.109
SA-1950	1	322	854.5	322	-1.109
STONEVILLE CLEAN SEED	1	322	854.5	322	-1.109
DELTAPINE 26	4	1621.5	3418	405.38	-1.874
AUBURN 623 RNR	4	1625	3418	406.25	-1.87
SEABROOK SEA ISLAND 12B2	4	1634	3418	408.5	-1.861
DELCOT 311	4	1678	3418	419.5	-1.815
CAHUGLBBCS-1-88	3	1295	2563.5	431.67	-1.527
ACALA 1517D	4	1729	3418	432.25	-1.761
DUNN HS 120	4	1729	3418	432.25	-1.761
LOCKETT 77	4	1729	3418	432.25	-1.761
ACALA 1517 WILT	4	1750	3418	437.5	-1.74
GACOT 79	4	1768	3418	442	-1.721
TX-2357	4	1768	3418	442	-1.721
ARKOT 9704	4	1786.5	3418	446.63	-1.701
CASCOT B-2	4	1786.5	3418	446.63	-1.701
TX-0195-1	4	1831	3418	457.75	-1.655
REX 713	4	1860.5	3418	465.13	-1.624
TX-1151	4	1860.5	3418	465.13	-1.624
FJA	3	1407	2563.5	469	-1.392
SA-0297	4	1885.5	3418	471.38	-1.598
COKER 100 WILT	4	1920	3418	480	-1.562
REBA B50	4	1934	3418	483.5	-1.548
MCNAIR 220	4	1949.5	3418	487.38	-1.531
REBA 288	4	1964	3418	491	-1.516
SA1643XNM12Y1005	4	1967	3418	491.75	-1.513
CHACO 510 INTA	4	1996	3418	499	-1.483
TX-2349	4	2022	3418	505.5	-1.456
DUNN 219	3	1523.5	2563.5	507.83	-1.252
TX-1003-1	4	2035.5	3418	508.88	-1.442
TX-0135-2	4	2062.5	3418	515.63	-1.414
TX-2321	4	2062.5	3418	515.63	-1.414
TX-1042-1	4	2074.5	3418	518.63	-1.401
TX-1283-3	4	2074.5	3418	518.63	-1.401
DEMETER II	4	2125	3418	531.25	-1.348
DUNN 400	4	2133.5	3418	533.38	-1.339
TX-0681-3	4	2137.5	3418	534.38	-1.335

TX-2359	4	2149	3418	537.25	-1.323
GA 2016111	4	2185	3418	546.25	-1.286
HYPERFORMER HYOO7	4	2185	3418	546.25	-1.286
AUBURN BR-1	3	1639.5	2563.5	546.5	-1.112
TX-0043	4	2191	3418	547.75	-1.279
GA 2017046	3	1645	2563.5	548.33	-1.105
CD3HCHULBH-1-88	4	2196.5	3418	549.13	-1.274
NM 13P1117	4	2199	3418	549.75	-1.271
TX-1364-2	4	2214	3418	553.5	-1.255
TX-2139	4	2234.5	3418	558.63	-1.234
TX-1171-1	4	2251	3418	562.75	-1.217
TX-2420-1	4	2251	3418	562.75	-1.217
TAMLBBFOV16	4	2272	3418	568	-1.195
TX-0202	4	2272	3418	568	-1.195
TX-0495-1	4	2290	3418	572.5	-1.176
CIANO ALAMOS 92	4	2310	3418	577.5	-1.155
TX-0183-1	4	2337.5	3418	584.38	-1.127
GA 2017112	4	2353	3418	588.25	-1.11
TAM 94L-25	4	2359.5	3418	589.88	-1.104
TX-2381	4	2373	3418	593.25	-1.09
TX-1609	4	2377.5	3418	594.38	-1.085
TX-0072-2	4	2380	3418	595	-1.082
TX-1147	3	1793	2563.5	597.67	-0.927
GA 2015072	4	2396.5	3418	599.13	-1.065
TX-0621-1	4	2403.5	3418	600.88	-1.058
TX-0072-1	4	2404.5	3418	601.13	-1.057
SA1643XNM12Y1004	4	2446.5	3418	611.63	-1.013
GA 2017091	4	2453.5	3418	613.38	-1.006
ARKOT 0305	4	2455	3418	613.75	-1.004
LAMBRIGHT 2020	4	2476.5	3418	619.13	-0.982
GA 2017053	4	2502	3418	625.5	-0.955
TX-2365	4	2517	3418	629.25	-0.939
EMPIRE WR 61	4	2524	3418	631	-0.932
NM 12Y1004XSA-0550	4	2533.5	3418	633.38	-0.922
TX-0650-1	4	2572	3418	643	-0.882
TX-2377	4	2591.5	3418	647.88	-0.862
GA 2016025	4	2602	3418	650.5	-0.851
TRICE 2A	4	2602	3418	650.5	-0.851
DP 1646	40	26122	34180	653.05	-2.687
SIOKRA	4	2612.5	3418	653.13	-0.84
TX-0647	4	2612.5	3418	653.13	-0.84
TX-2353	4	2615	3418	653.75	-0.837
TX-1914-1	4	2616.5	3418	654.13	-0.836
GA 2017106	4	2619	3418	654.75	-0.833
GA 2017050	4	2620	3418	655	-0.832

TX-1585-1	4	2620	3418	655	-0.832
ARKOT 9623	4	2631.5	3418	657.88	-0.82
TX-0650-2	4	2636.5	3418	659.13	-0.815
TX-2319	4	2641.5	3418	660.38	-0.81
TX-0570-2	4	2651.5	3418	662.88	-0.799
TX-1305	3	2003	2563.5	667.67	-0.674
TX-0093	4	2687.5	3418	671.88	-0.762
M-315 (CHECK)	4	2692	3418	673	-0.757
NM 12Y1004XSA-2390	4	2696	3418	674	-0.753
SA-1643XNM 12Y1004	4	2696.5	3418	674.13	-0.752
NM 12Y1004XSA-1759	4	2698.5	3418	674.63	-0.75
GA 2016016	4	2699.5	3418	674.88	-0.749
NM240162	4	2711	3418	677.75	-0.737
COKER 315	3	2044.5	2563.5	681.5	-0.624
REBA	4	2766	3418	691.5	-0.68
NM 12Y1004XSA-1555	3	2076.5	2563.5	692.17	-0.586
LA HG-063	4	2777	3418	694.25	-0.668
TX-0400	4	2782.5	3418	695.63	-0.662
TX-0095	4	2790	3418	697.5	-0.655
NM 12Y1004XSA1177	3	2104	2563.5	701.33	-0.553
KEKCHI CLEAN SEED	2	1404.5	1709	702.25	-0.448
AUBURN 56-24R	3	2112	2563.5	704	-0.543
TX-2373	3	2115	2563.5	705	-0.539
SPEARS UPLAND EARLY LONG STAPLE	4	2825	3418	706.25	-0.618
MISCOT 7803-52	4	2827.5	3418	706.88	-0.615
GA 2016110	4	2829	3418	707.25	-0.614
GA 2017101	4	2829.5	3418	707.38	-0.613
TX-1003-2	4	2852	3418	713	-0.59
TX-1718	4	2852	3418	713	-0.59
TX-0141-2	4	2858	3418	714.5	-0.584
TX-0704-1_WHITE	4	2871	3418	717.75	-0.57
TAMLBBFOV45	3	2156	2563.5	718.67	-0.49
TX-2144	4	2889.5	3418	722.38	-0.551
TX-2346	4	2893.5	3418	723.38	-0.547
GA 2016099	4	2894	3418	723.5	-0.546
TX-0498-3	4	2908	3418	727	-0.532
AU90084	4	2908.5	3418	727.13	-0.531
SA-1148XNM 12Y1005	4	2913	3418	728.25	-0.526
NEW MEXICO ACALA	4	2920.5	3418	730.13	-0.518
TX-1114-3	4	2920.5	3418	730.13	-0.518
TX-2369	4	2925	3418	731.25	-0.514
TX-2361	4	2928.5	3418	732.13	-0.51
TX-1418	4	2930	3418	732.5	-0.509
GA 2017035	4	2939.5	3418	734.88	-0.499

PAYMASTER 54	4	2947.5	3418	736.88	-0.49
TX-0681-2	3	2213	2563.5	737.67	-0.421
BALLARD CLEAN SEED HIGH LINT	4	2966	3418	741.5	-0.471
TX-0932	4	2972.5	3418	743.13	-0.464
ARKOT 9208	4	2973	3418	743.25	-0.464
M-315	20	14898	17090	744.9	-1.027
TX-2322	4	2982	3418	745.5	-0.454
TX-2403-1	3	2242	2563.5	747.33	-0.387
TX-1941-2	4	3001	3418	750.25	-0.434
STROMPROOF CA 119-1/29	4	3009.5	3418	752.38	-0.426
TX-0122-2	4	3019.5	3418	754.88	-0.415
TX-0294	4	3025	3418	756.25	-0.409
ACALA SJ-2	4	3034.5	3418	758.63	-0.4
TX-1178	3	2276.5	2563.5	758.83	-0.345
ARKOT 8717	4	3035.5	3418	758.88	-0.399
GA 2017016	4	3040	3418	760	-0.394
COKER'S CLEVEWILT #3	4	3044.5	3418	761.13	-0.389
TX-1306	4	3048	3418	762	-0.385
DELTAPINE 1646 (CHECK)	4	3063.5	3418	765.88	-0.369
MEBANE	4	3066	3418	766.5	-0.367
TX-2420-2	4	3068	3418	767	-0.365
GA 2016024	4	3071.5	3418	767.88	-0.361
TX-0373	3	2304	2563.5	768	-0.312
GA 2017073	4	3085	3418	771.25	-0.347
FIBER MAX 832	4	3093.5	3418	773.38	-0.338
TX-1302	4	3114.5	3418	778.63	-0.316
GA 2015092	3	2339	2563.5	779.67	-0.27
TX-2374	4	3119.5	3418	779.88	-0.311
TX-2356	4	3121	3418	780.25	-0.309
TX-0644-1	4	3133.5	3418	783.38	-0.296
SA-2381	1	784	854.5	784	-0.146
TAMLBBFOV30	4	3162	3418	790.5	-0.267
TX-0122-1	4	3162	3418	790.5	-0.267
SA-0404	4	3170	3418	792.5	-0.258
QUAPAW	4	3171.5	3418	792.88	-0.257
TX-0040	4	3177	3418	794.25	-0.251
TX-1211	4	3189.5	3418	797.38	-0.238
TX-0195-2	4	3210	3418	802.5	-0.216
TX-0933	4	3210	3418	802.5	-0.216
TX-1210-1	4	3217.5	3418	804.38	-0.209
TX-0117-1	4	3220.5	3418	805.13	-0.206
TX-1154-3	4	3226	3418	806.5	-0.2
TX-1300-2	4	3227.5	3418	806.88	-0.198
TX-0616-3	4	3229.5	3418	807.38	-0.196
SA-1049	4	3236	3418	809	-0.189

TX-1205-1	4	3244.5	3418	811.13	-0.18
TX-1003-3	4	3251.5	3418	812.88	-0.173
TX-0704-2	4	3266.5	3418	816.63	-0.158
TX-2335-2	3	2457.5	2563.5	819.17	-0.127
TX-1321	4	3281.5	3418	820.38	-0.142
TX-1585-3	4	3283.5	3418	820.88	-0.14
STONEVILLE 603	3	2465	2563.5	821.67	-0.118
TX-0490-1	4	3287.5	3418	821.88	-0.136
TX-2390-3	4	3288	3418	822	-0.135
AU91083	4	3315	3418	828.75	-0.107
NM 13R1015	4	3354	3418	838.5	-0.066
TX-2384	4	3361.5	3418	840.38	-0.058
TX-2402	4	3372.5	3418	843.13	-0.047
TX-2315	4	3374	3418	843.5	-0.045
ROWDEN (CHECK)	4	3378	3418	844.5	-0.041
TX-1270-1	4	3378	3418	844.5	-0.041
TX-1462-3	4	3378	3418	844.5	-0.041
TX-2403-3	4	3391.5	3418	847.88	-0.027
ROWDEN #3	4	3397.5	3418	849.38	-0.021
TX-1008	4	3404	3418	851	-0.014
GA 2016056	4	3413.5	3418	853.38	-0.004
TX-0135-3	4	3415	3418	853.75	-0.003
TX-1425-1	3	2565.5	2563.5	855.17	0.002
SEALAND 883	4	3444	3418	861	0.027
TX-2074	4	3450.5	3418	862.63	0.033
TX-0616-1	4	3452	3418	863	0.035
TX-2324	4	3457	3418	864.25	0.04
TX-0002-2	4	3464.5	3418	866.13	0.048
TX-1076	4	3467.5	3418	866.88	0.051
TX-1212	4	3475	3418	868.75	0.059
TX-1054-2	4	3477	3418	869.25	0.061
TX-1171-2	4	3477.5	3418	869.38	0.062
TIFCOT 56	4	3484	3418	871	0.068
TX-2362	4	3491.5	3418	872.88	0.076
FELISTANA UA-7-18	4	3498	3418	874.5	0.083
TX-0180-1	4	3502	3418	875.5	0.087
TX-0490-2	4	3522	3418	880.5	0.108
NM 12Y1005	3	2644	2563.5	881.33	0.096
GA 2017038	4	3525.5	3418	881.38	0.112
TX-0616-2	4	3531.5	3418	882.88	0.118
TX-1364-1	4	3542.5	3418	885.63	0.129
TX-0021	4	3544.5	3418	886.13	0.131
TX-0180-3	4	3546	3418	886.5	0.133
FTA	4	3546.5	3418	886.63	0.134
COKER 100A (WR)	3	2665.5	2563.5	888.5	0.122

TX-1154-2	4	3560.5	3418	890.13	0.148
TX-1308	4	3568	3418	892	0.156
TIDEWATER (SEABROOKS) (G.B.X G.H.)	4	3570.5	3418	892.63	0.159
MCNAIR TH 149-20 (TRIPLE HYBRID)	4	3584	3418	896	0.173
MAR5PD208S-4-90	3	2691.5	2563.5	897.17	0.154
TX-0240	4	3598	3418	899.5	0.187
TX-0709	4	3608.5	3418	902.13	0.198
TX-0597-1	4	3612	3418	903	0.202
TX-2308	4	3628	3418	907	0.219
NM 13G1018	3	2724	2563.5	908	0.193
TX-2323	4	3633.5	3418	908.38	0.224
TX-0135-1	4	3639	3418	909.75	0.23
TX-2311	3	2732.5	2563.5	910.83	0.203
TX-2335-1	3	2733.5	2563.5	911.17	0.204
TX-0002-1	4	3659.5	3418	914.88	0.251
TX-1409-2	4	3667.5	3418	916.88	0.26
STONEVILLE 2B (ORIGINAL)	4	3668.5	3418	917.13	0.261
TX-0101	3	2752	2563.5	917.33	0.226
TX-1000-3	4	3673	3418	918.25	0.265
TX-1213	4	3674.5	3418	918.63	0.267
TX-2355	4	3675	3418	918.75	0.268
TX-0141-1	4	3683	3418	920.75	0.276
WASHINGTON	4	3684	3418	921	0.277
MARSHALL	4	3690.5	3418	922.63	0.284
TX-2372	4	3691	3418	922.75	0.284
TX-0180-2	4	3704.5	3418	926.13	0.298
TX-1094	4	3704.5	3418	926.13	0.298
TX-2364	4	3712.5	3418	928.13	0.307
TX-0681-1	3	2784.5	2563.5	928.17	0.266
TX-0597-2	4	3718.5	3418	929.63	0.313
TX-0814	4	3719.5	3418	929.88	0.314
SA0460XNM12Y1005	4	3722	3418	930.5	0.317
TX-0244	4	3729.5	3418	932.38	0.324
GA 2017126	4	3734.5	3418	933.63	0.33
GA 2016060	3	2802	2563.5	934	0.287
TX-2375	3	2810	2563.5	936.67	0.296
AUBURN 56	4	3749	3418	937.25	0.345
TX-2394	3	2812.5	2563.5	937.5	0.299
TX-0495-2	4	3757	3418	939.25	0.353
TX-0703_WHITE	4	3774.5	3418	943.63	0.371
TX-2366	4	3785	3418	946.25	0.382
TX-0931-2	4	3789.5	3418	947.38	0.387
TX-1364-3	4	3822.5	3418	955.63	0.421

DNWC 1324 (REGISTERED AS GVS 6 ARS	4	3824	3418	956	0.423
NM 13P1121	4	3825.5	3418	956.38	0.425
TX-1196	4	3843	3418	960.75	0.443
TX-0931-1	4	3848.5	3418	962.13	0.449
GA 2016052	4	3855	3418	963.75	0.455
LA HG-660	4	3861.5	3418	965.38	0.462
REBA P 279 (REBA B-50 X DPL SMO.)	4	3863.5	3418	965.88	0.464
TX-1042-2	4	3872	3418	968	0.473
TX-0108	4	3873	3418	968.25	0.474
TX-0665-1	3	2906	2563.5	968.67	0.412
ARKOT 9811	4	3881.5	3418	970.38	0.483
COKER'S FOSTER #300	4	3881.5	3418	970.38	0.483
TX-1941-1	4	3887	3418	971.75	0.489
TX-0460-1	4	3893.5	3418	973.38	0.496
TX-2320	4	3913.5	3418	978.38	0.516
TX-2363	3	2944	2563.5	981.33	0.458
TX-1045	4	3934	3418	983.5	0.538
TX-2354	4	3936	3418	984	0.54
TX-1964	4	3946.5	3418	986.63	0.551
TX-0085	4	3947.5	3418	986.88	0.552
GA 2017136	4	3949.5	3418	987.38	0.554
CEDIX	3	2975	2563.5	991.67	0.495
TX-0062	4	3973	3418	993.25	0.578
TX-1322	4	3974.5	3418	993.63	0.58
STONEWILT	4	3993	3418	998.25	0.599
TX-2360	4	3997	3418	999.25	0.603
TX-1114-2	4	3998	3418	999.5	0.605
TX-1311	4	4002	3418	1000.5	0.609
AUBURN BR-2	4	4006.5	3418	1001.63	0.613
TX-0073-4	4	4011	3418	1002.75	0.618
TX-1122	4	4013	3418	1003.25	0.62
TX-1270-2	3	3011.5	2563.5	1003.83	0.539
TX-1149-2	4	4025.5	3418	1006.38	0.633
TX-1125	4	4040.5	3418	1010.13	0.649
TX-1303	4	4041	3418	1010.25	0.649
TX-1109	3	3038.5	2563.5	1012.83	0.571
DELTATYPE WEBBER	4	4055	3418	1013.75	0.664
NM 13P1088	4	4057	3418	1014.25	0.666
TX-1054-1	4	4057	3418	1014.25	0.666
NAIR GREEN SEED	4	4082.5	3418	1020.63	0.693
COKER'S WILDS #9	4	4094.5	3418	1023.63	0.705
ROWDEN	20	20529.5	17090	1026.48	1.612
MD 25	4	4108.5	3418	1027.13	0.72

TX-0625	4	4112.5	3418	1028.13	0.724
MISSDEL W.R. 1	4	4116.5	3418	1029.13	0.728
GA 2015026	4	4119	3418	1029.75	0.731
TX-1307	4	4124	3418	1031	0.736
TX-0681	4	4129	3418	1032.25	0.741
TX-0672	4	4151	3418	1037.75	0.764
TX-1300-1	4	4154	3418	1038.5	0.767
TX-1304	4	4164.5	3418	1041.13	0.778
TX-1201	4	4166	3418	1041.5	0.78
TX-0122-3	4	4173	3418	1043.25	0.787
TX-0183-2	4	4193	3418	1048.25	0.808
TX-1197	4	4203.5	3418	1050.88	0.819
TX-1301	3	3161	2563.5	1053.67	0.719
TX-2352	4	4219	3418	1054.75	0.835
TX-0931-3	4	4220.5	3418	1055.13	0.837
TX-1870	4	4235.5	3418	1058.88	0.852
TX-2389-1	4	4240	3418	1060	0.857
TX-0749-2	4	4243.5	3418	1060.88	0.861
TX-2022-1	4	4255.5	3418	1063.88	0.873
TX-1194-2	3	3195.5	2563.5	1065.17	0.76
TX-2358	4	4263	3418	1065.75	0.881
TX-1215	4	4267	3418	1066.75	0.885
TX-1300-3	4	4293	3418	1073.25	0.912
TX-0691-1	4	4299.5	3418	1074.88	0.919
TX-1194-1	4	4303	3418	1075.75	0.923
TX-1115	4	4305	3418	1076.25	0.925
TX-0644-2	4	4310.5	3418	1077.63	0.931
TX-0984	4	4314	3418	1078.5	0.934
WEST TEXAS ROUGH	4	4316	3418	1079	0.936
TX-0117-2	4	4341	3418	1085.25	0.962
TX-0621-2	4	4344	3418	1086	0.965
TX-1412	4	4371	3418	1092.75	0.994
TX-1063	4	4374	3418	1093.5	0.997
TX-0768-1	3	3288	2563.5	1096	0.872
BR 69-120	4	4392	3418	1098	1.016
TX-0251	4	4392.5	3418	1098.13	1.016
TX-2316	4	4393.5	3418	1098.38	1.017
TX-2317	4	4394.5	3418	1098.63	1.018
TX-0704-1_BROWN	4	4398	3418	1099.5	1.022
TX-0073-2	4	4403.5	3418	1100.88	1.028
TX-0762-1	4	4417	3418	1104.25	1.042
TX-1283-2	4	4418.5	3418	1104.63	1.043
GA 2017005	3	3315.5	2563.5	1105.17	0.905
TX-0286-1	3	3318.5	2563.5	1106.17	0.909
MACHA WR 2 (J. GANNAWAY)	4	4443	3418	1110.75	1.069

GA 2015083	4	4448	3418	1112	1.074
TX-2313	4	4448	3418	1112	1.074
TX-1462-1	4	4452.5	3418	1113.13	1.079
TX-1419	4	4477.5	3418	1119.38	1.105
TX-2376	4	4492	3418	1123	1.12
TX-0488	4	4498	3418	1124.5	1.126
TX-0206	4	4500	3418	1125	1.128
DURANGO	4	4537.5	3418	1134.38	1.167
TX-2389-2	4	4544.5	3418	1136.13	1.175
TX-2403-2	4	4557.5	3418	1139.38	1.188
TX-1154-1	4	4560	3418	1140	1.191
TX-2390-1	3	3420	2563.5	1140	1.031
TX-0073-1	4	4582.5	3418	1145.63	1.214
TX-1326	4	4590	3418	1147.5	1.222
TX-2335-3	4	4591.5	3418	1147.88	1.224
TX-0768-3	4	4599.5	3418	1149.88	1.232
TX-1409-3	4	4606	3418	1151.5	1.239
BR 70-111	4	4613.5	3418	1153.38	1.247
TX-1210-2	4	4618.5	3418	1154.63	1.252
TX-1199-1	3	3467	2563.5	1155.67	1.087
TX-1085-1	4	4658	3418	1164.5	1.293
TX-2022-2	3	3511	2563.5	1170.33	1.14
TX-0665-2	4	4741.5	3418	1185.38	1.38
TX-2347	4	4743.5	3418	1185.88	1.382
GA 2017092	4	4785	3418	1196.25	1.426
TX-1192	4	4787	3418	1196.75	1.428
TX-0762-3	4	4793	3418	1198.25	1.434
UA 7-9	4	4812	3418	1203	1.454
TX-0460-2	4	4830.5	3418	1207.63	1.473
TX-1199-2	3	3626	2563.5	1208.67	1.279
TX-1000-1	4	4864	3418	1216	1.508
TX-1000-2	4	4881	3418	1220.25	1.526
TX-2119	4	4884.5	3418	1221.13	1.529
TX-1975	3	3676.5	2563.5	1225.5	1.34
TX-2348	4	4907	3418	1226.75	1.553
TX-0768-4	4	4908.5	3418	1227.13	1.554
TX-1344	4	4934.5	3418	1233.63	1.581
TX-1327	4	4942	3418	1235.5	1.589
TX-1233	4	4963	3418	1240.75	1.611
TX-0498-1	3	3740	2563.5	1246.67	1.416
TX-0239-2	4	4991.5	3418	1247.88	1.641
SIKES W.R. STAPLE	4	5020.5	3418	1255.13	1.671
TX-0203	4	5072	3418	1268	1.725
TX-0064	3	3840.5	2563.5	1280.17	1.537
TX-1114-1	4	5138.5	3418	1284.63	1.794

TX-2385	4	5173.5	3418	1293.38	1.831
AU14809	3	3881.5	2563.5	1293.83	1.587
TX-0226	3	3909	2563.5	1303	1.62
TX-1409-1	4	5218	3418	1304.5	1.877
TX-0768-2	4	5231	3418	1307.75	1.891
DELFOS 4	4	5246.5	3418	1311.63	1.907
TX-0239-1	4	5253.5	3418	1313.38	1.914
TX-2328	4	5269	3418	1317.25	1.93
TX-2391	4	5272.5	3418	1318.13	1.934
TX-1323	3	3958	2563.5	1319.33	1.679
TX-2382	4	5280	3418	1320	1.942
TX-2318	4	5296.5	3418	1324.13	1.959
TX-0763	4	5298.5	3418	1324.63	1.961
TX-2386	4	5313	3418	1328.25	1.976
TX-0703_BROWN	4	5323.5	3418	1330.88	1.987
TX-0691-2	3	4019.5	2563.5	1339.83	1.753
TX-1003-4	3	4023.5	2563.5	1341.17	1.758
TX-1283-1	4	5472	3418	1368	2.142
WHITE GOLD WILT	4	5551.5	3418	1387.88	2.225
TX-2383	4	5793.5	3418	1448.38	2.478
TX-0762-2	4	5907.5	3418	1476.88	2.597
TX-1403	4	6121	3418	1530.25	2.819
TX-2390-4	2	3075	1709	1537.5	2.013
Kruskal-Wallis Test, Chisquare Approximation					
Chisquare	***Df***	***Prob> chisq***			
462.8406	431	0.1397			

Table 2.11. 2018 Vascular Discoloration (%) in Block 2 with no observable FW incidence

Germplasm	Block 2		
	Total	***Disc.***	***%***
Acala 1517D	33	10	30.3
Chaco 510	34	9	26.5
Delcot 277	62	8	12.9
DELTAPINE 55 (652-679)	27	7	25.9
Dunn 400	23	2	8.7
FJA	30	2	6.7
GA 2015046	27	2	7.4
GA 2016090	23	5	21.7
GA 2016110	18	1	5.6
GA 2017138	21	1	4.8
HART	45	18	40.0
HYC79-6	26	10	38.5
Lockett 77	16	4	25.0
MD 15	12	2	16.7
NM 12Y1004	29	11	37.9
SA-1643xNM 12Y1004	29	11	37.9
SA-1643xNM 12Y1005	9	2	22.2
TERRA 207	33	2	6.1
TIDEWATER #5	85	21	24.7
TIDEWATER 29	44	4	9.1
TX-0135-2	40	6	15.0
TX-0202	30	0	0.0
TX-0373	28	6	21.4
TX-0616-3	36	9	25.0
TX-1151	27	7	25.9
TX-1171-1	32	2	6.3
TX-1409-2	33	13	39.4
TX-2321	26	11	42.3
TX-2357	30	4	13.3

Table 2.12. 2018 ranking of germplasm based on *Fusarium oxysporum* f. sp. *vasinfectum* incidence (%)- Block 2

GERMPLASM	Mean FOV DI (%)
DELTAPINE 55 (652-679)	0
TX-1409-2	0
ACALA 1517D	0
TX-2321	0
HYC79-6	0
TX-0202	0
DUNN 400	0
SA-1643XNM 12Y1004	0
CHACO 510 INTA	0
GA 2015046	0
TX-0616-3	0
LOCKETT 77	0
MD 15	0
TX-1171-1	0
DELCOT 277	0
TX-1151	0
NM 12Y1004	0
TIDEWATER #5 (G. BARB. X G. HIR.)	0
TX-0373	0
TIDEWATER 29	0
TERRA 207	0
FJA	0
SA1643XNM12Y1005	0
TX-2357	0
GA 2016090	0
GA 2016110	0
GA 2017138	0
HART	0
TX-0135-2	0
CAHUGLBBCS-1-88	1.06
DELTAPINE 26	1.12
AUBURN 623 RNR	1.18
CD3HCHULBH-1-88	1.32
REBA 288	1.36
SEABROOK SEA ISLAND 12B2	1.48
AUBURN BR-1	1.52
DP 1646	1.7
DELCOT 311	2.25

TX-2359	2.28
NM 12Y1004XSA1177	2.33
NM 13P1121	2.33
DELTAPINE 1646 (CHECK)	2.33
TX-0043	2.39
DUNN HS 120	2.39
ACALA 1517 WILT	2.44
TX-2369	2.44
GACOT 79	2.5
TX-2322	2.5
NM 12Y1004XSA-2390	2.5
TX-0072-1	2.5
TX-1308	2.5
ARKOT 9704	2.57
TX-0141-2	2.57
CASCOT B-2	2.57
TX-2420-1	2.64
GA 2017112	2.71
TX-0195-1	2.71
MISSDEL W.R. 1	2.71
DUNN 219	2.78
TX-2390-3	2.78
REX 713	2.86
SPEARS UPLAND EARLY LONG STAPLE	3.04
SA-0297	3.04
TX-1003-2	3.13
GA 2017101	3.23
AU90084	3.23
COKER 100 WILT	3.51
NM240162	3.58
TX-0644-1	3.58
REBA B50	4
MCNAIR 220	4.42
TAMLBBFOV45	4.45
GA 2017046	4.55
TX-1270-1	4.66
TX-2373	4.77
NM 13G1018	4.77
TX-0093	4.88
TX-2349	5
COKER 315	5.13
SA-1148XNM 12Y1005	5.13
TX-1003-1	5.13
LAMBRIGHT 2020	5.13

M-315	5.18
TX-0665-1	5.27
TX-0294	5.27
TX-2377	5.27
TX-0180-3	5.27
GA 2017050	5.41
TX-1300-2	5.41
TX-1585-1	5.41
TX-2346	5.56
TX-1042-1	5.56
TX-1283-3	5.56
FELISTANA UA-7-18	5.72
M-315 (CHECK)	5.72
TX-0650-2	5.89
TX-1045	5.89
COKER'S CLEVEWILT #3	5.94
AUBURN 56-24R	5.96
TX-0616-2	6.07
GA 2017091	6.07
TX-1147	6.25
TX-2144	6.25
TX-1085-1	6.46
DEMETER II	6.53
DNWC 1324 (REGISTERED AS GVS 6 ARS	6.67
TX-0681-3	6.9
GA 2016099	7.15
EMPIRE WR 61	7.15
TX-2356	7.5
GA 2017005	7.7
TX-0095	7.7
GA 2016111	7.9
TX-1197	7.9
TX-0400	7.9
HYPERFORMER HYOO7	7.9
TX-0226	8.11
TX-1914-1	8.11
NM 13P1117	8.11
TX-0814	8.11
STONEVILLE 2B (ORIGINAL)	8.34
TX-1462-1	8.34
ARKOT 9623	8.58
TX-0040	8.58
TX-0002-1	8.58
TX-1364-2	8.58

REBA P 279 (REBA B-50 X DPL SMO.)	8.75
GA 2017016	8.83
TX-2352	8.89
TX-2139	9.1
TX-1283-2	9.1
TX-0762-3	9.1
TX-1419	9.31
TX-1054-1	9.38
TX-2119	9.53
TX-0703_WHITE	9.53
TX-0073-1	9.53
TX-2308	9.68
TX-2335-2	9.68
TX-1149-2	9.68
NM 12Y1004XSA-1759	9.68
NAIR GREEN SEED	10
TAMLBBFOV16	10
ROWDEN	10.05
ARKOT 9208	10.35
TX-0495-1	10.53
TX-2360	10.53
PAYMASTER 54	10.53
TX-1196	10.53
TX-1409-1	10.82
GA 2016016	10.82
TX-2354	10.87
TX-0141-1	11.12
MCNAIR TH 149-20 (TRIPLE HYBRID)	11.25
CIANO ALAMOS 92	11.33
TX-0495-2	11.43
NM 13R1015	11.54
TX-1302	11.63
TX-2311	11.77
TX-0570-2	11.91
REBA	11.91
TX-1305	11.91
TX-0621-2	11.91
TX-2358	12.13
COKER 100A (WR)	12.5
TX-0183-1	12.5
GA 2016024	12.5
MD 25	12.5
ACALA SJ-2	12.77

STONEWILT	12.8
SA0460XNM12Y1005	12.83
TX-0597-1	12.83
TX-1003-3	12.83
TX-0932	12.91
AUBURN BR-2	12.91
TX-2386	13.16
TX-1941-1	13.34
TAM 94L-25	13.34
AU91083	13.34
LA HG-063	13.34
TX-0108	13.64
TX-0002-2	13.64
TX-0180-2	13.64
TX-2381	13.89
TX-1199-1	13.89
TX-2420-2	13.96
NEW MEXICO ACALA	14.29
TX-1609	14.29
TX-0931-1	14.64
TX-0072-2	14.64
TX-2391	14.71
TX-1300-3	14.71
TX-0117-1	14.71
TX-2372	14.71
TX-1718	15
COKER'S FOSTER #300	15.16
TX-2323	15.39
TX-0073-2	15.63
GA 2015072	15.63
NM 12Y1004XSA-1555	15.79
TX-1114-3	15.79
TX-0498-1	16.13
TX-0621-1	16.67
TX-0616-1	16.67
TX-1364-3	16.67
TX-0931-3	17.03
TX-0762-1	17.15
GA 2017092	17.15
TX-0704-1_WHITE	17.15
TX-0933	17.4
TX-2376	17.5
TX-2348	17.5
GA 2017035	17.5
TX-1205-1	17.5

STONEVILLE 603	17.57
TX-1304	17.65
TX-1306	17.86
TX-1300-1	17.86
TX-0498-3	17.95
TX-1042-2	18.19
TX-1418	18.19
TX-0122-2	18.43
GA 2017136	18.75
TX-2385	18.92
DELFOS 4	19.15
GA 2016056	19.36
SA1643XNM12Y1004	20
TX-0183-2	20
TX-1425-1	20
GA 2015026	20.59
ARKOT 0305	20.84
TX-2362	21.06
TX-2022-2	21.06
TX-0117-2	21.22
MISCOT 7803-52	21.43
TX-2402	21.43
TX-1412	21.63
WEST TEXAS ROUGH	21.63
TX-2384	21.63
TX-1283-1	21.88
NM 13P1088	21.96
BR 69-120	22.23
TX-1215	22.5
TX-1210-1	22.59
TX-0763	22.59
TX-1201	22.86
TX-1000-3	22.86
TX-1364-1	23.08
SA-1049	23.08
GA 2017073	23.34
SA-0404	23.34
TX-0931-2	23.69
TX-1270-2	23.81
TX-2324	24.25
GA 2016052	24.33
TX-0625	25
GA 2016060	25
STROMPROOF CA 119-1/29	25
TX-2315	25

TX-2361	25
TX-1870	25
ARKOT 9811	25
TX-1154-3	25.72
MACHA WR 2 (J. GANNAWAY)	26.05
TX-0122-1	26.09
TX-1321	26.32
TX-1585-3	27.03
AUBURN 56	27.28
TX-0244	27.78
TX-2389-1	27.78
TX-0749-2	27.78
GA 2017053	27.78
TX-0665-2	27.78
TX-0703_BROWN	28.13
AU14809	28.95
MEBANE	28.95
TX-1327	29.17
TX-2363	29.42
CEDIX	29.73
MAR5PD208S-4-90	29.86
TX-2365	30
TX-1000-1	30
TX-1054-2	30
TX-0240	30.31
ROWDEN #3	30.44
TX-0681-1	30.56
WHITE GOLD WILT	30.77
TX-1975	30.77
TX-1114-2	30.77
NM 12Y1005	31.04
TX-0239-2	31.25
TX-1307	31.43
TX-0251	31.43
TIDEWATER (SEABROOKS) (G.B.X G.H.)	31.58
TX-0101	31.71
TX-2074	32.36
NM 12Y1004XSA-0550	32.44
TX-2335-1	32.44
TX-0681-2	32.5
ROWDEN (CHECK)	33.34
TX-1964	33.34
TX-0490-2	33.34

QUAPAW	34.38
TX-0195-2	34.38
TX-0672	35.14
TX-2366	35.14
TX-0085	35.3
TX-0286-1	35.56
TX-2347	35.72
COKER'S WILDS #9	35.9
TX-0709	36.12
TAMLBBFOV30	36.12
TX-1076	36.37
TX-0691-1	36.67
TX-1213	36.67
TX-0488	37.5
GA 2017038	37.94
TX-2403-1	38.3
TX-0704-1_BROWN	38.64
TX-0597-2	38.89
TX-1311	38.89
TX-2328	38.89
TX-2317	39.4
TX-2390-4	39.48
TX-0768-4	40
TX-0650-1	40
TX-1301	40
TX-1115	40.55
TX-0135-3	40.63
UA 7-9	40.91
TX-2394	41.18
TX-0490-1	42.43
TX-0984	42.43
TX-0062	42.86
BALLARD CLEAN SEED HIGH LINT	42.86
WASHINGTON	42.86
TX-1154-2	43.25
TX-1303	44
TX-2403-2	44.12
FTA	44.27
TX-0021	44.45
TX-0206	45.46
GA 2017126	45.46
TX-1154-1	46.43
TX-1409-3	47.37
TX-0681	47.5

FIBER MAX 832	47.83
TX-0460-1	48.15
TX-1125	48.15
TX-1178	48.65
TX-1008	48.65
TX-1171-2	48.72
GA 2016025	50
TRICE 2A	50
TX-1344	51.52
TX-0180-1	51.62
TX-2316	52.78
TX-0064	53.49
TX-1094	54.06
TX-1233	54.06
TX-2375	54.06
TX-0647	54.29
SIOKRA	54.29
LA HG-660	54.35
TX-2353	54.55
TIFCOT 56	56.1
TX-1941-2	56.53
ARKOT 8717	57.9
GA 2017106	58.63
TX-0460-2	58.83
SIKES W.R. STAPLE	58.83
TX-0704-2	59.53
TX-1003-4	60.53
TX-1212	60.61
TX-2335-3	60.72
TX-1192	61.12
SEALAND 883	61.77
BR 70-111	62.86
TX-1322	62.86
GA 2015083	62.97
TX-1211	63.42
TX-0768-1	63.64
TX-1000-2	63.64
TX-0073-4	64.29
TX-0135-1	65.52
TX-0691-2	65.63
TX-2382	65.72
TX-2319	66.67
TX-2022-1	66.67
TX-0644-2	66.67
TX-1326	66.67

TX-1114-1	67.5
TX-1199-2	67.65
TX-1063	68.97
TX-0239-1	69.45
TX-2364	69.45
TX-1323	69.45
TX-2355	70
TX-1403	70.28
DELTATYPE WEBBER	71.43
TX-1109	71.43
TX-0768-2	73.08
DURANGO	74.36
TX-1194-1	75
TX-2374	76.93
TX-2403-3	76.93
TX-1194-2	78.38
TX-2318	78.58
GA 2015092	80
TX-2390-1	80.56
TX-1210-2	81.09
TX-0762-2	81.58
TX-0203	81.82
TX-2313	85.3
TX-1122	86.67
TX-0768-3	86.67
TX-2383	86.85
TX-1462-3	87.1
TX-0122-3	90.63
MARSHALL	94.12
TX-2320	96.78
TX-2389-2	100

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CHAPTER 3

FIELD EVALUATION OF COTTON GERMPLASM FOR RESISTANCE TO FOLIAR DISEASES TARGET SPOT AND AREOLATE MILDEW[1]

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Abstract

Areolate mildew (*Ramulariopsis* spp.) and target spot (*Corynespora cassiicola*) are emerging and important foliar diseases of cotton, particularly in regions like Brazil, India, and the southeastern United States. This study evaluates 251 diverse cotton genotypes, including wild, obsolete, and elite lines, for resistance to both foliar diseases during field trials in 2018 and 2019. Using a 1 to 5 severity scale, two lines were identified as being resistant to areolate mildew, Brazilian cultivar BRS 372, identified as having resistance in Brazil, and a Texas A&M breeding line 7-7-1020CT. No lines were identified to be resistant to target spot, but germplasm varied in response, providing moderate resistance at best. TX 1008 and TX 0762-2 exhibited moderate resistance to both areolate mildew and target, suggesting potential in future breeding. The results from this study provide knowledge not previously known about cotton germplasm regarding target spot and areolate mildew resistance and identifies lines resistant to areolate mildew not previously known in the United States. This offers a foundation of knowledge regarding cotton germplasm by providing insight on diverse levels of foliar disease resistance. This will provide information for further research to focus on identifying genetic markers associated with resistance, aiding in the development of new, resistant cotton varieties to enhance productivity in cotton growing regions that experience yield loss from target spot and areolate mildew.

Introduction

Upland cotton (*Gossypium hirsutum.*) is predominantly cultivated in tropical and subtropical regions, where the hot, humid environment provides ideal conditions for various foliar diseases. These diseases can severely impact cotton productivity by damaging the leaves, which are critical for photosynthesis, the plant's primary energy source. Prolonged wet periods combined with warm temperatures accelerate the disease cycle of many fungal and bacterial

pathogens, increasing both the severity and incidence of foliar diseases. Fungal diseases caused by *Corynespora cassiicolla* (target spot), *Ramulariopsis* spp. (areolate mildew), and bacterial disease *Xanthomonas citri* subsp. *malvacearum* (bacterial blight) represent the three most important foliar diseases in cotton production. Bacterial blight, also known as angular leaf spot, is a serious and widespread disease, but the planting of resistant varieties has largely mitigated yield loss in most countries (Wheeler et al. 2007; Wheeler and Dever 2020). However, resistant varieties are not widely available or yet to be developed for areolate mildew and target spot, two of the most economically important foliar diseases of upland cotton that reduce yield in Upland cotton (*Gossypium hirsutum* L.) (Galbieri et al., 2014; Bowen et al., 2018, Crop Disease Loss Calculator, 2024).

Areolate mildew, sometimes referred to as grey mold or Ramularia leaf spot, is caused by two *Ramulariopsis species, R. pseudoglycines* (Synonyms: *Ramularia areola* Atk., *Ramulariopsis gossypii* (Speg.) U. Braun and *R. gossypii* (Speg.) Ciferi, *Cercosporella gossypii* Speg.). First identified in the United States in 1890 (Atkinson, 1890), Areolate mildew has now been identified in most major cotton production countries such as Brazil, India, and East Africa. Infections of areolate mildew typically appear on lower leaves initially as irregular lesions, but as lesions mature in high humidity environments, they darken and take on a necrotic appearance, leading to reduced photosynthesis, defoliation, and premature boll opening. (Bell, 1981; da Silva, 2019). Target spot was first identified in Alabama in 1959 on upland cotton (Jones, 1961). Target spot affects cotton by producing lesions that start in the lower canopy, progressively moving up the plant. Lesions from target spot appear to be irregular in shape and initially start as small specks that progress to a large ‘target’ shape as maturity of infection progresses. Mature lesions are typically dark brown and, in some cases, produce a light

green to yellow halo around the infected area. Severe infection from both areolate mildew and target spot causes premature defoliation resulting in yield loss (Galbieri et al., 2014; Bowen et al., 2018).

In the southeastern United States, both areolate mildew and target spot commonly appear later in the season, during or near boll maturity, hence contributing limited impact on yield (Hagan et al. 2018). However, in recent years, early season infection that resulted in defoliation and yield loss from areolate mildew equated to 34,385 bales in 2022 ($15,105,242) and 32,145 bales in 2023 ($11,983,004), whereas yield loss from target spot was 30,798 bales in 2022 ($13,529,359) and 14,374 bales in 2023 ($5,358,357) have been reported according to the Cotton Disease Loss Calculator (Crop Protection Network, 2024). Further, areolate mildew has now spread across the entire Southeast and recently into the Mid-south cotton fields (Conner et al., 2013; Faske, 2013; Price et al., 2015; Butler et al.; 2016; Shultz et al., 2017; Sumabat et al., 2018, Conner et al., 2023). The shift of these diseases from being non-existent to becoming significant pests targeted in cotton disease management programs is not fully understood. However, this may be related to a combination of factors such as changes in commercially grown varieties, a shift in breeding objectives throughout the last few decades, alterations in disease cycles due to climate change, and pathogen species shift or evolution.

While areolate mildew and target spot in previous years have only caused occasional damage and yield loss in the Unites States, they historically have been the two major foliar diseases responsible for significant yield loss in South America. For example, the Cerrado region of Brazil, which accounts for approximately 80% of the country's 1 million hectares of cotton production, has a sub-tropical climate that is conducive for target spot and areolate mildew, making these diseases critical concerns in cotton production management (da Silva et al., 2019;

Suassuna et al., 2019; Souza et al., 2020). Areolate mildew currently requires as many as eight fungicide applications each growing season, resulting in costs of approximately $160 million in input cost (da Silva et al., 2019). Due to the susceptibility of most commercial cotton varieties grown in Brazil, areolate mildew is currently regarded as the most prominent disease in the country's cotton production (Suassuna et al., 2008; Tormen et al., 2019; da Silva et al., 2023). Target spot also has recently been shown to cause yield loss in Brazil but can be managed with fungicides. Key management is protecting the lower canopy and managing plant height (Galbieri et al., 2014; Souza et al., 2020). Due to the costs associated with fungicide applications, resistant or tolerant varieties to these foliar diseases are important within integrated pest management practices (Hagan, 2014; Hagan et al. 2018).

Since both target spot and areolate mildew have historically been considered insignificant problems in the United States, breeding efforts have not specifically focused on these diseases. Currently, there are no commercial varieties that are resistant to target spot, but there are varying levels of tolerance and susceptibility among different elite cultivars (Hagen et al., 2015; Hagan et al., 2018; Hagan et al., 2020). For example, PhytoGen brand PHY 499 WRF (PHY 499; PhytoGen Cottonseed; Dow AgroSciences, Indianapolis, IN) has historically shown susceptibility to target spot, whereas varieties like Deltapine brand DP 1252 B2RF (Deltapine Cottonseed; Bayer, St. Louis, MO) exhibited better tolerance (Hagen et al., 2013, Hagen et al, 2014; Hagen et al., 2015). While no commercial cotton varieties in the United States are currently marketed as resistance to areolate mildew, cotton germplasm with high levels of resistance have been identified (Beasley et al. 2024). Cotton breeding in Brazil has emphasized the development of resistance varieties to areolate mildew due to its devastating impact on cotton

production in that country. According to Suassuna et al. (2020), a few commercial cultivars have resistance to areolate mildew including BRS 372 and BRS 416.

Resistant varieties play a crucial role in managing plant diseases in cotton production. (Egan and Stiller, 2022). However, the limited understanding of resistant germplasm sources for areolate mildew and target spot has hindered breeding efforts for resistance in the United States. The objective of this study was to conduct field evaluations to identify resistance in a diverse set of cotton germplasm, including wild, obsolete, and elite genotypes of *Gossypium hirsutum*, against areolate mildew and target spot.

Materials and Methods

Cotton genotypes.

The genotypes evaluated for resistance to areolate mildew (AM) and target spot (TS) include 251 cotton lines, representing a genetically diverse set of cotton germplasm (Table 3.1 and 3.2). They include 190 wild *G. hirsutum* (TX) accessions from the USDA Cotton germplasm collection, 23 obsolete upland varieties, and 31 elite breeding germplasm lines from the Regional Breeding Testing Network (RBTN; http://rbtn.cottoninc.com/files/), and three *G. barbadense* lines were included as an outgroup in the experiment. Finally, three commercial conventional varieties DP 393, DP 493, and UA 222, and one susceptible commercial check cultivar Phytogen 499 WRF were included in the total.

Field evaluations

The evaluation for resistance to TS and AM was conducted at the University of Georgia Gibbs Farm in Tifton, GA in 2018 and 2019 under natural infection. In 2018, all 251 genotypes were planted in a randomized complete block design (RCBD) with three replications. The plots

were single rows, spaced 1 m apart, and approximately 7 m long, planted at a seeding rate of approximately 60 seeds per plot. A severity scale from 1 to 5 was used in both disease ratings as follows: 1- no symptoms (lesions-target spot or spots-areolate mildew); 2- lesions or spots on the leaves in the lower third of the plants; 3-25% lesions or spots on leaves in the middle third of the plants; 4- 50% leaf covered with lesions or spots on the leaves in the upper third of the plants and defoliation begins, and 5- 95% leaf covered with lesions or spots with intense defoliation throughout the plant (Fig. 3.1). Based on the rating outlined above, genotypes were classified as resistant when the average rating was 1-2, moderately resistant or tolerant when the average rating was $2 \geq 3$, moderately susceptible when the average rating was $3 \geq 4$, and susceptible when the average rating was ≥ 4. Disease ratings for both TS and AM were taken on September 26 and 27 of 2018. All germplasm lines were compared to the susceptible check, PHY 499 WRF, to assess which lines to cull and which to advance to the second year of screening.

The 2019 screening included two separate trials for each disease; the target spot trial, which included 26 lines, and the areolate mildew trial, which consisted of 52 genotypes. Again, the commercial cultivar Phytogen 499 WRF was included in each trial as the susceptible check. In both trials, the plots for each genotype were single rows spaced 1m apart and 8.3 m long, planted at a seeding rate of approximately 75 seeds per plot. The plots were planted in a randomized complete block design (RCBD) with six replications. To promote disease development, a row of the susceptible check, Phytogen 499WRF, was planted in the first and every fourth rows thereafter, such that it appeared in the adjacent left or right rows of each experimental lines. Also, the cultivar BRS 372 developed by EMBRAPA (Brazil) was included in the areolate mildew trial as a putative resistant check (Suassuna et al., 2020). Each plot was rated according to the same severity scales used in 2018. Disease ratings were made initially on

September 14, 2019, and continued approximately every two weeks until November 14, 2019, accounting for five total evaluations.

Data analysis

JMP®, Version 17.0 SAS Institute Inc., Cary, NC, 1989–2023 was utilized to perform a one-way ANOVA with blocking and a Student's t-test was used for multiple comparisons of mean disease ratings to determine whether there is a significant difference between genotypes. A p value of 0.05 was used to compare differences. In the 2018 data set, the final severity assessment was used to rank the resistance of each germplasm. A similar analysis was conducted in 2019, again using the final severity assessment to evaluate and rank the germplasm lines.

Results

Target spot

In the preliminary screening conducted in 2018, germplasm responses to TS were compared to the susceptible check Phytogen 499, which has a rating of 4.15, indicating that the levels of inoculum in the field was sufficiently high to produce a susceptible reaction. In the ANOVA, the germplasm source showed a significant impact ($p<0.05$), as reflected by an F ratio of 2.4082 and a p-value of less than 0.0001 (Table 3.5). This indicates that differences in germplasm had a highly significant effect on the target spot rating. The block effect was not statistically significant, with an F ratio of 1.4837 and a p-value of 0.2277, indicating that replications did not have a meaningful influence on the rating results. Among the wild accessions, 13 were rated as resistant (1-2), 130 lines rated as moderately resistant ($2\underline{\geq}3$), 46 lines were rated as moderately susceptible range ($3\underline{\geq}4$), and 1 line was rated as susceptible ($\underline{\geq}4$). In the elite breeding lines, one was rated as resistant, 19 were rated as moderately resistant, 14 as

moderately susceptible, and one line as susceptible. In the obsolete lines, none were rated as resistant, 9 were rated as moderately resistant, 14 were moderately susceptible, and no lines rated as susceptible. Of the three *G. barbadense* genotypes, one line was rated as moderately resistant and two rated as moderately susceptible (Table 3.1; Figure 3.3). Based on this classification, the preliminary screening showed that 14 genotypes, which were primarily from the wild TX-germplasm, were resistant to target spot. All the elite lines were moderately resistant to susceptible with no substantial host plant resistance related to target spot except for one line, TAM LBB150824 which had a severity rating between 1-2. Twenty-six lines with observed resistance were selected to be evaluated in the 2019 experiment.

In the 2019 screening, the susceptible check Phytogen 499 WRF had the highest mean severity rating of 4.17, indicating that the levels of inoculum in the field were sufficient to produce a susceptible reaction (Table 3.3). The ANOVA revealed a significant genotypic effect ($p<0.05$), as reflected by an F ratio of 6.4827 and a p-value of less than 0.0001, demonstrating that differences in germplasm had a significant impact on the response to target spot. The block effect was not statistically significant, with an F ratio of 0.2618 and a p-value of 0.2618. Of the 26 genotypes tested, 18 were moderately susceptible (ROWDEN, TX 2394, TX 2386, TX 2402, TX 0180-3, TX 1197, TX 0062, TX 0762-2, TX 2139, TX 2315, TX 0490-1, TX 0681, TX 1054-2, TX 1063, TX 1114-2, TX 1302, TX 1311, TX 1718). These genotypes exhibit a noticeable level of disease but show slightly better performance than the susceptible group. Eight lines were in the moderately resistant or tolerant category (TX 0002-2, TX 2335-2, TX 1154-2, TX 1008, TX 1045, TX 1270, TX 1283-2, and TX 2357). There were no lines within the study that were observed to have resistance (Table 3.3).

Areolate Mildew

In the 2018 preliminary screening for areolate mildew resistance, germplasm responses to areolate mildew were compared to the susceptible check Phytogen 499, which had a rating of 3.8, indicating that the levels of inoculum in the field were sufficiently high to produce a susceptible reaction. In the ANOVA, the germplasm source showed a significant impact ($p<0.05$) related to the variation observed, as reflected by an F ratio of 5.2853 and a p-value of less than 0.0001. The block effect was not statistically significant, with an F ratio of 0.3132 and a p-value of 0.7312 (Table 3.6). Among the wild accessions, 37 were classified as highly resistant (1-2), 77 lines were moderately resistant or tolerant ($2\underline{\geq}3$), 69 lines were moderately susceptible ($3\underline{\geq}4$), and 7 lines were susceptible (>4). Elite germplasm had only one line rated as resistant, three lines were moderately resistant, 24 were moderately susceptible, and 7 lines fell into the susceptible category. Obsolete germplasm showed no lines as resistant, five lines were moderately resistant, 13 lines were moderately susceptible, and five lines were susceptible. The *G. barbadense* germplasm demonstrated resistance entirely, with all 3 lines rated 1-2. (Table 3.2 and Figure 3.4). In total, 41 genotypes were identified as resistant sources. These were primarily from wild accessions, *G. barbadense* lines, and one elite breeding line, 7-7-1020-CT. There were a portion of wild accessions more susceptible than Phytogen 499, which indicates a wide range of genetic diversity within that class of germplasm.

In the 2019 screening, the susceptible check Phytogen 499 WRF had the highest mean severity rating of 4.5, indicating that the levels of inoculum in the field was sufficient to produce a susceptible reaction. The ANOVA revealed a significant genotypic effect (when $p<0.05$), as reflected by an F ratio of 8.6873 and a p-value of less than 0.0001. The block effect was statistically significant, with an F ratio of 3.7904 and a p-value of 0.0025, suggesting that block

variation may have had an influence on the results. Mean disease severity ranged from 3.05- 3.33 across blocks. The results identified 43 genotypes with moderate susceptibility. Seven lines were rated as moderately resistant (TX 0762-2, TX 1212, Pima S-6, TX 0768-2, TX 1008, GB-0853 and GB-1049). One elite breeding line, 7-7-1020-CT, with a rating of 2.0, showed excellent resistance. Finally, BRS 372 had the lowest severity rating of 1.25 deeming it the most resistant line among those tested (Table 3.4).

Cross resistance

A total of 78 genotypes were tested in both the 2019 target spot and areolate mildew nurseries. Based on the screening results, two were moderately resistant to both diseases. For example, TX 1008 stands out with a target spot severity mean of 2.83 and an areolate mildew severity mean of 2.75, alluding to moderate resistance to both diseases. Similarly, TX 0762-2 shows consistent tolerance to both foliar diseases with a target spot severity of 3.08 and an areolate mildew severity of 2.92. These two accessions are of interest given they exhibit moderate disease severity ratings for both pathogens.

Discussion

Currently, areolate mildew and target spot are two of the most economically important foliar diseases affecting upland cotton in both Brazil and the United States. Areolate mildew has become a major challenge for cotton production in Brazil, especially in the Cerrado region, which accounts for most the country's cotton output (da Silva et al., 2019). The high susceptibility of popular commercial cotton varieties in the region has exacerbated the problem, making both diseases a key concern for growers. Controlling areolate mildew with fungicide applications, can reach $160 million of annual input costs in this geography (da Silva et al.,

2019). The dependence on fungicides raises concerns, not only about economic sustainability, but also the possibility for resistance development in the pathogen population (Mathioni et al., 2022; da Silva et al., 2023). Efforts to develop resistant cotton varieties are ongoing, but the widespread use of susceptible varieties continues to pose challenges for managing this disease in Brazil (Morello et al., 2010; Morello et al., 2012; Morello et al., 2015; Suassuna et al., 2020). This situation emphasizes the need for integrated disease management strategies, which should include new resistant varieties that deploy multiple sources of genetic resistance to reduce the impact of areolate mildew (Suassuna et al., 2008; Tormen et al., 2019). By focusing on breeding for resistance and combining it with good cultural practices, fungicide resistance management, and timely fungicide applications, growers can better manage target spot and areolate mildew to mitigate economic impact.

Comparatively in the United States, efforts to control foliar diseases has primarily been achieved through the use of fungicides (Hagan et al. 2019; Kichler and Kemerait 2022; Hagan et al. 2022). Fungicides, predominantly strobilurins and SDHIs, have been shown to significantly reduce target spot and areolate mildew progression, which in return provides growers with the ability to protect yields and improve fiber quality compared to untreated cotton. In areas with significant disease pressure, two or more fungicide applications may be necessary to provide adequate yield protection (Hagan et al. 2022; Kichler and Kemerait 2022). In 2019, foliar diseases, primarily areolate mildew, with a smaller contribution from target spot, resulted in an estimated $4.3 million in economic losses for Georgia cotton growers (Kichler and Kemerait 2022). While fungicides are effective in managing both target spot and areolate mildew, the development and use of resistant cultivars offers the most economical and environmentally benign management tool for their control. Because germplasm resistance to areolate mildew and

target spot have not been reported in the United States, this study identifies important genetic resistant germplasm sources as well as sources that are not applicable to breeding for disease resistance because of susceptibility.

Observations from the 2018 and 2019 disease nursery showed that resistance to target spot resistance did not seem as profound as areolate mildew. For target spot, the range of severity was 2.83 to 4.17 with TX 1008, TX 1045, TX 1270, TX 1283-2, and TX 2357 having the lowest severity ratings in 2019. All the genotypes tested in 2019 had lower target spot severity than the check Phytogen 499 WRF. For areolate mildew resistance, the second-year evaluation in 2019 validated the preliminary evaluation, which suggested wild accessions and an elite breeding line, 7-7-1020CT, were resistant to areolate mildew. This germplasm line was derived from a reselection of CA 3084, consisting of a complex pedigree that may include Del Cerro, Acala, and Pima backgrounds, which was developed in 1987 by the TAES cotton breeding program at El Paso, TX. Areolate mildew resistance was identified 7-7-1020CT by chance through these disease screening experiments, and also unexpected as areolate mildew resistance was not originally a focus when this line was developed. This unexpected resistance exemplifies the value of screening a diverse group of germplasm. Efforts from this study have contributed to 7-7-1020CT being jointly released as areolate mildew resistant germplasm by the University of Georgia and Texas A&M AgriLife Research (Beasley et al. 2024), offering a potential novel source of genetic resistance.

The cultivar BRS 372, developed by the Brazilian Agricultural Research Corporation (EMBRAPA), is known for providing resistance to the Brazilian biotypes of the *Ramulariopsis pseudoglycines* (Suassuna et al. 2020). This line was included in the 2019 areolate mildew nursery when seeds became available. The addition of this line provided a valuable opportunity

to determine if resistant traits identified in Brazilian cotton germplasm perform similarly in the United States. The results indicate that BRS 372 exhibits resistance levels superior but similar to 7-7-1020CT (Table 3.4). Because BRS 372 and 7-7-1020CT do not share common parentage (Suassuna et al. 2020; Beasley et al., 2024), it is possible that they carry different resistance genes or different alleles for the same gene. Future work will focus on determining whether the resistance observed in these lines, particularly in 7-7-1020CT, is conferred by unique resistance genes through genetic mapping. Should 7-7-1020CT prove to be a novel source of resistance, stacking these resistance genes would enhance the development of cotton varieties with durable resistance to areolate mildew.

Resistance to target spot was classified primarily as moderately resistant and was observed only in the wild TX accessions (Table 3.3). The level of resistance identified was not as notable as areolate mildew resistance. However, the wild TX lines offer promising utility for cotton breeding programs, especially given the limited target spot resistance observed in the improved and elite germplasm. Because the TX wild accessions are highly genetically distinct from elite cotton varieties, introducing resistant traits from this germplasm into elite genetic background may require extensive breeding efforts to maintain fiber quality and yield while incorporating target spot resistance.

This study identified unique genotypes from the wild accessions, TX 1008 and TX 0762-2, that showed tolerance to both foliar diseases. While the resistance observed in these two accessions are not as strong as BRS 372 or 7-7-1020CT, they are promising candidates for breeding efforts aimed at developing cotton varieties with improved tolerance to both target spot and areolate mildew. This could be beneficial when incorporating with other management

variables such as fungicides that, in return, could limit the disease severity of both diseases when grower application timing is not adequate.

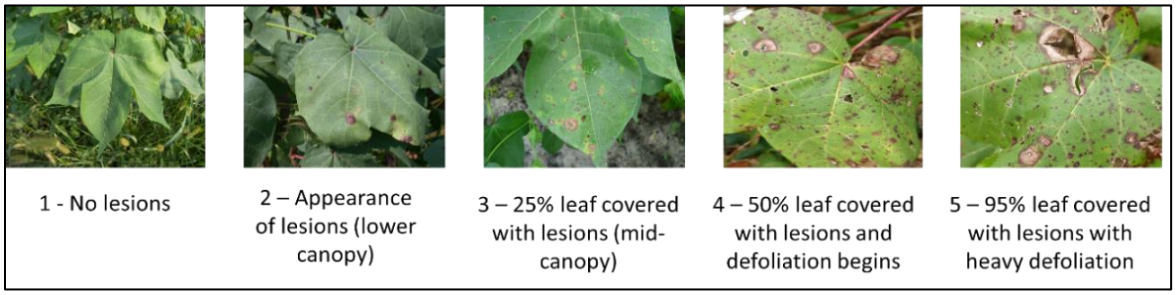

Figure 3.1. Target spot severity rating scale

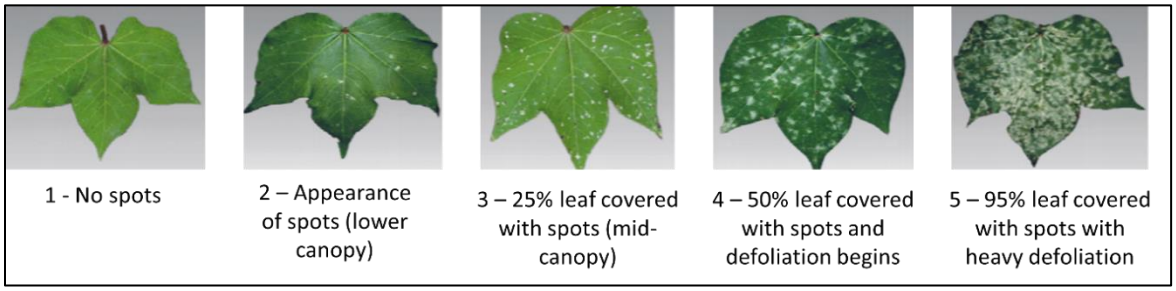

Figure 3.2. Areolate mildew severity rating scale

Table 3.1: 2018 classification of germplasm lines based on target spot ratings.

	1-5 Disease Rating Scale for Target Spot Severity			
Genotypes	(1-2) **R***	(2≥3) **MR**	(3≥4) **MS**	(4≥5) **S**
Wild/Unimproved (TX-)	13	130	46	1
Elite	1	19	14	1
Obsolete	0	9	14	0
G. barbadense	0	1	2	0

*R-Resistant, MR-Moderately Resistant, MS-Moderately Susceptible, S-Susceptible

Table 3.2: 2018 classification of germplasm lines based on areolate mildew ratings.

	1-5 Disease Rating Scale for Areolate Mildew Severity			
Genotypes	(1-2) **R***	(2≥3) **MR**	(3≥4) **MS**	(4≥5) **S**
Wild/Unimproved (TX-)	37	77	69	7
Elite	1	3	24	7
Obsolete	0	5	13	5
G. barbadense	3	0	0	0

*R-Resistant, MR-Moderately Resistant, MS-Moderately Susceptible, S-Susceptible

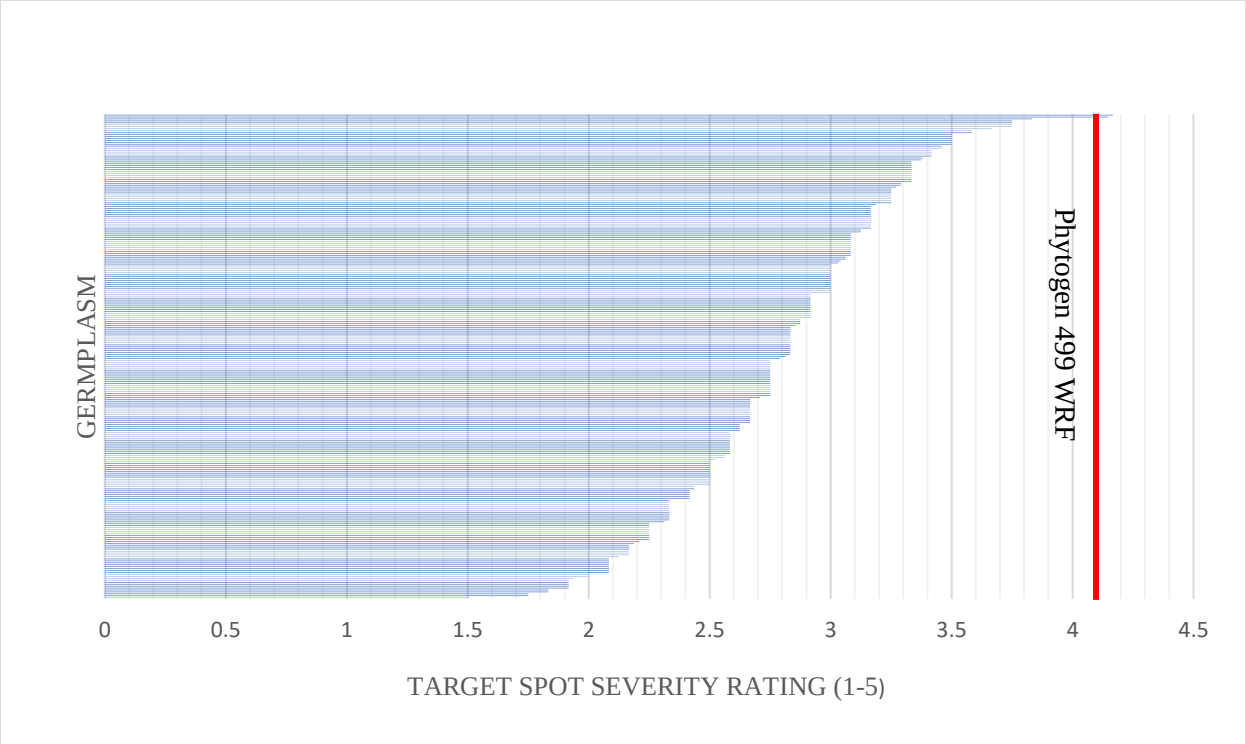

Figure 3.3 Target spot severity by germplasm, 2018.

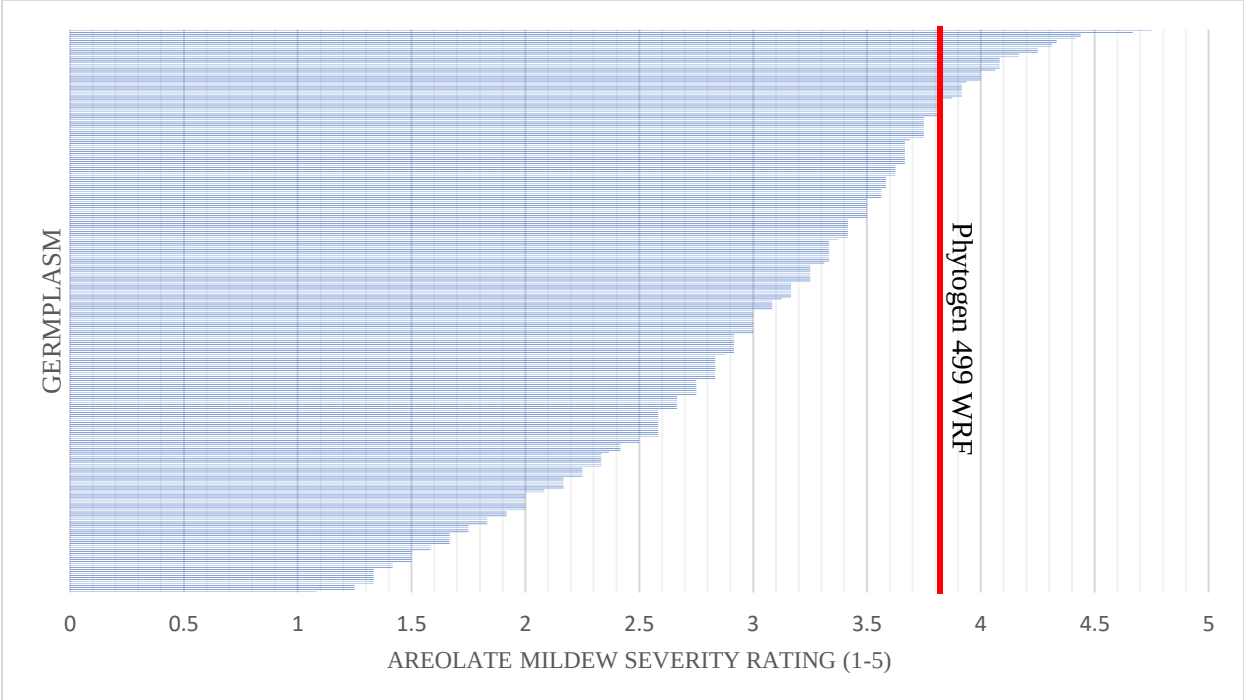

Figure 3.4 Areolate mildew severity by germplasm, 2018.

Table 3.3. 2019 germplasm screening for resistance to target spot

GERMPLASM	***LSD=0.333***	***TS Severity Mean***
PHYTOGEN 499 WRF	A	4.17
ROWDEN	B	3.75
TX 2394	C	3.42
TX 2386	CD	3.33
TX 2402	CDE	3.25
TX 0180-3	CDEF	3.17
TX 1197	CDEF	3.17
TX 0062	DEFG	3.08
TX 0762-2	DEFG	3.08
TX 2139	DEFG	3.08
TX 2315	DEFG	3.08
TX 0490-1	EFG	3.00
TX 0681	EFG	3.00
TX 1054-2	EFG	3.00
TX 1063	EFG	3.00
TX 1114-2	EFG	3.00
TX 1302	EFG	3.00
TX 1311	EFG	3.00
TX 1718	EFG	3.00
TX 0002-2	FG	2.92
TX 2335-2	FG	2.92
TX 1154-2	FG	2.90
TX 1008	G	2.83
TX 1045	G	2.83
TX 1270	G	2.83
TX 1283-2	G	2.83
TX 2357	G	2.83

Target spot severity ratings taken in 2019. Values with the same letter are not statistically different. Lower value indicates less disease**.**

Table 3.4. 2019 germplasm screening for areolate mildew resistance

GERMPLASM	*LSD=0.41667*	*Mean AW Severity*
PHYTOGEN 499WRF	A	4.5
GA 230	B	3.9
TEXAS RED	BC	3.67
TX 0704-B	BCD	3.5
TX 0203	BCD	3.5
TX 2403-2	CDE	3.42
TX 2369	CDE	3.42
SA-0297	CDE	3.42
SA-0401	CDE	3.42
TX 1063	CDE	3.42
TX 0616-2	CDE	3.42
TX 1941-2	CDE	3.4
TX 2372	CDE	3.4
TX 0073-1	CDE	3.33
TX-1283-3	CDE	3.33
TX 1419	CDE	3.33
SA-1269	CDE	3.33
TX 2311-1	CDE	3.33
TX 0183-2	CDE	3.33
TX 0226	CDE	3.33
TX 1210-2	CDE	3.33
TX 0621-2	CDE	3.33
TX 0665-2	CDE	3.33
TX-1364-2	CDE	3.33
TX-2335-3	CDE	3.33
TX 0621-1	CDEF	3.3
TX 0763	DEF	3.25
TX 2390	DEF	3.25
TX 0085	DEF	3.25
TX 0570-2	DEF	3.25
TX 0135-2	DEF	3.2
TX-1205-1	DEF	3.2
TX 1114-2	DEF	3.17
TX 1122	DEF	3.17
TX 0239	DEF	3.17
TX 1283-2	DEF	3.17
TX 1311	DEF	3.17
TX 1364	DEF	3.17
TX 1964	DEF	3.17
SA-2379	DEF	3.17

TX 0931-1	DEF	3.17
TX 1364-2	DEFG	3.1
TX 1718	EFG	3.08
TX 0814	EFG	3.08
TX 0762-2	FGH	2.92
TX 1212	FGH	2.92
PIMA S-6	FGH	2.92
TX 0768-2	FGH	2.9
TX 1008	GH	2.75
GB-0853	GH	2.75
GB-1049	H	2.67
7-7-1020-CT	I	2
BRS 372	J	1.25

Ramularia spp. (areolate mildew) severity ratings taken in 2019. Values with the sample letter are not statistically different. Lower value indicates less disease.

Table 3.5. ANOVA results for 2018 target spot evaluation**.**

Analysis of Variance

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	252	185.92831	0.737811	2.4082
Error	609	179.53206	0.30639	Prob > F
C. Total	838	365.46037		<.0001

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Germplasm	250	250	184.92633	2.4144	<.0001
Block	2	2	0.90909	1.4837	0.2277

Table 3.6. ANOVA results for 2018 areolate mildew evaluation.

Analysis of Variance

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	252	561.96820	2.23003	5.2853
Error	568	247.25281	0.42193	Prob > F
C. Total	838	809.22101		<.0001

Effect Tests

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Germplasm	250	250	561.77597	5.3257	<.0001
Block	2	2	0.24628	0.3132	0.7312

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CHAPTER 4

REGISTRATION OF CA 4011 COTTON GERMPLASM LINE WITH RESISTANCE TO AREOLATE MILDEW AND TOLERANCE TO THRIPS[1]

[1] Beasley, E. D., Wann, D., Shanbhad, S., Lubbers, E., Suassuna, N. D., Jones, D. C., Kelly, C. M., Dever, J. K., & Chee, P. W. Accepted by the *Journal of Plant Registrations.* Reprinted here with permission of the publisher, Wiley Publishing.

Abstract

CA 4011 (Reg. no. GP-1149, PI 705597) is a noncommercial breeding line of cotton (*Gossypium hirsutum* L.) jointly released by Texas A&M AgriLife Research and the Agricultural Experiment Station at the University of Georgia-Tifton. This cotton germplasm is a selection from CA 3084, a germplasm line released by Texas Agricultural Experiment Station in 1987. CA 3084 was derived from a cross of EPSM-75-AAAA-3 and EPSM-1224-1-74-2-4-2-1, historical breeding lines developed by the cotton breeding program at Texas Agricultural Experiment Station in El Paso. Progeny row of CA 4011 was selected for 2008 preliminary yield testing in Southern High Plains by the Texas A&M AgriLife Research cotton breeding program in Lubbock. In 2012, CA 4011 had less damage from thrips feeding injury than 22 other genotypes tested in a greenhouse assay but was equal to the resistant check TX110 (PI 163608). Performance testing for yield, fiber quality, and other related agronomic properties was conducted under organic management and on certified organic farms during 2012, 2013, and 2014 growing seasons. CA 4011 showed comparable yield and fiber quality to standard check cultivars grown in the Southern High Plains. Subsequent testing was done at the University of Georgia Coastal Plains Experiment Station in Tifton, GA, to evaluate for resistance to the foliar diseases areolate mildew and target spot. Disease ratings were taken in 2018 and 2019 growing seasons. CA 4011 showed favorable resistance to areolate mildew in comparison to susceptible checks, providing less leaf infection and defoliation.

Introduction

Cotton (*Gossypium hirsutum* L.) is an important crop around the world, grown for its lint fiber and seed. Cotton belongs to the hibiscus family *Malvaceae* that includes other crops such as okra (*Abelmoschus esculentus*) and cacao (*Theobroma cacao*) (Fryxell, 1979). The cotton genus

(*Gossypium* L.) has long been viewed as important because of its value to the world economy. As a commodity, cotton fiber is used in many products, primarily clothing, but also secondary products as oil and animal feed. According to the USDA Economic Research Service, "one bale of cotton can make more than 200 pairs of jeans or 1,200 t-shirts" (Meyer, 2022). The primary species of cotton grown in the United States is *Gossypium hirsutum*, commonly known as upland cotton. Pima cotton (*Gossypium barbadense*) is also grown in the United States but makes up less than 3% of production.

Upland cotton has broad adaptation across the United States, grown in 17 Cotton Belt states from Virginia to California and from Kansas to the Lower Rio Grande Valley of Texas. Given the broad geography of cotton acres, cotton growers are burdened with managing a plethora of pests including insects, weeds, and diseases. A considerable portion of pest management prior to 1995 relied on the use of insecticides, which subsided significantly with the introduction of *Bt* cotton. Currently, about 19 transgenic cotton events which encompass herbicide and insecticide tolerance have been approved since the introduction of genetically modified (GMO) cotton in 1994 (Bayer CropScience, 2023; Vulchi et al., 2022). Popular insect resistant traits include those for lepidopteran pests such as tobacco budworm (*Heliothis virescens*) (Bollgard II, Bollgard III [Monsanto Company] and WideStrike trait packages [Dow AgroSciences LLC]) as well as novel technology for thrips (*Frankliniella fusca* and *Frankliniella occidentalis*) and plant bugs such as ThryvOn from Bayer CropScience. The continued reliance on transgenic technology deployed in the market has significantly reduced the reliance on pesticides used. Despite efforts to develop new transgenic cotton cultivars, other biotic issues in cotton cultivation also demand a classical breeding approach to enhance tolerance to common insect pests (Vulchi et al., 2022).

Cotton diseases drastically reduce yields in the United States. According to the Faske and Sisson (2023), cotton yields were reduced by 8.7% (1.4 million bales). The percent average of disease loss over a 22-year period (2000–2021) was 12.2%. While major diseases of cotton remain, such as southern root-knot nematode (Meloidogyne incognita) and many soilborne pathogens, incidence of foliar diseases are on the rise. Currently, areolate mildew (sometimes referred to as grey mold or Ramularia leaf spot [RLS], is caused by *Ramulariopsis pseudoglycines* [Synonyms: *Ramularia areola* Atk., *Ramulariopsis gossypii* (Speg.) U. Braun, *Ramularia gossypii* (Speg.) Ciferi, *Cercosporella gossypii* Speg.] or *Ramulariopsis gossypii*; Videira et al., 2016) and target spot [caused by *Corynespora cassiicola* (Berk. & Curt.)] are two of the most economically important foliar diseases of upland cotton in the US Cotton Belt, with losses recorded at 34,385 and 30,798 bales, respectively (Faske & Sisson, 2023). In Central Brazil, *R. pseudoglycines* is prevalent and sources of resistance were identified (Suassuna et al., 2020). First identified in the United States in 1890 (Atkinson, 1890), areolate mildew has now been identified in most major cotton production countries such as Brazil, India, and East Africa (Bahadur & Dutta, 2024). The disease causes early defoliation, photosynthetic area reduction, and premature boll opening, reducing lint yield, and negatively affecting fiber quality (Bell, 1981; da Silva et al., 2019).

Target spot was first identified in the southeast United States in Alabama in 1959 on upland cotton (Jones, 1961). Brazil reported *Corynespora cassiicola* causing disease on cotton in 1995 (Mehta et al., 2005). Target spot was not observed to be an issue in the United States until recently, where it was first reported in Georgia in 2012. (Fulmer et al., 2012). Other states in the southeastern United States soon reported *Corynespora cassiicola* in commercial cotton fields including Alabama, Louisiana, and Tennessee (Butler et al., 2016; Campbell et al., 2012; Conner

et al., 2013; Price et al., 2015). China and Brazil also observed reemergence of this pathogen in 2014 (Galbieri et al., 2014; Wei et al., 2014). Target spot affects cotton by producing lesions that primarily start in the lower canopy, progressively moving up the plant, which if persists, causes defoliation resulting in yield loss (Galbieri et al., 2014).

Identifying disease resistance in cotton germplasm is more important now than historically because germplasm resistance to foliar diseases such as areolate mildew and target spot have not been reported in the United States. As such, the breeding line described here for public release represents the first germplasm resistant to areolate mildew within the United States while also highly tolerant to thrips and possessing acceptable agronomic and fiber quality traits. This germplasm should be useful to cotton breeding programs in providing native resistance to areolate mildew and thrips, which ultimately would impact grower's management practices positively by minimizing inputs related to fungicide and insecticide applications.

Materials and methods

Early Generation Population and Line Development

CA 4011 (Reg. no. GP-1149, PI 705597), evaluated as "07-7-1020CT" (explained below), originated from a single plant selection from CA 3084 in 1991 at the Texas A&M AgriLife Research and Extension Center in Lubbock, TX (LREC). CA 3084 was developed through three cycles of plant to row selection from a cross between El Paso source material EPSM-75-AAAA-3 and EPSM-1224-1-74-2-4-2-1. CA 3084 was released informally as germplasm for improving fiber quality in 1987 through a Texas Agricultural Experiment Station (TAES) plant material release procedure. Parent lines were developed in the TAES cotton breeding program at El Paso. Germplasm sources contained within the TAES-El Paso program

were developed by John R. Gannaway and the late Paul J. Lyerly. Selections out of the El Paso source materials had insufficient records to permit accurate pedigrees. It is assumed that Pima, Del Cerro, and Acala germplasm were used in developing these materials.

CA 3084 was planted in a seed increase block at Texas A&M AgriLife Research in Pecos in 1992. Bulk selection for breeder seed maintenance was conducted in Pecos for 2 years. CA 4011 was developed following plant to row selection in a cold tolerance nursery from 1995 to 2007 except 1996 and 2003 when the nursery was lost to hail and planted the next year from remnant seed. The cold tolerance nursery was planted in early April each year into suboptimum soil temperature and conditions recommended for seed germination in the Southern High Plains. Nursery objectives were germination in cool-temperature, early season seedling survival, and for fiber quality, plant type, and boll type. Individual plants were selected after full maturity in the field based on visual assessment of productivity, plant type, and boll type. In 2007, row 07-7-1020CT, which later becomes CA 4011, was selected as a uniform line and the seed bulk-harvested for field performance trials. In 2008, seed from 07-7-1020CT was planted in preliminary yield tests at four locations in the Texas Southern High Plains (LREC irrigated, LREC rainfed, Lamesa irrigated, and Halfway irrigated). After the first year of yield testing in 2008, 07-7-1020CT did not advance to intermediate testing based on relative yield performance. In 2010, the LREC cotton breeding program added the objective to develop cultivars for organic cotton production with host plant resistance to thrips feeding injury to its priority list. In 2012 to 2014, 07-7-1020CT underwent greenhouse thrips screening according to the assay developed by Arnold et al. (2012). 07-7-1020CT then underwent replicated performance testing from 2012 to 2014 at two locations each year, both on a certified organic farm, and on conventional land

managed with organic practices. 07-7-1020CT was recommended as appropriate germplasm to develop cotton cultivars for organic production systems. (Wann et al., 2017).

Pest and disesease resistance evaluation

Thrips feeding injury

Greenhouse screening for thrips resistance was conducted in three experiments from 2012 to 2014 at LREC in Lubbock, TX. The screening assay has been described in Arnold et al. (2012), which first uses wheat (*Triticum aestivum* L.) as a host crop to increase thrips population followed by termination the growth with glyphosate application at cotton planting to force the thrips to move to cotton. Table 4. lists the 22 lines evaluated, which include 16 breeding lines from the Texas A&M AgriLife Research Cotton Improvement Program at Lubbock, and five resistant and two susceptible checks. The susceptible checks are FM 958 and FM 989. The resistant checks are Tamcot 73 (PI 662044), Atlas (PI 561579), Tam 04WB-33s (PI 662041), 'Cobalt Pima' (*Gossypium barbadense* L.; PI 638527, US PVP 200500112) and TX 110 (*Gossypium barbadense*; PI 163608). The trials were conducted in a randomized complete block design with 4 blocks, each consisting of six plant experimental units. Damage ratings were assessed by using a novel 1–9 scale that indicates: 1 = complete plant necrosis, no observable green true leaf material; 2 = severely-reduced leaf area, large necrotic lesions, very little green leaf material; 3 = significantly-reduced leaf area, upward cupping of leaves, small necrotic lesions, severe chlorotic "bubbling" on leaves, observable green leaf tissue; 4 = reduced leaf area, some upward cupping of leaves, spotty necrotic lesions, observable chlorotic bubbling of leaf tissue; 5 = minor reductions in leaf area, little or no necrosis, some chlorotic bubbling of leaf tissue, no visible leaf cupping; 6 = little observable reduction in leaf area, some chlorotic

Table 4.1. Leaf area reduction, visual thrips injury ratings, and thrips densities of 18 cotton genotypes in a greenhouse evaluation near Lubbock, TX, in 2012.

Entry	*Thrips Density*[a]	*Thrips Injury Ratings*[b]	*Leaf Area Reduction*
	no. plant^{-1}	1–9	%
06-21-519FQ	1.2 a	3.5 b-d	61 b-e
06-45-1104D	2.4 a	3.0 cd	71 a-c
07-7-519CT	1.7 a	4.5 ab	54 de
07-7-1001CT	1.9 a	4.0 b-d	66 a-d
07-7-1020CT	1.5 a	4.5 ab	65 a-d
07-7-1303CT	1.3 a	3.5 b-d	62 b-e
07-7-1407CT	0.9 a	2.8 d	73 ab
07-14-205FS	1.4 a	3.3 b-d	80 a
07-14-510FS	1.6 a	4.0 b-d	57 c-e
07-20-1304D	0.7 a	4.3 a-c	68 a-d
09-1-1116FQ	1.1 a	4.3 a-c	71 a-c
ATLAS	1.1 a	4.0 b-d	74 ab
COBALT	1.3 a	3.0 cd	70 a-c
FM 958	2.0 a	3.8 b-d	49 ef
FM 989	2.1 a	3.8 b-d	75 ab
TAM 04WB-33S	1.0 a	3.8 b-d	78 a
TAMCOT 73	0.8 a	3.5 b-d	59 b-e
TX 110	0.8 a	5.5 a	34 f

Means within a column followed by the same lowercase letter are not significantly different according to pairwise t-tests at $P = 0.05$. FM 958 and FM 989 are susceptible checks; Tamcot 73, Atlas, Tam 04WB-33s, Cobalt, and TX 110 are resistant checks.

[a] Mean thrips density was approximately 1.4 thrips plant^{-1}.

[b] Visual ratings were based on a 1–9 scale (1 = plant death and 9 = no damage).

bubbling; 7 = no observable reductions in leaf area, patchy chlorotic lesions; 8 = no observable reductions in leaf area, very few chlorotic lesions; and 9 = no observable damage. Thrips densities and damage ratings were assessed in all three experiments (Wann et al., 2017).

Foliar Disease Resistance

07-7-1020CT and over 200 diverse *G. hirsutum* germplasm accessions were evaluated for resistance to foliar diseases under natural infection in 2018 and 2019 at the University of Georgia Coastal Plains Experiment Station in Tifton, GA. Both experiments were designed in a random complete block design. A severity scale from 1 (immune) to 5 (highly susceptible) was used in disease rating, assigning grades as follows: 1 = no symptoms observed; 2 = lesions with sporulation only in the leaves on lower third of the plants; 3 = lesions with sporulation reaching leaves on the middle third of the plants; 4 = lesions on the leaves in the upper third of the plants and moderate defoliation; and 5 = intense defoliation throughout the plant. In 2018, three replications were used to screen for resistance to areolate mildew and target spot. All germplasm lines were compared to a known susceptible check PHY 499 WRF (PHY 499; PhytoGen Cottonseed; Dow AgroSciences). The germplasm lines evaluated include wild accessions, obsolete varieties, and public breeding lines. Ratings were taken at the end of the season in 2018 to assess which germplasm to cull and which to advance to the second year of screening. Germplasm with ratings ≤ 2.5 were selected to be evaluated in the following growing season. In 2019, PHY 499 WRF was also used as a susceptible check and a known resistant line from Brazil, 'BRS 372' (Suassuna et al., 2020), was used as a resistant check. Among the germplasm tested, the line TX 1718, TX 0768-2, SA-2379, and a *G. barbadense* Pima S-6 were included herein for comparison to 07-7-1020CT for areolate mildew resistance. After reviewing severity ratings of target spot, 07-7-1020CT was not tested in 2019 due to it not having significant resistance.

Preliminary field performance test evaluation

07-7-1020CT was entered in strains testing in 2008 at four locations; LREC furrow-irrigated, LREC rain-fed, Lamesa pivot irrigated, and Halfway furrow irrigated. The field design for all performance tests was a randomized complete block with four replications. Entries were arranged in two-row plots, 1.02-m row width with plot length ranging from 8.2 to 9 m depending on location. The Lubbock tests were planted in either Amarillo (fine-loamy, mixed, superactive, thermic, Aridic Paleustalf) or Olton (fine, mixed superactive thermic Paleustoll) soils, the Halfway test in Pullman clay loam soil (fine, mixed, superactive, thermic Torrertic Paleustoll), and the Lamesa test in Amarillo fine sandy loam soil (fine, mixed, superactive, thermic Aridic Paleustalf). Planting dates ranged from May 9 to May 17, while harvest dates ranged from October 28 to December 2. Plots were harvested with a two-row mechanical stripper, and lint yield was determined by applying the percentage by weight of lint from a 600-g grab sample to the stripped plot weight and adjusting for land area as described by Dever et al. (2013). A 30-g subsample of lint was submitted for fiber quality measurement by High Volume Instrument to Fiber and Biopolymer Research Institute in Lubbock, TX. Three commercial check cultivars common across all tests were FiberMax brand ‘FM 958’ (PI 619096; PVP 200100208) and ‘FM 989’ (PI 603958; PVP 009800259), and All-Tex ‘Atlas’ (PI 561579; PVP 9200188). FM 958 and FM 989 are prominently used in Southern High Plains organic cotton production, and All-Tex Atlas is a historical, typical stripper-type cotton cultivar that was used as a susceptible check in greenhouse and field thrips response tests.

Field Performance Evaluations for Organic Production

Field tests were conducted from 2012 to 2014 to evaluate several upland cotton lines, including 07-7-1020CT and check cultivars FM 958, FM 989, and All-Tex Atlas under USDA-

certified organic management, for potential use in organic production. Trials were conducted at LREC and a certified organic farm at Lamesa, TX, in 2012; Texas A&M AgriLife Research Station in Halfway and a certified organic farm Meadow, TX, in 2013; and certified organic farms in Meadow and Lamesa in 2014. All certified organic sites were located at grower cooperators' farms.

Individual tests were planted on May 22 and 23, 2012, at the Lamesa and Lubbock locations, respectively; May 15 and 22, 2013, at the Halfway and Meadow locations, respectively; and June 4 and May 16, 2014, at the Lamesa and Meadow locations, respectively. All locations were planted in a randomized complete block experimental design, with four replications. Plots were two rows wide, measuring 2.0 m by 9.1 m. All tests, except the 2 years at Lamesa, were planted with a four-row John Deere 7100 MaxEmerge cone planter (John Deere and Co.) to achieve a plant stand of approximately 9.7–13.0 plants m^{-1}. The Lamesa locations in 2012 and 2014 were planted with the grower cooperator's 16-row equipment and seed was hand-dribbled into the individual row units.

Each location was managed according to USDA certified-organic guidelines where possible, with fertility maintained with composted manure and weed control by mechanical cultivation and hand-weeding as needed. All locations were irrigated with overhead pivot irrigation, except for the 2012 Lubbock site, which was furrow-irrigated, and the 2012 and 2014 Lamesa sites, which were rainfed. Plots were harvested with a two-row mechanical stripper, and lint yield was determined by applying the percentage by weight of lint from a 600-g grab sample to the stripped plot weight and adjusting for land area as described by Dever et al. (2013). A 30-g subsample of lint was submitted for fiber quality measurement by High Volume Instrument to Fiber and Biopolymer Research Institute in Lubbock, TX.

Characteristics

Response to thrips feeding injury

In 2012, resistant check TX 110 (PI 163608) displayed the least amount of visual injury due to thrips feeding, along with 07-7-519CT and 07-7-1020CT (Table 4.1). In 2013, thrips densities were higher than in 2012, which resulted in a narrower range of injury rating and leaf area reduction values. 07-7-1020CT showed more damage than TX 110, but less than check cultivar All-Tex Atlas (Table 4.2). Thrips injury ratings were statistically different between genotypes in 2012 and 2013 evaluations ($P < 0.05$), but not in 2014. This was likely contributed by an overwhelming quantity of thrips in 2014 (9.0 thrips plant^{-1}). It is very possible that with high thrips density levels in 2014, natural mechanisms for resistance could have been overcome, given that proven resistant lines such as TX 110 also showed significant injury.

Table 4.2. Leaf area reduction and visual thrips injury ratings of 22 cotton genotypes in a greenhouse evaluation near Lubbock, TX, in 2013.

Entry	***Thrips Injury Ratings[a,b], [a,b]***	***Leaf Area Reduction***
	1–9	%
06-21-519FQ	2.1 b-e	98.1 a-d
06-45-1104D	1.8 de	96.3 a-g
07-7-519CT	2.6 bc	96.4 a-g
07-7-1001CT	1.8 de	98.6 a-d
07-7-1020CT	1.9 c-e	97.6 a-e
07-7-1303CT	2.6 bc	94.9 d-g
07-7-1407CT	1.9 c-e	97.4 a-f
07-14-205FS	2.0 c-e	97.7 a-e
07-14-510FS	2.0 c-e	97.7 a-e
07-20-1304D	2.3 b-e	99.0 a-c
09-1-1030FQ	1.6 e	98.8 a-d

09-1-1116FQ	2.5 b-d	96.1 b-g
11-2-802GD	2.3 b-e	96.9 a-f
11-2-1103GD	2.3 b-e	95.9 c-g
11-14-507V	2.1 b-e	98.0 a-e
ATLAS	1.8 de	99.0 a-c
COBALT	2.9 b	94.2 e-g
FM 958	2.6 bc	93.5 fg
FM 989	2.1 b-e	97.2 a-f
TAM 04WB-33S	1.8 de	99.7 ab
TAMCOT 73	1.6 e	99.8 a
TX 110	3.8 a	93.0 g

Means within a column followed by the same lowercase letter are not significantly different according to pairwise t-tests at $P = 0.05$. FM 958 and FM 989 are susceptible checks; Tamcot 73, Atlas, Tam 04WB-33s, Cobalt, and TX 110 are resistant checks.

[a] Visual ratings were based on a 1–9 scale (1 = plant death and 9 = no damage).

[b] Mean thrips density was approximately 3.9 thrips plant$^{-1.}$

Disease Resistance

For areolate mildew screening in 2018, 07-7-1020CT was superior to PHY 499 with a score of 1.4 compared to 3.67 in a scale of 1–5, with 1 being the most resistant and 5 being highly susceptible (Table 4.3). In 2019, the resistance for 07-7-1020CT was confirmed with a score of 2 whereas PHY 499 received a 4.5. When compared to other germplasm such as TX 1711, TX 0768-2, SA-3792, and Pima S-6, 07-7-1020CT showed better resistance based on severity ratings (Table 3.4). BRS 372 provided better resistance than 07-7-1020CT with slightly lower rating. Hence, 07-7-1020CT can provide a level of resistance needed in commercial germplasm to aid in control of areolate mildew. In 2018, 07-7-1020CT was also screened for target spot resistance in comparison to PHY 499. The score average on a 1–5 scale (where 1 is immune and 5 is highly

susceptible) was 3.34 for 07-7-1020CT, which was equivalent to 4.17 for PHY 499. Other germplasm including TX 1711, TX 0768-2, SA-3792, and Pima S-6 had numerically lower rating to target spot than 07-7-1020CT; only TX 1711 was statistically different. Hence, 07-7-1020CT was not evaluated for target spot resistance in 2019 due to it not providing acceptable control of this foliar disease (Table 4.5).

Table 4.3. Disease evaluations of areolate mildew on 07-7-1020CT compared to other germplasm and a known susceptible check 2018.

Germplasm	***Rep1***	***Rep2***	***Rep3***	***Mean***	***SD***
			1–5		
PIMA S-6	1.5	1	1.5	1.34a	0.29
07-7-1020CT	1.3	1.5	1.5	1.41a	0.32
TX 1718	1.5	1.5	1.5	1.5a	0
TX 0768-2	1.5	2.75	1.25	1.83a	0.80
PHY 499	3.5	3.8	3.8	3.7b	0.14
SA-2379	4.75	4.75	4.75	4.75c	0

Disease assessments were recorded on a scale from 1 (immune) to 5 (highly susceptible). Ratings were taken by judging the severity of leaves infected along with defoliation. Means followed by the same lowercase letter are not significantly different according to Fisher's Least Significant Difference test at $P \leq 0.05$. Testing locations in 2018 were Tifton, GA, at the Gibbs farm.

Table 4.4. Disease evaluations of areolate mildew on 07-7-1020CT compared to known susceptible and resistant germplasm 2019.

Germplasm	***Rep1***	***Rep2***	***Rep3***	***Rep4***	***Rep5***	***Rep6***	***Mean***	***SD***
				1–5				
BRS 372	1	1.5	1	1.5	1	1.5	1.25a	0.27
07-7-1020CT	1.5	2	2	2.5	2	2	2b	0.32
TX 0768-2	3	3	–	2.5	3.5	2.5	2.9c	0.42
PIMA S-6	1.5	3	3	3.5	3	3.5	2.92c	0.74
TX 1718	2.5	3	3	3.5	3	3.5	3.08c	0.38
SA-2379	3.5	3	3	3	3.5	3	3.16c	0.26
PHY 499	4.5	4.5	4.5	4.5	4.5	4.5	4.5d	0

Disease assessments were recorded on a scale from 1 (immune) to 5 (highly susceptible). Ratings were taken by judging the severity of leaves infected along with defoliation. Means followed by the same lowercase letter are not significantly different according to Fisher's Least Significant Difference test at $P \leq 0.05$. Testing locations in 2018 were Tifton, GA, at the Gibbs farm.

Table 4.5. Disease evaluations of target spot on 07-7-1020CT compared to other germplasm and a susceptible check 2018.

Germplasm	*Rep1*	*Rep2*	*Rep3*	*Mean*	*SD*
			1–5		
TX 1718	2	2.25	2	2.08a	0.14
TX 0768-2	1.75	2	3.5	2.42ab	0.96
SA-2379	2.5	3	2.75	2.75ab	0.25
PIMA S-6	3.25	2.875	3	3.04abc	0.19
07-7-1020CT	4.5	2.5	3	3.33bc	1.04
PHY 499	4.3	4.8	3.5	4.17c	0.63

Disease assessments were recorded on a scale from 1 (immune) to 5 (highly susceptible). Ratings were taken by judging the severity of leaves infected along with defoliation. Means followed by the same lowercase letter are not significantly different according to Fisher's Least Significant Difference test at $P = 0.05$. Testing locations in 2018 were Tifton, GA, at the Gibbs farm.

Yield, lint percent, and fiber properties

Analysis of variance for preliminary performance tests across four locations in 2008 showed significant difference in yield among locations and lines and significant difference in lint percent among lines. Lines also differed in micronaire, length, and strength, with significant differences among locations for length and strength. No significant interaction was observed (Table 4.6). 07-7-1020CT produced less lint yield than FM 958 and had lower lint percent than both FM 958 and FM 989. Micronaire was lower than FM 958, but fiber length was longer than FM 958 and All-Tex Atlas and equal to FM 989. Fiber strength was better than All-Tex Atlas and equal to FM 958 and FM 989. 07-7-1020CT length uniformity was similar to all the check cultivars. Since 07-7-1020CT did not exceed current production standard cultivars, no further testing in conventional performance tests was conducted. Analysis of variance for performance tests conducted under organic management at six site-year-locations from 2012 to 2014 showed differences in yield, micronaire, length uniformity, and strength among locations, but not lines (Table 4.7). Percentage lint was different among lines, and fiber length showed differences among both locations and lines. Fiber strength had a line × location interaction. 07-7-1020CT

produced similar yield to check cultivars with lower percent lint compared to FM 958. Fiber length was similar to check cultivars, but fiber strength was lower than both FiberMax cultivars. Micronaire and length uniformity was similar among all lines (Table 4.7)

Table 4.6. Lint yield, percent lint, micronaire, upper half mean length, length uniformity and strength for 07-7-1020CT and three check cultivars grown at four locations in Texas in 2008.

Designation	*Lint Yield*	*Percent Lint*	*Micronaire*	*Length*	*Uniformity*	*Strength*
	kg ha^{-1}	*%*	*units*	*mm*	*%*	*kN m kg^{-1}*
07-7-1020CT	1132b	35.8b	3.9b	30.0a	82.6a	298.9a
ALL-TEX ATLAS	1043b	35.9b	4.3ab	28.2b	82.7a	278.3b
FM 958	1435a	40.7a	4.4a	29.5b	81.9a	298.9a
FM 989	1359ab	39.4a	3.9b	30.0a	81.8a	305.8a

Means within a column followed by the same lowercase letter are not significantly different according to Fisher's Least Significant Difference test at $P = 0.05$. Locations included Lubbock, TX, furrow irrigated; Lubbock, TX, rain-fed; Halfway, TX, furrow-irrigated; and Lamesa, TX, pivot-irrigated

Table 4.7. Lint yield, percent lint, micronaire, upper half mean length, length uniformity, and strength for 07-7-1020CT and three check cultivars grown at six site-year-locations under USDA-certified organic management in 2012–2014.

Designation	*Lint Yield*	*Percent Lint*	*Micronaire*	*Length*	*Uniformity*	*Strength*
	kg ha^{-1}	*%*	*Units*	*mm*	*%*	*kN m kg^{-1}*
07-7-1020CT	989a	34.3b	3.8a	27.9ab	80.5a	300.9b
ALL-TEX ATLAS	1028a	35.3b	4.1a	27.2b	80.8a	305.8ab
FM 958	1059a	36.8a	4.0a	28.2a	80.1a	314.6a
FM 989	1031a	36.4ab	3.9a	28.4a	80.9a	317.5a

Means within a column followed by the same lowercase letter are not significantly different according to Fisher's Least Significant Difference test at $P = 0.05$. Site-year-locations included Lubbock, TX, in 2012; Halfway, TX, in 2013; Meadow, TX, in 2013; and Meadow, TX, in 2014.
† Site-year-locations included Lubbock, TX in 2012; Halfway, TX in 2013; Meadow, TX in 2013; and Meadow, TX in 2014.

Availability

Inquiries regarding seed for potential commercial use should be directed to Texas A&M University System Office of Technology and Commercialization, 800 Raymond Stotzer

Parkway, Suite 2020, College Station, TX, 77845 (main phone: (979) 847-8682). Small quantities of CA 4011 for research purposes can be requested from J. K. Dever, Texas A&M AgriLife Research, 1102 E. Drew, Lubbock, TX 79403. Unless specifically approved by Texas A&M AgriLife Research, CA 4011 may not be used as a parent or recurrent parent in a breeding program. Seed of CA 4011 has been deposited in the National Plant Germplasm System, where it will become available for distribution 20 years after the date of publication.

Author contributions

Edward D. Beasley: Data curation; formal analysis; investigation; writing—original draft. Dylan Wann: Data curation; investigation; methodology. Shreya Shanbhad: Data curation; formal analysis; methodology. Edward Lubbers: Investigation; methodology; project administration. Nelson Dias Suassuna: Conceptualization; investigation; methodology; writing—review & editing. Don C. Jones: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; writing—review & editing. Carol M. Kelly: Data curation; formal analysis; investigation; methodology; project administration; writing—review & editing. Jane K. Dever: Conceptualization; data curation; formal analysis; funding acquisition; writing—review & editing. Peng W. Chee: Conceptualization; funding acquisition; investigation; resources; supervision; writing—review & editing.

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Conflict Of Interest Statement

The authors report no conflicts of interest.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

Cotton (*Gossypium hirsutum*) is a globally valued crop that is cultivated for both lint fiber and seed. As a member of the hibiscus family (*Malvaceae*), it shares lineage with crops like okra and cacao (Wendel et al., 1992). Cotton's domestication involved four main species, with *G. hirsutum* and *G. barbadense* originating in the Americas, while *G. arboreum* and *G. herbaceum* are native to Africa-Asia (Wendel and Cronn, 2003). Over 50 other species within the *Gossypium* genus contribute to its extensive genetic diversity, which dates back millions of years (Fryxall et al.,1992).

Recent cotton production has ranged from 90 to 125 million bales globally, with the U.S. producing about 22.5 million bales in 2019 (USDA, 2019). Cotton is an important crop in the southern United States. Beyond textiles, cotton fiber and seed contribute to many products other than textiles, including cosmetics, medical supplies, and even food products.

Cotton breeding throughout history have focused on several key traits which are primarily lint yield and fiber quality (Constable et al., 2015). Host plant resistance to important diseases have also been an important focus of cotton breeding. (Bowman, 2000). Diseases within that focus include common cotton pathogens, such as bacterial blight, fusarium wilt, root-knot nematode, and verticillium wilt. Breeding efforts have successfully integrated resistance traits to these diseases into modern cultivars (Zhang and Boopathi, 2022). With the increasing evidence of fusarium wilt in commercial fields and the novel understanding of FOV diversity, breeding

initiatives are needed to combat native fusarium species as well as new emerging foliar diseases such as areolate mildew and target spot.

Fusarium wilt, caused by the pathogen *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), is a significant challenge in cotton production due to its ability to inhibit plant growth, or in extreme cases, plant mortality (Davis et al., 2006). This inevitably results in severe yield loss across Upland cotton species. FOV is a common soilborne pathogen that can persist and spread for years through contaminated equipment, plant debris, and irrigation (Davis et al., 2006). Once established, it can form aggregated areas within fields that expand over time. Control of fusarium wilt has become increasingly difficult, especially since fungicide or fumigant applications have limited efficacy against the disease. This trend has increased the value of host resistance making integrated management approaches necessary (Cianchetta and Davis 2015).

Different FOV races affect cotton that usually become more successful in combination with plant parasitic nematodes. FOV4 does not, but it has yet to be identified in the Southeast (Kim et al., 2005; Halpern et al., 2018; Zhu et al., 2020). In the United States, FOV management traditionally focused on host resistance to root-knot nematode due to the association between FOV and nematode infestations in cotton fields (Hillocks, 1984; Shepard and Kappelman; 1986). However, new research indicates that focusing solely on root-knot nematode resistance may be insufficient in the presence of highly virulent FOV strains, which can cause severe infections even in nematode-free conditions (Holmes et al., 2009; Cianchetta et al., 2015, da Silva et al. 2019a).

In Georgia, research by Da Silva et al. (2019a) has confirmed that Race 1 is the primary race associated with fusarium wilt, but other less common races also present need further investigation. Similarly, the virulence of different strains among pathogenic races are also a

concern (Halpern et al., 2020). Also, another contributing factor, sting nematode, has also been correlated with FOV severity, suggesting that existing control measures might be inadequate for Georgia's cotton growing regions (da Silva et al., 2019a).

Target spot, caused by *Corynespora cassiicola,* identified on Upland cotton in Alabama in 1959, has emerged as a significant concern in U.S. cotton due to its recent spread across southeastern United States, as well as in Brazil and China (Jones, 1961; Fulmer et al., 2012; Galbieri et al., 2014; Wei et al., 2014). Control relies on fungicides, but resistance development is concerning, especially given the pathogen is classified as high-risk for resistance (Bowen et al., 2018; FRAC, 2019). Several studies provide evidence that timely fungicide applications at disease onset, could prevent 4–6% yield loss, but results are inconsistent (Mehl et al., 2017; Bowen et al., 2018). Common fungicides include azoxystrobin, pyraclostrobin, and others, yet ongoing resistance issues in tomato and soybean indicate possible future challenges in cotton as well (MacKenzie et al., 2020; de Mello et al., 2021). Effective management should include resistant varieties, but definitive genetic resistance to target spot has not been identified in commercial cotton varieties (Hagen et al., 2015). This offers importance in identifying diverse cotton germplasm sources for resistance.

Areolate mildew, caused by *Ramulariopsis areola* and *Ramulariopsis gossypii,* also known as grey mildew or Ramularia leaf spot, is a significant disease affecting cotton. Initially identified in the U.S. in Alabama in 1890 (Atkinson, 1890; Bell, 1981). Historically this disease was categorized as a minor issue in the United States but is increasingly becoming a valid concern due to its spread and impact on yield. In cotton producing countries like Brazil, India, and parts of East Africa areolate mildew is a large threat.

In the United States, management strategies primarily include fungicides. In Georgia, fungicide applications are recommended between the first and sixth week of bloom, depending on disease onset (Kemerait, 2021). In Brazil, where areolate mildew is the predominant yield limiting diseases, growers may use up to eight fungicide applications per season using various chemistries (da Silva et al., 2019b). However, there is concern of fungicide resistance given the CYTB-G143A mutation associated with reduced sensitivity to QoI fungicides (strobilurins) was identified in 100% of Brazilian isolates tested (Mathioni et al., 2022). Azoxystrobin is widely used for areolate mildew in the U.S. provided it being a cheaper option. If QoI resistance is identified in the United States, it could confound current methods of control. *Ramulariopsis pseudoglycines,* which carries the CYTB-G143A substitution was recently identified in Mississippi (Conner et al., 2023). It is probable to assume that this species is widespread in the United States. Host resistance will be essential for sustainable management and has been utilized in Brazil and India (Rathaiah, 1976; Morello et al., 2010; Morello et al., 2012; Morello et al., 2015, Suassuna et al. 2020). However, in the U.S., this area remains unexplored since breeding efforts were not focused on areolate mildew resistance.

This goal of this research was to assess cotton disease resistance by evaluating a diverse selection cotton germplasm against endemic diseases affecting the southeastern U.S. This study provides valuable insight regarding sources of disease resistance among wild, obsolete, and elite lines. These results are intended to support cotton genetic research and breeding efforts in the effort to introgress resistance into commercial cultivars and offering cotton researchers identification of germplasm sources that have resistance or susceptibility to fusarium wilt, target spot and areolate mildew.

In 2018 an initiative was made to screen cotton germplasm for resistance to endemic diseases of cotton in the southeastern United States. In July, an experiment was made to evaluate a large selection of cotton germplasm for resistance to fusarium wilt in Georgia. In this study, 430 cotton germplasm lines were screened for resistance to fusarium wilt, including 264 from the unimproved or wild cotton from the USDA National Cotton Germplasm Collection in College Station, Texas, 117 from obsolete cultivars and 49 public breeding lines from New Mexico and Georgia. Germplasm was evaluated under natural conditions in a commercial field to assess resistance to fusarium wilt. All cotton lines were evaluated by their ability to overcome or tolerate FOV infection which indicated levels of resistance or susceptibility. Three subsequent tests followed in 2019-2021 to better characterize germplasm that were observed to be resistant. Over the four years of testing fusarium wilt resistance, germplasm lines showed varying levels of susceptibility and resistance. Georgia breeding lines provided consistent levels of resistance to FOV as well as some obsolete varieties. Single plant selections from resistant lines demonstrated to be equivalent or superior to their parent lines performance during 2020-2021 trials. Much of the wild germplasm appears to be variable in resistance indicating a truly diverse germplasm pool but also provides resources that could help identify novel genes for FOV resistance (Chapter 2). Some obsolete lines were identified as having resistance (DELTAPINE 55, DELCOT 277, TIDEWATER #5, and FJA), which was not surprising, but many did not provide expected results, as the obsolete lines screened in this study were identified previously to have fusarium wilt resistance. However, it was not expected to identify resistance in elite material given that breeding efforts for these lines were not focused on fusarium wilt resistance as a phenotypic selection in advancement (GA 2016090, GA 2016110, GA 2017138, and GA

2015046). We now can conclude from Chapter 2 specifically what is susceptible, with further emphasis on what has resistance to fusarium wilt.

Simultaneously in 2018, an experiment was conducted to evaluate cotton germplasm for resistance to areolate mildew and target spot, which included 251 cotton lines, representing a genetically diverse set of cotton germplasm (Chapter 3) consisting of 190 wild *G. hirsutum* (TX) accessions from the USDA Cotton germplasm collection, 23 obsolete upland varieties, 35 elite breeding germplasm lines from the Regional Breeding Testing Network (RBTN), three conventional standard varieties, and three *G. barbadense* lines were included as an outgroup in the experiment. The following year (2019) screening included a trial for each disease; the TS trial, which evaluated 26 lines, and the AW trial, which consisted of 52 genotypes. Two consecutive years of testing showed that cotton germplasm had varying levels of resistance to target spot and areolate mildew. Among genotypes with lower target spot severity were TX 1008, TX 1045, TX 1270, TX 1283-2, and TX 2357, all of which outperformed the check variety, Phytogen 499 WRF. In contrast, areolate mildew resistance was significant, particularly in the wild accessions and elite line 7-7-1020-CT, which showed resistance even though it wasn't originally selected for disease resistance (Beasley et al., 2024). The cultivar BRS 372 from EMBRAPA, a known resistant line to Brazilian strains of areolate mildew, exhibited comparable resistance, which warrants further investigation (Suassuna et al., 2020).

Chapter 4 discusses release of areolate mildew resistant germplasm CA 4011 (tested as 7-7-1020CT). originating from CA 3084 (1987), developed and released as a noncommercial cotton breeding line by Texas A&M AgriLife Research in collaboration with the University of Georgia's Agricultural Experiment Station through the research efforts correlating with Chapter 2. The 2018 and 2019 testing in Georgia revealed notable resistance to areolate mildew, which

establishes CA 4011 as a valuable resource for developing areolate mildew resistant cotton varieties (Beasley et al., 2024). Likewise, areolate mildew resistance of CA 4011 could provide novel information for exploring unique areolate mildew resistance genes. Future research should evaluate if CA 4011 and BRS 372 carry the same resistance genes or if they are different. (Chapter 3 and 4).

This research gives new knowledge regarding significant genetic diversity in resistance to fusarium wilt, areolate mildew, and target spot, granting information for breeding improved disease resistance in elite cotton varieties, as well as utilizing the results of susceptibility in germplasm. The results are particularly valuable, as they reveal novel sources of resistance within diverse cotton germplasm, including elite breeding lines, obsolete lines, and wild germplasm. These findings will serve as practical resources to strengthen cotton disease resistance against endemic cotton pathogens in the Southeast United States. Future direction of research should be to:

1. Utilize screening data from FW, TS, and AM evaluations to identify and characterize genetic resistance mechanisms.
2. Advance and release resistant lines with FW resistance to provide robust germplasm sources for breeding programs.
3. Investigate the resistance of CA 4011 and BRS 372 to identify novel genes related to AM resistance.
4. Explore combining resistance genes through breeding from CA 4011 and BRS 372 to provide enhanced AM resistance.
5. Further evaluate wild germplasm for their future improvement of disease resistance regarding FW, TS, and AM.

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