# BREEDING FOR HOST PLANT RESISTANCE TO FOV4 IN COTTON

A Thesis

by

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# ABSTRACT

*Fusarium oxysporum f. sp. Vasinfectum* race 4 (FOV4) is a vascular disease identified in multiple cotton producing areas in the United States. Host plant resistance (HPR) is currently the most effective control measure available to the cotton industry to combat this disease. A breeding project was initiated in 2018 by the Cotton Improvement Lab at Texas A&M University to screen germplasm and create novel breeding lines with HPR for FOV4. The program involved dual nurseries and trials in an infested field in El Paso County, TX, and a non-infested field near College Station, TX. Lines were evaluated for disease reaction, seed size, and yield components as well as fiber qualities. Analysis was conducted to determine which variables and interactions play the largest role when selecting for tolerance. Improvements have been made in the efficacy of phenotypic screening methods and trial designs since the inception of the program. In addition, measurable genetic HPR gains have been made in germplasm. As the project matured, fewer differences in resistance were found between genotypes indicating successful selection methods over the years. An incremental root stain trial conducted in 2022 gives insight on how the pathogen moves through the plant in susceptible and resistant germplasm. Based on steady improvements in both HPR to FOV4 and other agronomic qualities, it is possible to develop commercially viable cultivars for the US cotton industry.

# DEDICATION

I would like to dedicate this thesis to my family. To my dad, who instilled my passion for agriculture at an early age and taught me the importance of hard work. To my mom, who taught me to dream big and encouraged me to get out of my comfort zone. Finally, to Camden, who made me the competitive individual that I am which has always driven me to see what I can accomplish next. I would not be where I am today without their continuous support and encouragement.

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CONTRIBUTORS AND FUNDING SOURCES

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NOMENCLATURE

| | |
|---|---|
| AST | Advanced Strain Trial |
| ATCC | American Type Culture Collection |
| CIL | Cotton Improvement Lab |
| CS | College Station |
| ELS | Extra-long Staple |
| EST | Elite Strain Trial |
| f.sp. | Formale specialis |
| FOV | *Fusarium oxysporum* f. sp. *vasinfectum* |
| FOV1 | *Fusarium oxysporum* f. sp. *vasinfectum*- Race 1 |
| FOV2 | *Fusarium oxysporum* f. sp. *vasinfectum*- Race 2 |
| FOV3 | *Fusarium oxysporum* f. sp. *vasinfectum*- Race 3 |
| FOV4 | *Fusarium oxysporum* f. sp. *vasinfectum*- Race 4 |
| FOV5 | *Fusarium oxysporum* f. sp. *vasinfectum*- Race 5 |
| FOV6 | *Fusarium oxysporum* f. sp. *vasinfectum*- Race 6 |
| FOV7 | *Fusarium oxysporum* f. sp. *vasinfectum*- Race 7 |
| FOV8 | *Fusarium oxysporum* f. sp. *vasinfectum*- Race 8 |
| FW | Fusarium Wilt |
| HPR | Host Plant Resistance |
| IPS | Individual Plant Selection |
| NCGC | National Cotton Germplasm Collection |
| NDVI | Normalized Difference Vegetative Index |

| | |
|---|---|
| PR | Progeny Row |
| PSO | Preliminary Strain Observation |
| PST | Preliminary Strain Trial |
| RKN | Root Knot Nematode |
| RSS | Root Stem Stain |
| SJV | San Joaquin Valley |
| TX | Texas |
| US | United States |
| USDA | United States Department of Agriculture |
| VRS | Vascular Root Stain |

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# CHAPTER I

# INTRODUCTION

Globally, cotton is the most important natural fiber crop. The United States is the world's third largest producer of cotton, producing about 20 million bales per year (USDA, 2022) and the leading exporter of the fiber. A large percentage of that cotton comes from the High Plains of Texas. Two species of cotton are currently commercially grown in the United States, Upland cotton (*Gossypium hirsutum*) and Pima cotton (*Gossypium barbadense*). Upland cotton accounts for 97% of all cotton grown in the United States (USDA, 2020). Upland cotton generally is used for medium to low-quality textile products. Pima cotton is an extra-long staple (ELS) cotton used in higher value textile products because of its fine and long fibers. However, due to climate and management requirements, Pima cotton production is limited to longer-season production areas in California, Arizona, New Mexico and far West Texas. Most of the United States Pima production is produced in the San Joaquin Valley, California (USDA, 2022). Extensive research has gone into breeding cotton to increase yield, improve fiber quality, and enhance pest resistance. Host plant resistance to bacterial blight (*Xanthomonas citri pv. Malvacearum*), root-knot nematode (*Meloidogyne incognita*), and Verticillium wilt (*Verticillium dahlia*) have all come from the primary germplasm pool of cotton, which is well-adapted to commercial production (Ulloa et al., 2020). The cotton industry has overcome many threats through the breeding of improved germplasm.

Cotton growers across the western United States are facing a new challenge that is posing a threat to the cotton industry. Fusarium wilt is a fungal disease caused by *Fusarium oxysporum f. sp. vasinfectum* (FOV) that can reduce yield and fiber quality. Prior to 2001, only FOV races 1 and 2 were known to be in the United States (Kim et al., 2005). Disease pressure from FOV was mainly concentrated in the sandy acidic soils of the southeast. Damage from these races could partially be mitigated by control of root-knot nematodes (RKN) (*Meloidogyne spp.*) since RKN presence increased disease incidence. In 2001, growers in the San Joaquin Valley (SJV) of California observed symptoms of Fusarium wilt in clay soils where root-knot nematodes were not present. This strain of Fusarium was identified as FOV race 4 and was much more virulent and difficult to control than other races of Fusarium, presumably because it is nematode-independent. FOV4 was confirmed in El Paso County, Texas, in 2017 (Halpern et al., 2018), and in southern New Mexico near Las Cruces in 2019 (Zhu et al., 2021). Current practices such as sanitation and limiting the use of seed from infested fields have reduced the spread of this disease.

FOV4 has thick-walled survival structures called chlamydospores which can survive for long periods without a host in the soil or plant residue and are impenetrable by fungicides (Ulloa et al., 2020). For this reason, crop rotation may not be beneficial in controlling this pathogen. This disease can cause mortality in seedlings shortly after emergence. In heavily infested fields, 90-100% mortality has been observed prior to midseason (Zhu et al., 2021). With little to no success in controlling FOV4 with crop rotation or fungicides, breeding for host plant resistance appears to be the best option.

Since the identification of FOV4, breeding programs have been initiated to screen thousands of breeding lines for tolerance or resistance. High levels of resistance were discovered in a few Pima cotton lines but resistance in Upland cotton has proven to be more elusive to date.

FOV can be transported through contaminated seed, plant residue, and soil moved with equipment or irrigation water. This can cause local spread, but if custom harvesting equipment is used or infected seed is used for planting, the area of spread can be much larger. Races of FOV tend to be geographically restricted within places they can readily become established. This suggests that either the pathogen has difficulty becoming established or is not easily moved around (Davis et al., 2006). It is also possible that different races have not been detected yet because the crops planted could be asymptomatic or nematode populations are too low to facilitate damage. In 2021, the cotton industry experienced an overall 11.2% yield loss due to plant diseases, but only a 0.35% loss due to Fusarium wilt alone (Blasingame and Patel, 2022). The disease incidence in 2021 was higher than in most previous years which was likely due to the above average rainfall across most of the US Cotton belt. Texas suffered a 0.4% yield loss attributed to Fusarium wilt (Blasingame and Patel, 2022).

The objectives of this project are:

1. Improvement of the precision of screening methods for FOV4 symptoms. Currently, several plant breeders and pathologists rank tissue and root damage on a scale from 0 to 5. This scale can be interpreted differently between individuals during the assessment process.

2. Enhance FOV4 resistance in germplasm.

With ineffective cultural practices and no known fungicide, breeding for host plant resistance is currently the best method to combat this plant disease.

3. Simultaneously improve fiber and yield potential along with HPR in germplasm.

While the primary focus of this breeding effort was to improve host plant resistance, fiber quality and yield were important criteria when making advancement selections.

# CHAPTER II

# LITERATURE REVIEW

## 2.1 Life Cycle and Survival of FOV

*Fusarium* belongs to the class Ascomycetes and is a filamentous fungus that attacks the water conducting vessels of host plants (Pitt et al., 1994). *Fusarium* wilts affecting important crops worldwide are mainly caused by *F. oxysporum.* Strains can also be isolated from healthy plant roots which are termed non-pathogenic (Okungbowa and Shittu, 2012). The high level of host specificity of pathogenic strains led to the development of the "formae specials" concept which are distinguished by the ability of their members to cause disease on a limited range of host plants (Lievens et al., 2009). Understanding the life cycle and infection process of F. *oxysporum* can provide insight into the resistance mechanism of the host plants. Deficiencies in knowledge surrounding the biology of F. *oxysporum* is one reason why major gene resistance has been hard to identify. There has been research that suggests roots present barriers such as the structure of the hypodermis, composition of root exudates, and response of cortical tissue to the pathogen that influence the pathogen's success at infection (Gordon, 2017).

*Fusarium* species go through a dormant, parasitic, and saprophytic stage like many other vascular pathogens (Beckman, 1987). In the dormant stage, chlamydospores are commonly found in infested soils. These hardy spores can withstand extreme weather condition and survive years without a host. This ability to survive without regular host interactions explains why FOV can remain in soil almost indefinitely. For example, Smith et al. (2001) noted survival of the pathogen in soil that had not been

planted with cotton in over 10 years in California. These spores will remain dormant for years in the soil until they encounter root exudates in the rhizosphere of a host plant (Steinkellner et al., 2008). Sugars and amino acids released by roots are organic compounds that can stimulate spore germination and also support germ-tube growth (Gordon, 2017). This reversal of inhibition of the spores from germination can be caused by both host plants and non-host plants (Steinkeller et al., 2008). If the germ-tube does not come in contact with a plant root, it will either die or form new chlamydospores (Gordon, 2017).

Rodriguez Galvez and Mendgen (1995), studied the early phases of infection by FOV. The first step for plant infection occurs when a compact mycelium mat forms on the surface of the roots. During hyphal colonization, no damage occurs to the root, meaning it is an endotrophic or biotrophic phase (Rodriguez Galvez and Mendgen, 1995). Once the mat is established, branching and penetration of the hyphae occurs with high colonization of meristematic tissue (Rodriguez Galvez and Mendgen, 1995). This is when F. *oxysporum* enters the parasitic stage. Penetration usually occurs through root hairs, wounds, or cracks formed by emerging roots. The ideal temperature for FOV hyphal penetration of the root is between 28 and 30 ℃. Penetration is likely assisted by hydrolase-type enzymes secreted by *Fusarium* (Walter et al., 2009).

Once the pathogen has successfully infected the root's epidermis, the growing hyphae will come into contact with the root cortex. In some host plants, structural features including thicker radial cell walls are attributed to resistance to the pathogen (Gordon, 2017). From there they will penetrate the endodermis and move into the xylem

vessels. Infection of F. *oxysporum* in the xylem does not always result in disease (Gao et al., 1995). Beckman 1987 suggests that quantitative resistance may be based on how quickly and effectively the pathogen can move through the xylem. In the xylem, microconidia produced by mycelium move upward with the transpiration stream until they reach vessel end walls (Okungbowa and Shittu, 2012). At the end of the vessel, the microconidia germinated and penetrate the next vessels where they continue to grow and produce more microconidia. Symptom expression in the host plant occurs when hyphae spread into the cell apoplast (Walter et al., 2009). Vessels in the plant are clogged by mycelia and fungal spores resulting in wilting and eventually death of the plant. During the seedling stage, Fusarium wilt favors temperatures above 23 ℃ for infection and subsequent disease symptoms to occur. Cotton plants become more susceptible at flowering when temperatures reach the optimum range of 28 to 32 ℃ (Hillocks et al., 1992; Abdel-Raheem and Bird, 1968). Fusarium prefers warm, moist soil conditions that encourage root infection.

When infected plant residue is returned to the soil, the saprophytic fungus feeds on the detritus, allowing the fungus to grow and reproduce. Competitiveness as a saprophyte against other soilborne saprobes is essential because it influences the amount of inoculum produced. Recent research suggests the more organic material returned to the soil, the more fungal DNA can be identified. If this is true, inoculum levels in a field could be affected by the amount of organic material returned to the field the previous growing season. (Chappell, personal communication, February 14, 2023). As the plants

decay, conidia, hyphae, and chlamydospores are all produced and the lift cycle of the pathogen restarts.

## 2.2 Host Range of FOV

On occasion, FOV will infect the root epidermal and cortical cells of a wide range of plants but never cause disease, and usually will not enter the xylem tissue (Davis, 2006). This is one reason the pathogen can be successful since it can survive and multiply without causing wilt undetected. There have been reports of FOV infecting Yelredo soybean (*Glycine max*), flue-cured tobacco (*Nicotiana tabacum L.*), alfalfa (*Medicago sativa*), chili pepper (*Capsicum annuum*), cowpea (*Vigna unguiculata*), green gram (*Vigna radiata*), hollyhock (*Alcea*), lupine (*Lupinus*), and okra (*Abelmoschus*) (Davis, 2006). Finger millet (*Eleusine coracana*), pearl millet (*Pennisetum glaucum*), snapdragons (*Antirrhinum majus*), sorghum (*Sorghum bicolor*), sweet potato (*Ipomoea batatas*), and an assortment of weed species have all been found to be non-symptomatic hosts of FOV (Charudarran and Kalyanasundaram, 1966). In a 5-year study, a higher population of FOV was found following secondary hosts than after cotton (Davis et al., 2006). FOVs ability to multiply and survive on multiple hosts makes crop rotation ineffective as a management strategy.

## 2.3 Symptomology

FOV can infect cotton plants at all growth stages throughout the season. Early infection can cause seedling mortality only a few days post-emergence. Cotyledons of

infected seedlings will quickly wilt and fall off. These symptoms can be easily confused with seedlings suffering from damping-off caused by other plant pathogens. Symptoms throughout the season can include chlorosis and abscission of cotyledons, chlorosis and necrosis of older leaves, plant wilting, and even plant death. When inoculum is introduced into the field, small areas of wilting plants will appear. With help of irrigation water and machinery moving soil, the area of infected plants will expand each year. Since it may take years for the pathogen to spread throughout the field and because FOV symptoms can easily be confused with other cotton wilt diseases, growers may not recognize FOV4 in their field for several growing seasons. Similarly, if conidia levels are low or field conditions are not ideal for the pathogen, plants might not show any symptoms. With race 1, approximately 77,000 conidia per gram of soil were needed before symptoms were visible in plants but only 650 conidia per gram of soil were needed with the presence of root knot nematode (Garber et al., 1979). Currently, the best diagnostic symptoms are dark brown discoloration in the vascular system of the root and lower stem (Zhu et al., 2021). For this reason, end-of-season root stain ratings are an important method of measuring resistance in plants. Fusarium wilt symptoms are also almost identical to symptoms of Verticillium wilt (*Verticillium dahliae*). When cotton has Verticillium wilt, leaves will commonly have a red hue, and vascular discoloration is not as pronounced as with fusarium wilt. These subtle differences should not be relied upon when identifying the cause of cotton wilt disease. Instead, isolation of the pathogen is necessary (Davis et al., 2006). While greenhouse screenings can save time and space, results can vary since plants are not grown in field conditions. In previous studies, plant

death does not occur until two to four weeks post-inoculation in a greenhouse setting (Ulloa et al., 2020).

Like most plant pathogens, the environment influences and dictates the impact FW will have on a cotton crop. Seedlings under stress are more likely to be susceptible to FW. Disease pressure from FOV depends on numerous factors including virulence of the pathogen, soil type, presence of nematodes, climate, susceptibility of the cotton cultivar, soil fertility, temperature, inoculum level, and time of infection. Even in the same field, disease pressure can change from year to year based on weather conditions. Time of infection plays an important role since seedlings infected early in the season will die before ever producing bolls. Later in the season after flowering, infected plants that have survived usually mature earlier than non-infected plants with smaller bolls, lower yields, and poorer quality fiber (Davis, 2006).

### 2.4 Transmission

While substantial research has addressed the spread of FOV, long distance transmission is an ongoing area of interest because it involves the risk of seed transmission. Seed-borne pathogens allow the disease to spread and become established regionally and internationally. FOV can infect seeds through penetration of the seed coat when bolls are infested (Davis et al., 2006). Bennett et al. (2008) conducted a study to examine potential seed transmission of FOV4. Of the four cultivars they tested, seed from only one cultivar, ‘DP 744’ (Delta and Pine Land Company, Scott, MS), frequently tested positive for FOV 4. DP 744 is highly susceptible to FOV4 (Kim et al., 2005).

Other studies have examined the risk of spread through seed and shown various levels of inoculum emanating from infected seed. Results are erratic in part because of inconsistent protocols used by investigators (Sanogo, 2016).

Seed treatments with biocontrol agents have shown moderate promise in controlling FOV. When cotton seed was treated with *Gilocladium virens* and *Bacillua subtilis* and then planted in soil with FOV conidia, the incidence of FW was reduced (Zhang et al., 1996). Another biocontrol treatment was *Trichoderma harzianum* which was applied as a seed coat or in soil and reduced the presence of FW (Sivan and Chet. 2008). Dry heat and hot water treatments have also been used to disinfect cottonseed while maintaining seed germination and vigor (Bennett and Colyer, 2010); however, none of these methods are currently being used as a widespread management tool. So far, chemical seed treatments have been unsuccessful in reducing the presence of FW (Lawrence et al., 2007).

While results from studies conducted on seed transmission have been irregular, average seed infection rate is estimated at around 10% (Bennett et al., 2008). Studies completed before and after Elliott (1923) failed to demonstrate transmission through infected seeds (Gilbert, 1921; Fahmy, 1927; Neal, 1928). Due to the confirmation of FOV4 in multiple cotton producing regions of the world including the United States, India, and China, transmission of the disease through infected seed may be the best explanation for global dispersal. Seed-transmission of pathogens is an important mechanism of dispersal since it allows pathogens to travel great distances, find new

susceptible hosts, and seed lots allow for multiple primary infections in a field instead of a single introduction (Baker and Smith 1966).

### 2.5 Management

FW management can be approached in multiple ways including control of long-lived chlamydospores in the soil and plant residue, the broad range of host plants, the interactions between the pathogen and nematodes, and transmission through seeds (Sanogo, 2015). For nematode-dependent races of Fusarium, success in controlling wilt has been achieved by controlling nematodes with nematode-resistant cultivars or crop rotation with crop species that are non-hosts to nematodes. However, this approach will not necessarily reduce the number of Fusarium spores in the soil. Inoculum levels will continue to increase each time infested plant residue is returned to the soil (Wang et al., 1999). Leaving fields fallow is not a practical option since many weed species can be hosts for nematodes and Fusarium, allowing the FOV population to increase. Plus, landowners need to be able to generate revenue from their cropping systems on a regular basis. The hardy chlamydospores will allow FOV to persist even without a host. While some soil fumigants have been found to reduce the population of FOV, the cost can be prohibitive for most cropping systems. Since FOV has a broad range of host plants, crop rotation is also not an effective way to reduce disease pressure.

When residue was ground into mulch and left on top of the surface instead of being plowed into the soil, disease incidence of FOV in seedlings was reduced by 31% in cotton in Australia (Allen and Lonegran, 2000). The hypothesis behind this system is

when chlamydospores are exposed to sunlight and weathering, survival rates decline without the protection of soil. Leaving spores on top of soil also physically separates them from the root rhizospheres and exudates which are needed for germination. Tillage may also assist in spreading the pathogen through the field since it is moving the soil around. Most early management studies in the United States were most likely conducted with FOV race 1, but control strategies are not necessarily effective for other FOV races (Davis et al., 2006). Current commercial fungicides are ineffective because they are unable to penetrate the thick walls of the chlamydospores residing in the woody plant tissue. Being nematode independent makes FOV4 even more difficult to control since nematode control has no impact upon the incidence or severity of FOV4.

In relation to seedling stress, higher disease pressure is often found in areas with higher weed pressure (Kochman et al., 2002). This is likely due to stress from competition but also because many weed species have been found to be asymptomatic hosts of FOV. Proper weed management may be a potential method to mitigate Fusarium wilt. Overall, maintaining seedling health and reducing stress is key in combating FW.

### 2.6 Races of FOV

*Formae specialis vasinfectum* came from the identification of F. *oxysporum* on cotton (Sanogo, 2015). Races began to be assigned to FOV when it was noted that it was infecting other plant species beyond cotton. Traditionally, the term race is used to denote the differential response of a set of plants within the same species to a pathogen. The

way race is used regarding FOV has been deemed invalid since it relies of the response of plants across species. Literature in relation to FOV still uses the term race to maintain consistency (Holmes et al., 2009).

The original grouping of FOV was done by Fahmy (1927) who established three groups based on the pathogenicity on American Upland (*Gossypium hirsutum),* Egyptian (*G. barbadense),* and Asiatic (*G. arboretum.* and *G. herbaceum)* cotton. This work has been criticized for using isolates with low virulence and not being specific enough since the species of cotton were being impacted differently (Armstrong and Armstrong, 1960). Armstrong and Armstrong (1960) investigated Fusarium isolates from the United States, Egypt, and India on different cotton species. Sea Island cotton (*G. barbadense*) was considered resistant to the Indian strain (Armstrong and Armstrong, 1960). The *G. hirsutum* cultivars were mostly resistant to all FOV races. In the end, it was determined the amount of FOV races that could be detected were dependent upon the varieties of cotton included in the screening. In the end, Armstrong and Armstrong (1960) classified four races of FOV. FOV1 and FOV2 are from the United States and can only be distinguished based on FOV2 being infectious to tobacco and soybean since they expressed the same reactions on cotton. FOV3 is from Egypt and FOV4 from India.

Armstrong and Armstrong (1960) conducted their initial study in a greenhouse. When they planted cotton species in an FOV-infested field, 10% of the resistant varieties died. The field was likely infested with race 1 or 2. Armstrong and Armstrong (1960) considered a variety to be resistant if less than 50% of plants showed symptoms, and susceptible if more than 50 % of plants had symptoms. Factors such as quality of the

pathogen and environment could make the terms “resistant” and “susceptible” arbitrary if there were small changes in disease responses. This could explain why in some of Armstrong and Armstrong’s experiments they observed wilt in what they considered “resistant” cultivars. Due to inconsistent results of susceptible and resistant varieties, Armstrong and Armstrong (1960) concluded that irregular performances in different fields was most likely due to complex environmental factors that alter the infection incidence and severity of FOV. This makes a good argument as to why field nurseries are the most effective screening strategy to find resistant varieties suitable for a small and specific location. Their findings also suggest that greenhouse screenings, which don’t interact with the environment, may not be robust enough to identify cotton breeding lines with resistance to FOV over a wide range of growing conditions.

Prior to the early 2000s, only FOV race 1 and 2 were known to be in the United States. The fungus was mainly found in sandy soils where root knot nematodes were present. FOV was first identified in California in 1960 (Garber and Paxman, 1963). In the 1960s FOV was primarily held in check with host plant resistance to nematodes, crop rotation, and fumigation since wilt was only severe in the presence of nematodes. In the late 1990s, Australia discovered a virulent strain of FOV that did not seem to be dependent on root knot nematodes. This discovery sparked the research to classify the races of FOV present in California at the time because of the fear of a potential introduction of the Australian race of FOV. With the assistance of molecular markers and gene sequencing, isolates taken from California cotton fields were identified as races 3, 4, and 8 (Kim et al., 2005). The biotypes found in Australia were never assigned a

race since they did not match any of the previously known races (Davis et al., 1996). Therefore, it was concluded that FOV4 found in California did not come from Australian cotton seed.

Virulent isolates of race 4 were found in the San Joaquin Valley and showed severe symptoms and reduced plant yield in Pima cultivars. Upland cultivars had the same symptoms to race 4 as the other isolates from different races of FOV. Race 4 did not cause greater growth reduction in upland cotton than other FOV races (Kim et al., 2005). After this finding, more surveys across the United States were conducted including one by Holmes et al. (2009) that reported race 3 and 8 in a survey conducted in the Mid-South (Arkansas, Missouri, Georgia, and Louisiana).

*FOV Race 1*

FOV race 1 was first identified in the United States. Due to its widespread nature, it is most likely the race that was studied in Fusarium wilt research projects prior to the creation and differentiation of multiple FOV races (Armstrong and Armstrong, 1960). Race 1 isolates have been collected from Alabama, Arkansas, Georgia, North Carolina, South Carolina, Oklahoma, and Tennessee and most likely occurs in other cotton producing regions (Armstrong and Armstrong, 1960). FOV disease severity from race 1 increases with the presence of root knot nematodes and usually appears in sandy acidic soils, but also has been found in neutral pH soils in Oklahoma.

*FOV Race 2*

FOV race 2 has a similar impact on cotton as race 1 but can infect tobacco and Yelrado soybean (Armstrong and Armstrong, 1958, 1960). Like race 1, race 2 can most often be found in sandy soils with acidic or neutral pH (Hillocks, 1992). Race 2 is thought to be a mutant derivative of race 1 since pathogenicity on cotton is similar. However, FOV race 2 is more virulent on other crops than FOV1 (Armstrong and Armstrong, 1960).

*FOV Race 3*

Unlike FOV races 1 and 2, race 3 generally is found in clay soils (Hillocks, 1992). It is believed to have originated in Egypt (Armstrong and Armstrong, 1960) and has a low virulence in Pima and Upland cotton. It also does not cause symptoms in alfalfa, okra, or tobacco. Kim et al. (2005) determined race 3 would not be a likely threat to the United States commercial cotton production.

*FOV Race 4*

The designation for FOV race 4 was given to a strain of FOV that appeared to originate from heavy alkaline soils in India. At the time of its identification and classification, FOV4 did not cause severe symptoms on Upland cotton, alfalfa, okra, barley, or tobacco (Armstrong and Armstrong, 1960). In 2001, FOV4 was first identified in the United States in the San Joaquin Valley in California (Kim et al., 2005). It was isolated from fields of Pima cotton that were showing severe symptoms of wilt. The

fields from which this pathogen was isolated had clay loam soils with little to no presence of RKN. In the survey done in California by Kim et al. (2005), the predominant FOV race collected was FOV4. This was not because FOV4 is more common than other FOV races, but because FOV4 was highly virulent and caused severe economic damage in fields planted to the Pima cultivar DP 744 in the absence of nematodes. These isolates were collected from Tulare and Fresno County, California. FOV4 isolates were genetically identical to the American Type Culture Collection (ATCC) race 4; however, the ATCC race 4 did not cause any symptoms to Upland cotton in previous studies. Pathogenicity could have been lost during storage, but FOV4 caused disease symptoms in cotton in other studies. Fields in California infested with FOV4 had been reported as almost complete losses. It was recommended to plant fields known to have FOV4 with Upland varieties or resistant Pima varieties. FOV4 was later confirmed in fields of Pima cotton in El Paso County, Texas, in 2017 (Halpern et al., 2018), but had been suspected for a few years earlier than that. FOV4 was later confirmed to be in southern New Mexico near Las Cruces in 2019 (Zhu et al., 2020). Just like in California, fields in Texas and New Mexico infested with FOV4 were absent of RKN.

Long-distance transmission through seed is currently the best explanation for the present geographical distribution of FOV4 (Bell et al., 2019). Once FOV enters a field through seed, it can continue to spread throughout the field by other mechanisms that move soil such as equipment or irrigation water. Four genotypes of race 4, including N, T, MT, and MiT have been identified (Bell et al., 2019). While genotypes found in the San Joaquin Valley of California match those found in New Mexico, they do not match

the MT genotype found in the lower valley of El Paso, Texas (Wagner, 2022, Bell et al., 2019). This leaves room for discussion about how FOV4 made its way into Texas fields. If given a chance to spread farther east and into the High Plains, it could become an even bigger threat to the cotton industry.

*FOV Race 5*

FOV race 5 was originally identified in Sudan, but eventually determined to be identical to race 3 (Nirenberg, 1994). Nirenberg (1994) advocated to have race 5 withdrawn since he concluded that different pathogenicity results can be attributed to different inoculation methods. This incongruence in testing protocols may explain why FOV races 3 and 5 were originally thought to be unique. Race 5 (and 3) are distinct from all other races with their smaller conidia and differential symptom development on hosts (Davis et al., 2006).

*FOV Race 6*

Armstrong and Armstrong (1978) discovered a new race in Brazil which they classified as FOV race 6. The reaction from FOV6 in upland cotton and okra was closely related to the reactions caused by FOV1 and FOV2. However, unlike races 1 and 2, it did not cause wilt in *G. barbadense, G. arboreum,* alfalfa, tobacco, lupine, or soybean (Armstrong and Armstrong, 1978). Through greenhouse testing and isolation of additional race 6 pathogens, they reported that other plant species may be symptomless carriers.

*FOV Race 7*

FOV races 7 and 8 were first described by Chen et al., (1985) in China. These races are based on reactions to cotton, okra, tobacco, alfalfa, and soybean (Chen et al., 1985). FOV 4 and FOV7 have similar pathogenicity effects on cotton and other hosts and genomic sequencing suggests they may be closely related (Skovgard et al., 2001).

*FOV Race 8*

Race 8 was also fist described by Chen et al., (1985) in China. It was first observed in the United States by Kim et al. (2005) using genomic sequencing. This race caused mild symptoms on Pima and Upland cotton.

**2.7 Fusarium Wilt in India**

FOV4 is thought to have originated in India (Armstrong and Armstrong, 1960). India recently surpassed China and is currently the highest producer of cotton in the world (USDA, 2022). Cotton yield on a per hectare basis in India is below the world average, but the amount of land in cotton production far surpasses that of other cotton producing countries.

India is the only country in the world that cultivates four *Gossypium* species (Blaise and Kranthi, 2019). In the 1940s, most cultivated cotton was planted as 'tree cotton' (*G. arboreum)* which is native to India and 'Levant cotton' (G. *herbaceum*) which originated in the semi-arid regions of sub-Sahara Africa and Arabia. Even before the disease was officially described as FOV4, fusarium wilt posed a challenge for cotton

growers in India. In an early study conducted by Mundkur (1936), Indo-American cultivars seemed to be resistant to the wilt after growing in an infested field for eight years and showing no symptoms while the native cultivars were susceptible. Mundkur (1936) grew Indian and American cotton in sandy acidic soils (similar to the soil in the southeastern US) and inoculated them with the American and Indian fusarium. The Indian cotton was not affected by the American race and similarly the American cotton was not affected by the Indian race (Mundkur, 1936). It was concluded that environment plays a large role and makes the pathogen incapable of infecting the potential host plant or perhaps environmental conditions make the host plants resistant to infection from FOV4.

In the United States, most fields that were thought to be infested with fusarium in the early 20$^{th}$ Century had sandy soils with a pH range of 5.5-5.9 (Taubenhaus, 1928). In the same era in India, soils where cotton was produced had heavy clay with a pH range of 6.6-8 (Mundkur, 1936). These large differences in soil texture and pH could explain why a highly virulent strain can be observed as a passive saprophyte to a susceptible host when placed in a different soil (Mundkur, 1936).

With the commercialization of Bt cotton, India began to plant more Upland cotton developed from US germplasm. By 2013, Upland cotton accounted for 96% of India's cotton cultivation (Blaise and Kranthi, 2019). Cotton growing regions of India include a large range of soil types and climates. Blaise and Kranthi (2019) listed the top 14 challenges India's cotton industry faces; however, neither Fusarium wilt, nor any other wilt for that matter, was on their lists of major concerns. In fact, FW does not even

make it onto the list of top diseases Indian growers encounter on a yearly basis. Bacterial blight is listed as the most common disease. Fusarium wilt is only mentioned briefly in years where weather conditions are unique enough to create FW disease symptoms. Even though FOV4 originated from India, Indian growers don't appear to be struggling with FOV4 as much as growers in some areas of California, New Mexico and Texas. One reason could be that less than 1% of India's cotton area is planted in *G. barbadense* which can be highly susceptible and shows more noticeable symptoms. It has been reported that FOV4 found in California is a more virulent biotype than the one in India (Bell et al., 2019).

### 2.8 Fusarium Wilt in Australia

Australia is one of the world's leading exporters of raw cotton. FOV was first identified in Australia in 1993 from wilting cotton plants in Darling Downs, Queensland (Kochman, 1995). In the following year, it was identified in New South Wales where Verticillium wilt was already a common disease, but plants began to exhibit slightly different symptoms than those of Verticillium wilt disease. These symptoms included wilting, dark brown discoloration of the vascular system, and seedling death. Affected plants appeared in patches in the fields, some a few meters long. Two different FOV genotypes have since been isolated through genomic sequencing and determined to be unique from other FOV races (Kochman et al., 2012). Even though the Australian FOV pathogen caused reactions in cotton similar to FOV race 1 on cotton differentials (Davis et al., 2006) and was like FOV race 6 in secondary host infections (Davis et al., 1996),

Kim et al. (2005) found it to be genetically unique. Since identification of these Australian strains, no other new strains have been identified in Australia. Due to its widespread presence across the cotton industry, fusarium wilt was considered by many to be the most important constraint to cotton production in Australia. Since the discovery of FOV, substantial research efforts have been made towards the development of management practices to reduce the spread and severity of FOV. Since FOV is a stress related pathogen, finding ways to mitigate stress was an initial strategy. It is not uncommon for many Australian cotton producing regions to experience a cold shock early in the growing season which severely stresses seedlings. In November 2001 after a cold shock, investigators found that 20-40% of dead seedlings had been infected by FOV while other dead seedlings were infected with a complex of *Rhizoctonia* and *Thielaviopsis* (Kochman et al., 2002). Adjusting planting dates and reducing stress along with identifying resistant cultivars were successful ways to mitigate FW incidence and severity. Currently, most commercial cultivars have partial resistance to FOV, but ongoing work is needed to transfer higher levels of FOV resistance found in wild cotton species into commercial Upland cotton varieties. Besides stress, factors such as conidia concentration, growing location, environmental conditions, and seed quality can affect Fusarium severity in Australia.

When the news of the highly virulent FOV strain in Australia brought global attention to the disease and the threats it posed to the industry, the U.S. Animal and Plant Health Inspection Service (APHIS) classified the Australian strains as prohibited pathogens. This threatened the export of 300,000 tons of Australian grown cottonseed

which represented $100 million in annual sales to dairies in California (Kochman et al., 2002). This economic threat prompted researchers to investigate potential transmission of the FOV pathogen through seeds.

Since over 5000 spores could be found in a gram of soil, the “Come Clean Go Clean” campaign in Australia was established to bring awareness and implement the practice of sanitation (Kochman et al., 2002). Australians hoped this would slow the spread of FOV spores found in soil which could be carried by shoes, vehicles, or farm equipment that moved between fields. Even though these provisions were broadly and quickly adopted by most people in the Australian cotton industry, FOV was still appearing in new fields. Many of these fields were downstream of infested fields and when rivers and other streams flooded, flood water brought new soil containing FOV spores. However, even fields that are in watersheds relatively isolated from FOV infested fields began showing wilt symptoms of Fusarium. It is theorized that since many of the high-yielding and popular commercial cultivarts were highly susceptible to FOV, they were allowing the pathogen to multiply each year until inoculum levels were high enough and environmental conditions were conducive for infection to take place and symptoms to appear.

Restriction of seed dispersal was an important strategy Australian growers took to slow the spread of FOV. Seed companies could only produce planting seed in areas not affected by FOV. As of 2002, FOV had only been isolated from seed produced from plants displaying FOV4 symptoms. In seed cotton taken from infected plants, the original infection level was near 50%. This decreased with storage time and after six

months, no FOV causal agents were found in seeds stored at room temperature. Delinted seed had even fewer infested seed. Therefore, it seemed reasonable that the spread of this FOV pathogen could be largely controlled through seed production systems.

### 2.9 The Nematode Complex

Interaction between nematodes and Fusarium wilt is one of the oldest and most well-known disease complexes in the world. FOV is generally associated with Root Knot Nematode (*Meloidogyne incognita* (Kofiod & White) Chitwood). Fumigation of nematode infested soil has been a successful method for controlling for Fusarium wilt, at least for the races that are nematode dependent. Traits for resistance to RKN have been found in cotton landraces (Shepherd, 1974; Shepherd, 1982) and have been critical tools in controlling FW.

In fewer cases, non-gall forming nematodes including the sting nematode (*Belonolaimus gracilis* Steiner and B. *longicaudatus* Rau) (Holderman and Graham, 1954), lance nematode (*Hoplolaimus seinhorsti* Luc) (Rajaram, 1979), lesion nematode (*Pratylenchus branchyurus* (Godfrey) Filipjev & Schuurmans -Stekhoven) (Michell and Powell, 1972), and reniform nematode (*Rotylenchulus reniformis* Linford & Oliveira) (Neal, 1954) can also contribute to wilt damage (Armstrong and Armstrong, 1960).

It is still unclear how the relationship between FOV and nematodes exists. The idea that the nematode is a vector for the fungus does not hold up because the stylet of the nematode is too small for the spores to enter (Davis et al., 2006). One hypothesis, which has not been verified, is that the nematode is able to carry spores on its cuticle,

allowing FOV spores to move through the soil and come in contact with roots (Mai and Abawi, 1987a). Another unverified hypothesis is that resistance genes for FOV and RKN are closely linked.

Another common hypothesis is that nematodes simply produce an entry point for the fungus from damage caused by feeding on roots. This theory was supported by the findings of Minton and Minton (1966) when they conducted a study showing FOV only affected older plants and not seedlings when both the nematode and pathogen were present. Injury to the root from the nematode had to occur before the pathogen could infect the plant (Minton and Minton, 1966). Michell and Powell (1972) had a similar hypothesis indicating a mechanical interaction between the nematode and FOV. Others disagree that the relationship would be so simple since not all nematodes increase the presence of wilt as much as RKN (Holdeman and Graham, 1954). Katsantonis et al. (2003) found that even when FOV and nematodes were separated (the stem was inoculated with Fusarium instead of the roots), disease incidence was still higher when nematodes were present. This would contradict hypotheses related to mechanical damage. While the relationship between FOV and nematodes is still questionable, it is clear that for nematode dependent races, increased populations of RKN lead to higher disease incidence.

### 2.10 Seed Index and Indirect Selection Methods

Indirect selection methods can be a useful approach for breeders to predict how a cultivar will perform. As seed costs keep rising for growers, it is important that growers

plant high quality seeds, especially if they plant at low seed rates. Many factors are indicative of high-quality seed including emergence rates, seedling vigor, and stand uniformity. While seed quality is not the only factor determining success of a cotton crop, it does play a critical role. Rathinavel et al. (2021) reported that seeds with a relatively high volume and weight result in more ideal plant population, lower pest and disease incidence, and are more likely to produce a higher yielding cotton crop than lower volume seeds.

Increasing yield has historically been the goal for most plant breeders. In cotton, higher lint yields have been achieved in genotypes that produce small seeds. Lint percent is the ratio of fiber weight to the cottonseed weight. Seed index (SI) is the mass of 100 seeds and the most common term when relating to seed size. Popular belief is that smaller seeds have lower seedling vigor and can cause problems for cotton gins. However, larger seeds tend to decrease lint percent and commonly have thinner seed coats than smaller seed (Groves et al., 2016). To eliminate disadvantages associated with small or large seeds, moderate seed indexes have been favored by many cotton breeders and attributed to higher yields (Main et al., 2013). These mid-size seeds have an advantage over smaller seeds when it comes to germination, and achieving a full stand (Minton and Supak, 1980). They also have a survivability similar to large seeds but produce yields more in line with smaller seeds. Later studies challenged the idea that larger seeds lead to lower lint yields. Snider et al. (2016) concluded that other factors such as environment play a larger role in lint yield than seed size. Therefore, selecting for larger seeds may not detract as much from potential yield as previously thought.

Only one environment in his study found yield to be negativity correlated with seed size. Aside from cotton, many crops have been shown to produce seedlings with more vigor when grown from larger seeds.

The association between seed size and disease resistance has been studied in many crops with many of those studies providing contradictory results. Root rot *(Cochliobolus sativus)* is a common fungal disease in wheat cause by a soil borne fungus. No significant differences between mid-season disease index or harvest disease index were found between the large and small seed (Piccinni et al., 2001). These findings concur with an early study by Ducsek and Piening (1982) involving common root rot of barley (*Hordeum vulgare*) which can be caused by Fusarium wilt. That study was done to further investigate one conducted by Ghobrial (1976) who found less disease on plants grown from larger seeds. It is possible that conflicting conclusions were reached due to differences in locations and other sources of variation that may have interacted with seed size. While adequate evidence is lacking to support the idea that seed size promotes host plant resistance, improving seedling vigor may play a role in reducing FW in seedlings and serve as a possible indirect selection criterion.

### 2.11 Breeding for Resistance

The most effective field nurseries for FOV research are heavily infested with FOV and are invaluable to plant breeders screening and selecting plants with host plant resistance. Crop residue from infected cotton plants is incorporated into the soil which, in turn, increases inoculum levels for the subsequent crop. The National Fusarium Wilt

Nursery at Auburn University in Tallassee, Alabama, which was initiated in 1952, is an example of a high-quality field nursery. There are advantages and challenges with using a field-based nursery for disease screening. Using a field-based nursery exposes cotton plants to similar conditions as they would encounter in commercial production fields. However, it is likely that the nursery will not have a uniform distribution of inoculum across the field. Screening for FW resistance can also be hindered by the confusion created by the presence of other FW pathotypes, soil pathogens, and experimental errors. Unreliable weather and field conditions also can affect crop performance. Multiple years and locations may be needed to measure stability of a germplasm's response to FOV (Sanogo, 2015). Controlled environment screening such as growth chambers and greenhouses also can be used for disease screening. This allows researchers to screen many entries in a small and well-controlled space. Inoculum level, type, and timing can also be regulated. A disadvantage of using growth chambers and greenhouses is the inability to expose cotton genotypes to the similar biotic and abiotic stresses that they would encounter in a field setting. While results from greenhouse screenings can be inexact from those using field-based nurseries, they often follow similar trends. Most studies conducted on FOV resistance screening include complementary field-based nurseries and controlled environment screenings.

After the identification of races 1 and 2, breeding Upland cotton for resistance mainly occurred in the southeastern United States where FOV was an economically important plant disease. Initially progress was slow and resistant breeding lines were not agronomically desirable. While breeders continued to make improvements in wilt

resistance in cotton, the rate of genetic gains for yield and fiber quality were not as successful since it is difficult to select concurrently for resistance and agronomic traits (Kappelman, 1980). After 1900, the rate of improvement for FOV resistance increased when hybridization allowed breeders to move higher yielding traits into wilt resistant cultivars. It wasn't until about 1942 that wilt resistant varieties were released that also possessed favorable agronomic traits (Presley, 1972). In 1960, it was determined that in Upland cotton host plant resistance to FOV was controlled by a major dominant gene with minor modifying genes, and host plant resistance in Egyptian cotton was controlled by two dominant genes (Smith and Dick, 1960). Kappelman et al. (1971) reached a similar conclusion that resistance in Upland cotton was quantitatively inherited and controlled by several major genes with minor modifying genes. While Acala cotton types tend to have a higher survival rate than other types of Upland cotton, there still have been lower levels of tolerance identified in Upland cotton compared to resistant Pima cotton (Ulloa et al., 2020).

After the discovery of FOV4 in California, host plant resistance in Pima cotton became a priority because of the severity of the economic impact to that industry. Both Pima and Upland cotton germplasm exhibit variable levels of host plant resistance to FOV4, but Pima more frequently demonstrates more complete resistance (Ulloa et al., 2020). Inheritance of FOV4 resistance in Pima cotton appears to be intermediate to dominant while most Upland cotton breeding lines have inheritance that ranges from recessive to intermediate (Ulloa et al., 2020). In Pima cotton, heritability of 0.64 to 0.95 allowed selections to be effectively made as early as the $F_2$ generation (Ulloa et al.,

2006). Pima cultivars with relatively high levels of resistance were identified in commercial Pima cotton ‘PHY 800’ (Phytogen Seed Co., Corteva Agriscience, IN), and ‘Pima S-6’. Today, there are several commercial Pima cultivars that show moderate to high resistance (Phytogen Seed Co., Corteva Agriscience, CA and Delta and Pine Land Co., Bayer CropScience, MS). While the number of acres planted in Upland cotton has declined in California, Pima cotton acreage remains steady and makes up about 50% of California’s cotton area (Geisseler and Horwath, 2016).

Asiatic cotton species (*G. arboreum* and *G. herbaceum)* could possibly possess sources of genetic resistance to FOV4 and be sources for Upland germplasm improvement since these species and FOV4 pathogens originated in the same region. Ideally, having multiple sources of resistance genes would allow breeders to pyramid genes which provides long-term protection against evolving FOV4 populations. Of all the available *G. arboreum* breeding lines available in the U.S. Nation Cotton Germplasm Collection (NCGC), all accessions were susceptible to FOV4 (Abdel-Raheem et al., 2019). These findings on *G. arboreum* were confirmed by Zhang (2020) and that genes related to FOV4 resistance are possibly heterogeneous in many germplasms.

# CHAPTER III

# METHODS AND MATERIAL

## 3.1 Previous Work

In 2018, the Texas A&M Cotton Improvement Lab started the FOV4 resistance project by preliminary screening of an extensive number of existing lines in single plots. These lines were initially screened for FOV4 resistance at two locations in El Paso County, Texas. These locations included a field near Clint, Texas, operated by O&A Enterprises, and the other near Fabens, Texas, managed by Texas A&M AgriLife. Plots at both locations were 3.3 meters in length and 1 meter in width with a planting rate of 20 seeds per meter. 'DPL 357' was used as a susceptible check and planted at regular intervals throughout the test. Plots in Clint were rated two weeks after first bloom for survival, and leaf chlorosis. Plots in Fabens were rated 30 days after planting for survival, and chlorosis ratings. Both locations were rated at the end of the season for productivity and root stains.

The same lines were planted in College Station in a non-infested field. After the mid-season disease ratings, many lines were eliminated in the College Station nursery based on susceptibility as measured in the El Paso County tests. The remaining lines were harvested as progeny rows and Individual Plant Selections (IPSs) were collected from the most promising lines. Fiber quality was measured with HVI™ from the harvested lines and IPSs. Decisions to advance IPSs to the 2019 nursery were made based upon fiber quality and lint percent.

In 2019, the most promising lines were entered in the O&A program in Clint, TX, with the same screening methods as the previous year. In Fabens, a preliminary strain trial (PST) including 60 lines was created using the most promising advanced breeding lines from the screening trial the previous year. This trial included three replications and the same plot size as 2018. The susceptible check DP 357 and resistant check 'FM2334GLT' were also included. Along with screening methods used in 2018, a normalized difference vegetation index (NDVI) measurement was taken of each plot at the seedling and first bloom growth stages using a hand-held Trimble Greenseeker. IPSs from the 2018 College Station nursery were planted as progeny rows (PR) in Fabens which included 30 lines and were screened the same way as the PST. The most promising lines of the PST were advanced to the 2020 advanced strain trial (AST). Likewise, the most promising PR lines were advanced to the 2020 PST.

All lines grown in El Paso Country were once again increased in College Station and IPSs were harvested from reselections of promising material. About 50% of IPSs were advanced to the 2020 nursery based on lint percent and fiber quality. Also in 2019, hybridizations were made using parents with the most promising HPR performance at Fabens in 2018 and from the evaluation made in July 2019 (Table 1). These hybridizations were then planted as $F_1$s in 2020 and would be evaluated in 2022 as $F_3$s.

**Table 1. Hybridizations made at College Station, Texas, in 2019.**

| Population | Pedigree |
|---|---|
| 19277 | 14B-72/13T-38 |
| 19278 | 14B-72/13V-57 |
| 19280 | Reba B50/13T-38 |
| 19281 | Blackarm ResistantX16/13T-38 |
| 19282 | 13T-38/Giza 70 |
| 19283 | 13T-38/Giza 75 |
| 19284 | 13T-38/Giza 80 |
| 19286 | 13V-57/Reba B50 |
| 19287 | 13V-57/Blackarm Resistant X16 |

In 2020, no lines were evaluated at the O&A Enterprises location in Clint, TX. The AST included 16 lines that were advanced from the 2019 PST. The PST included 26 lines and 85 progeny rows evaluated in Fabens, TX. The same evaluation methods were conducted as the previous years. All lines were once again grown in College Station, TX, for increases and fiber quality data.

### 3.2 2022 Disease Screening

The nursery for disease screening was planted in late April 2022 in an FOV4 infested field in El Paso County, Texas. The trial was planted in a complete randomized block design to block for varying levels of inoculum within the field. All plots were planted 3 meters long with 1.01meters row spacing. 50 seeds were planted per plot. This field was furrow irrigated throughout the season and managed by Texas A&M AgriLife Extension. A susceptible check, ‘DP1646B2XF', as well as a resistant check, FM2334GLT (Zhu et al., 2021), were included in each test. This nursey was used for

observations since plant material could not be removed from the field in order to prevent the spread of the disease.

The Elite strain trial (EST) and Advanced strain trial (AST) included four replications while the preliminary strain trial (PST) included three replications. The rest of the trials including the $F_3$s, preliminary strain observations (PSO), and the progeny rows (PR) were grown in non-replicated single plots.

**Table 2. Lines included in the Elite Strain Trial at Fabens, Texas, 2022.**

| 2022 Entry | 2022 Designation | Pedigree |
|---|---|---|
| XEST-FOV-1 | 16SHS-32 | 08 WZ-75/07 V-45 |
| XEST-FOV-2 | 16SHU-38 | 11323/11333 |
| XEST-FOV-3 | 17 SHJ_48 | 10 WA-07/[(08WZ-51/08WZ-39)F1] |
| XEST-FOV-4 | 17SHO_26 | 09 SIUP 120/11 HA-27 |
| XEST-FOV-5 | 17SHK_74 | 11 HA-27/09 WJ-37 |

**Table 3. Lines included in the Advanced Strain Trial at Fabens, Texas, 2022.**

| 2022 Entry | 2022 Designation | Pedigree |
|---|---|---|
| XAST-FOV-1 | 14 E-12-FOV-05 | DP90/07X-26 |
| XAST-FOV-2 | 16-SHU-11-FOV-02 | 08 WZ-87/08 WZ-83 |
| XAST-FOV-3 | 16-SHU-11-FOV-04 | 08 WZ-87/08 WZ-83 |
| XAST-FOV-4 | 16-SHU-11-FOV-05 | 08 WZ-87/08 WZ-83 |
| XAST-FOV-5 | 16-SHU-27-FOV-05 | Hyperformer HY007/08 WZ-87 |
| XAST-FOV-6 | 16-SHU-27-FOV-06 | Hyperformer HY007/08 WZ-87 |
| XAST-FOV-7 | 16-SHU-27-FOV-09 | Hyperformer HY007/08 WZ-87 |
| XAST-FOV-8 | 16-SHU-38-FOV-05 | 11323/11333 |
| XAST-FOV-9 | 16-SHU-38-FOV-14 | 11323/11333 |

**Table 4. Lines included in the Preliminary Strain Trail at Fabens, Texas, 2022.**

| 2022 Entry | Designation | Pedigree |
|---|---|---|
| XPST-FOV-1 | 206-20 | 11323/11333 |
| XPST-FOV-2 | 233-02 | 11 HA-27/09 WJ-37 |
| XPST-FOV-3 | 233-09 | 11 HA-27/09 WJ-37 |
| XPST-FOV-4 | 233-11 | 11 HA-27/09 WJ-37 |
| XPST-FOV-5 | 233-12 | 11 HA-27/09 WJ-37 |
| XPST-FOV-6 | 238-02 | 09 SIUP 120/11 HA-27 |
| XPST-FOV-7 | 238-03 | 09 SIUP 120/11 HA-27 |
| XPST-FOV-8 | 209-14 | TT07,11328(F1)/09WJ-37 |
| XPST-FOV-9 | 209-16 | TT07,11328(F1)/09WJ-37 |
| XPST-FOV-10 | 209-23 | TT07,11328(F1)/09WJ-37 |
| XPST-FOV-11 | BB-04 | 10X-64/13T-14 |
| XPST-FOV-12 | 17 MM-51-59-09 | 10X-63/6270 |
| XPST-FOV-13 | 17 NN-31-39-07 | 10X-63/K-7610 |
| XPST-FOV-14 | 17 PP-31-39-02 | 10X-78/6173 |
| XPST-FOV-15 | 17 PP-41-49-16 | 10X-78/Fiji Sea Island |
| XPST-FOV-16 | 17 QQ-31-39-02 | 10X-78/13X-51 |
| XPST-FOV-17 | 17 QQ-41-49-11 | 10X-78/K-7610 |

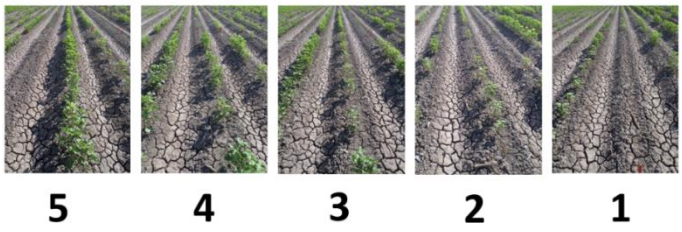
In late May, about two weeks post emergence, stand counts were conducted to determine early season mortality. This involved counting the number of dead and alive seedlings in each plot.

**Figure 1. Healthy seedlings adjacent to a dead seedling at the Fabens nursery in El Paso County TX, in 2021.**

Also in late May, foliar ratings were recorded based on a 1 to 5 scale with 1 representing an empty plot and 5 representing a full and healthy stand with no visible

signs of infection. Ideally, a mid-season foliar rating and normalized difference vegetation index would have been taken but due to inclement weather this was not possible. Once plants had reached maturity, a final trip was made to El Paso in November to rate foliar symptoms, productivity, and root stem stains. The productivity rating is based on how well the plants that survived performed from a yield potential perspective. This rating is also on a 1 to 5 scale. 0 represents a dead plot and 5 represents healthy looking plants that are producing a lot of cotton.

**Figure 2. Stand rating scale of 1-5.**




### 3.3 Vascular Root Stains

In mid-November when the plants had matured, five plants from each plot were dug up with a shovel to take vascular root stains. The taproots were sliced open vertically below the soil line to reveal the vascular system. Dark brown staining along the length of the root is one of the key symptoms of FOV4. These roots were rated on a 0 to 5 scale. On the scale, 0 represents a healthy root with no discoloration while 5 represents a completely dead root.

**Figure 3 Root stain scale.**


To observe Fusarium movement within the xylem tissue, root stains were analyzed in increments. Five plants from each plot were extracted from the soil with a hand shovel and then sliced horizontally at the cotyledon node. If staining was present in xylem tissue at this mark, another horizontal cut was made 20mm above the previous cut. This process was repeated until a clean stem was observed. If there was no staining at the initial cotyledon node, a new horizontal slice was made 20mm below the initial slice. This process was repeated down the tap root until vascular staining was observed.

**Figure 4. Cross-section of a cotton stem with xylem tissue staining.**

### 3.4 College Station Nursery

All lines that were grown in El Paso County were also grown at College Station, Texas. These plants were used to assess fiber quality potential as well as to increase seed. In 2021, plants were self-pollinated to generate seed for evaluation in 2022. Material for this project was also included in the crossing block.

A thirty-boll sample was collected from each plot grown at College Station for fiber and lint percent evaluation. A 40-gram sample of fiber from each line was sent to Texas Tech University's Fiber and Biopolymer Research Institute at Lubbock, Texas, for fiber measurements using HVI. Fiber data included fiber length, strength, length uniformity, micronaire, and elongation. From these results, decisions were made on which IPSs to advance to progeny rows.

### 3.5 Elite Strain Yield Trial

To evaluate the competitiveness of the most advanced breeding lines in the program, a yield trial was conducted at College Station. Five EST lines with FM2334GLT and DP1646B2XF serving as commercial checks were grown in a randomized complete block design with four replications in two row plots. Plots were 13.11 m in length and had 1.01 m row spacing width. The field was furrow irrigated. A thirty-boll sample was collected from the left row of the two row plots from the first and third replications for fiber analysis. Both rows were mechanically harvested and seedcotton weighed.

### 3.6 Seed Index

Seed index was calculated by weighing the mass of 100 fuzzy seeds. The objective of this project was to explore a potential relationship between seed index and resistance to FOV4, specifically earlier in the season due to seedling vigor. A Pearson's correlation coefficient was assigned to each correlation between seed index and disease screening data.

# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1 FOV4 Breeding Efforts 2018-2020

FOV4 breeding efforts and results from 2019-2020 by the Cotton Improvement Lab (CIL) at Texas A&M University were reported by Lakey (2020). Our study was a continuation of many of those efforts; however, to understand progress during the 2021 and 2022 seasons, it is important to summarize many of Lakey's original findings and strategies.

To prevent the spread of FOV4, a non-infested nursery was grown near College Station (CS), TX, for fiber quality and lint percent evaluations along with line maintenance. The strategy of the CIL was to quickly remove susceptible lines from the breeding program and put efforts into exploiting populations with putative resistance and/or tolerance to FOV4. Breeding lines that showed susceptibility in the screening nurseries in El Paso County, were not harvested from the CS nursery. The remaining lines were harvested, and individual plant selections (IPSs) were made as reselections from the most promising resistant lines. From the IPSs, selected lines were advanced within the program to progeny rows (PR) based on fiber quality and lint percent.

In 2018, we had the opportunity to screen a large portion of the germplasm pool from the CIL in a field nursery managed by O&A Enterprises. The screening site was in Clint, TX, and had been verified as having FOV4 for multiple years. Lines were evaluated in non-replicated plots surrounded by susceptible check cultivars as per the

protocol established by O&A Enterprise. Plots were rated on a scale of 1-5 with 1 representing high susceptibility which is characterized by poor stands, stunted growth, and chlorotic foliage. A score of 5 reflected a complete stand with no vegetative chlorosis.

**Table 5. Preliminary screening for FOV4 resistance of the germplasm pool from the Cotton Improvement Lab at Texas A&M University. These lines were screened at the O&A Enterprises field nursery in Clint, TX, in 2018.**

| Population | # lines | Mean rating | C.V., % |
|---|---|---|---|
| Obsolete germplasm | 196 | 1.28 | 43.8 |
| Recent germplasm | 48 | 1.50 | 43.0 |
| EST | 14 | 1.21 | 33.8 |
| AST | 30 | 1.03 | 17.4 |
| PST | 20 | 1.19 | 33.0 |
| PSO | 120 | 1.52 | 48.9 |
| F4 | 571 | 1.40 | 44.7 |
| **Mean** | - | 1.38 | - |
| **Standard deviation** | - | 0.62 | - |

**Lines were rated on a scale of 1-5 with 1=highly susceptible and 5=highly resistant.**

Results from this screening effort suggested that genetic diversity is an important factor to finding high levels of HPR to FOV4 (Table 5). For example, the advanced strain population (AST) had relatively few lines, 30, but more importantly, these lines were closely related with only a few parents represented within the pedigrees of this cohort. The narrow genetic base within the AST group resulted in no lines exhibiting functional HPR. Another important finding was the contrast between recent germplasm releases versus obsolete germplasm lines. Lines that were designated as recent germplasm were those lines that completed the CIL pedigree breeding program within the last five years while the obsolete germplasm represented lines released more than

five years prior to this screening including several lines released many decades earlier. On average, the more recent germplasm had a slightly higher mean rating than the obsolete germplasm (1.50 versus 1.28). This may be the result of indirect selection or improvement of traits that enabled the more modern lines to at least tolerate FOV4 infection.

Another fortunate observation was the relatively high levels of resistance in the early generation populations as represented by the preliminary strain observations (PSO) and the $F_4$ cohort groups. Incidentally, the $F_4$s were first-generation progeny row selections. Because these early-generation lines had yet to be closely evaluated and screened for yield, fiber, and other agronomic traits, they represented an opportunity for the CIL breeding program to initiate a divergent FOV4 breeding program which could emphasize FOV4 resistance as the highest priority with yield and fiber quality as secondary criteria.

Texas A&M AgriLife managed a different nursery in El Paso County near Fabens, TX, in 2018. In this field, the spread of FOV4 was likely not as profuse as the field at Clint, but it did allow us to conduct evaluations with replicated plots. Under these circumstances where FOV4 inoculum levels may be variable across the field, we hoped to see low disease incidence and severity along with low coefficients of variation within a line under evaluation. This would indicate the line had a similar performance with and without heavy disease pressure.

**Table 6. Summary of FOV4 disease resistance screening for seedling survivability and early-season foliage chlorosis and growth habit near Fabens, TX, in 2018. This trial included 86 strains from the Cotton Improvement Lab at Texas A&M University.**

| Value | Survival (%) | Foliage Rating (1-5) |
|---|---|---|
| **Mean** | 84.7 | 1.62 |
| **Maximum** | 100.0 | 3.33 |
| **Minimum** | 55.2 | 0.67 |
| **C.V., %** | 13.6 | 35.5 |

Lines in this trial were replicated three times. Overall, lines exhibited a high level of survivability, which suggested many lines had enough resistance to withstand the initial infection of FOV4 (Table 6). Two other factors contributed to the high seedling survival rate – seed quality and weather conditions. Planting seed used in 2018 was harvested from 2017. Seed produced in 2017 were generally high-quality seed. In fact, most seed grown in the 2017 CIL program had a germination rate of more than 95%. Moreover, early-season weather conditions were helpful in establishing a healthy stand. Daily temperatures were generally mild (22-30 ℃) for the first three weeks after planting. The field also received multiple light rainfall events that mellowed the soil and created favorable conditions for emergence.

Because of the advantageous conditions for emergence at this location in 2018, the breeding team felt the primary factor for seedling mortality was FOV4. Lines with the lowest seedling mortality rates could be removed from the trial at this point because they most likely did not have enough resistance to survive early-season FOV4 infection. Approximately 30% of the lines were no longer considered viable candidates for the program at this point in the program. It is also important to note that the coefficient of

variation for seedling survival was relatively low at 13.6%, which suggests lines were sufficiently challenged by FOV4 as they emerged across the entire trial.

By mid-season, plants were rated for foliar chlorosis, stunting, and mortality on a scale of 1-5 with 1 representing a plot with no surviving plants and 5 representing a plot with a full stand of plants with normal colored foliage and plant height. While this disease symptom rating was not as discriminating as the seedling survival measurements, it did provide valuable insight into mid-season expression of FOV4 symptoms. None of these lines showed levels of FOV4 resistance high enough for commercialization. The best line, ’16 SHU-38’ had a rating of only 3.33 while the worst line was rated at 0.67. The coefficient of variation for the foliar rating was 35.5%, which leads us to believe that inoculum levels that could infect plants well-after emergence were not well dispersed throughout the trial and that some lines were able to escape FOV4 by chance.

The 2018 growing season at El Paso County was probably the most critical season for the fledgling breeding program because we learned four important lessons:

1) FOV4 infections and disease expression of symptoms will come in waves throughout the growing season.
2) Seed quality and early-season emergence are critical to our ability to discern mortality due to FOV4 apart from other non-disease related factors. We would later experience how both threats severely compromised the 2021 field screening season.

3) Mid- and late-season diagnostic methods are needed to identify high levels of HPR for FOV4 because infections and symptom expressions can occur continuously throughout the life of the cotton plant.
4) We were able to identify candidate lines for re-selection and use as parents in new populations. We also observed the benefits of genetic diversity because HPR for FOV4 appeared to be quantitatively inherited with a continuous level of HPR within similar pedigrees.

**Table 7. Cotton fiber quality and lint percent of FOV4 breeding populations at College Station, TX, 2018-2020. These lines were grown in soil free from FOV4.**

| Trial | # of lines | Lint % | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|---|
| | | | Unit | mm | % | kNm/kg | % |
| **2018** | | | | | | | |
| PR | 70 | 39.6 | 4.2 | 29.3 | 83.5 | 303 | 5.3 |
| IPS | 270 | 42.0 | 4.3 | 30.5 | 83.5 | 302 | 5.4 |
| **Mean** | - | 40.1 | 4.3 | 30.5 | 83.5 | 302 | 5.4 |
| **2019** | | | | | | | |
| PR | 145 | 39.2 | 4.6 | 28.7 | 83.9 | 330 | 5.6 |
| IPS | 213 | 39.2 | 4.6 | 27.9 | 84.3 | 339 | 5.6 |
| **Mean** | - | 39.2 | 4.6 | 28.8 | 83.9 | 331 | 5.6 |
| **2020** | | | | | | | |
| AST | 6 | 38.0 | 4.5 | 29.8 | 84.0 | 333 | 5.6 |
| PST | 15 | 37.4 | 4.1 | 30.2 | 82.9 | 319 | 5.5 |
| PR | 27 | 37.0 | 4.1 | 30.2 | 83.1 | 335 | 5.4 |
| PSO | 368 | 37.0 | 4.2 | 29.3 | 82.8 | 312 | 5.5 |
| **Mean** | - | 37.0 | 4.2 | 29.4 | 82.8 | 314 | 5.5 |

At the start of the project in 2018, the lint percent and fiber length potential in most breeding lines was competitive with commercial lines (Table 7). These populations had been originally developed for fiber quality and yield potential. When reselections

were made in 2018 based solely upon FOV4 resistance potential, there was a slight overall decrease in lint percent, fiber length, and other quality traits. This was to be expected since selections were no longer being made based on fiber quality and the genetic diversity of our FOV4 candidate line shrank. It is important to note that fiber strength trended upwards within the program across these formative years, probably a serendipitous occurrence that some of the most important parents for FOV4 HPR also possessed alleles for strong fibers. Maintaining variation in the populations is essential to maintain and improve fiber quality while simultaneously selecting for disease tolerance.

In 2019, 60 lines were screened in a replicated preliminary strain trial (PST). These lines had shown the most promising levels of resistance in 2018 at both testing sites in El Paso County. The 30 best individual plant selections based on fiber quality in 2018 from the nursery near College Station were planted as single replication progeny rows (PR) in 2019 at the Fabens location. NDVI readings were taken of every plot in May (early season) and July (mid-season). Early season survival counts, mid-season foliage rating, and end-of-season root stem stain ratings were also collected for all plots.

The highest performing lines from the 2019 PST were advanced to the 2020 advanced strain trial (AST) which included three replications of 16 entries. To better understand the end of season performance of the plants, a productivity rating (1-5) was collected at maturity. A score of 1 represented a stunted plant with few bolls while 5 represented a plant producing multiple bolls per fruiting branch with ten or more fruiting branches.

**Table 8. Disease screening evaluations of advanced (AST), preliminary (PST) and progeny rows (PR) at Fabens, TX, in 2019 and 2020. Values reported are the means of each population.**

| Trial | # Lines | Survival | Foliar rating | Early-season NDVI | Mid-season NDVI | Root Stem Stain | Productivity |
|---|---|---|---|---|---|---|---|
| | | (%) | (1-5)[a] | (%) | (%) | (0-5)[b] | (1-5)[c] |
| **2019** | | | | | | | |
| PST | 60 | 86.4 | 3.2 | 7.0 | 47.0 | 1.3 | - |
| PR | 30 | 75.8 | 2.1 | 5.0 | 23.0 | 1.9 | - |
| **Mean** | - | 84.8 | 3.0 | 7.0 | 43.0 | 1.4 | - |
| **2020** | | | | | | | |
| AST | 16 | 70.4 | 3.5 | - | 22.7 | 2.6 | 3.7 |
| PST | 26 | 77.0 | 3.7 | - | 28.3 | 2.6 | - |
| PR | 114 | 61.0 | 2.6 | - | 20.0 | - | - |
| **Mean** | - | 65.5 | 3.0 | - | 21.8 | 2.6 | - |

[a]1 = high susceptibility, poor stands, and chlorotic foliage; 5= a healthy stand with no vegetative chlorosis or wilting
[b]0=a healthy root with no visible discoloration; 5 = a dead and rotten root with severe staining.
[c]1 = a poor preforming plant with no bolls; 5 = a healthy high yielding plant.

Results from screening evaluations in 2019 and 2020 suggest that inoculum levels increased at the testing site (Table 8). HPR trends within these breeding cohorts suggest that the first cycle of selection for FOV4 HPR was critical to improve the mean HPR performance. The PST cohort appeared to be better than the progeny rows that had yet to be screened for HPR to FOV4. Meanwhile, the PST and the AST that were tested in 2020 appeared to favor the PST in all measures of HPR. This suggests that the average breeding values of these populations were improving with each generation.

**Table 9. Mean squares of FOV4 disease screening for genotypes within strain trials at Fabens, TX, in 2019 and 2020.**

| Trial | df | Survival (%) | Early NDVI | Mid-season NDVI | Foliar rating (1-5) | Root Stem Stain (0-5) | Productivity (1-5) |
|---|---|---|---|---|---|---|---|
| **PST-2019** | | | | | | | |
| Genotype | 59 | 165.6* | 0.0002 | 0.02** | 1.2 | 0.54 | - |
| Reps | 2 | 5,701.1** | 0.0050** | 0.85** | 4.3** | 0.19 | - |
| **AST-2020** | | | | | | | |
| Genotypes | 15 | 0.0008** | - | 110.8** | 2.09** | 0.72 | 1.95** |
| Reps | 2 | 0.33** | - | 834.89** | 8.27** | 6.55** | 3.08** |
| **PST-2020** | | | | | | | |
| Genotypes | 24 | 0.07** | - | 232.49** | 3.19** | 1.95** | - |
| Reps | 2 | 0.26** | - | 420.25** | 7.43** | 6.89** | - |

*significant at the 0.05 probability level
**significant at the 0.01 probability level

One important observation were differences among blocks in all trials in 2019 and 2020 (Table 9). These results suggest inoculum levels were not uniform throughout the field. Even so, there were significant differences found among genotypes for survival rates and at the mid-season NDVI measurement for all trials. Root stem staining and foliar ratings, which are standard practices for many FOV4 breeding programs, were not always discriminating enough to identify HPR for FOV4 in 2019 and 2020. These observations led us to believe that more reliable and fastidious screening methods for FOV4 resistance are needed.

**Table 10. Pearson rank correlations of disease screening methods in FOV4 trials and nurseries grown at Fabens, TX in 2019 and 2020.**

| Trial | Survival (%) | Foliar rating (1-5) | Early NDVI | Mid-season NDVI | Root Stem Stain (0-5) |
|---|---|---|---|---|---|
| **PST-2019** | | | | | |
| Foliar rating (1-5) | 0.53** | - | - | - | - |
| Early NDVI | 0.65** | 0.51** | - | - | - |
| Mid NDVI | 0.78** | 0.66** | 0.72** | - | - |
| Root Stem Stain (0-5) | -0.11 | -0.13 | -0.21 | -0.18 | - |
| **PR-2019** | | | | | |
| Foliar Rating (1-5) | 0.38* | - | - | - | - |
| Early NDVI | 0.23 | 0.67** | - | - | - |
| Mid NDVI | 0.35 | 0.90** | 0.63** | - | - |
| Root Stem Stain (0-5) | -0.49 | -0.82** | -0.36 | -0.69* | - |
| **AST-2020** | | | | | |
| Foliar Rating (1-5) | 0.76** | - | - | - | - |
| Early NDVI | 0.80** | 0.85** | - | - | - |
| Root Stem Stain | -0.41** | -0.40** | -0.43** | - | - |
| Productivity | 0.70** | 0.78** | 0.68** | - | -0.40** |
| **PST-2020** | | | | | |
| Foliar Rating (1-5) | 0.78** | - | - | - | - |
| Early NDVI | 0.72** | 0.89** | - | - | - |
| Root Stem Stain (0-5) | -0.65** | -0.64** | 0.55** | - | - |
| **PR-2020** | | | | | |
| Foliar Rating (1-5) | 0.83** | - | - | - | - |
| Early NDVI | 0.79** | 0.90** | - | - | - |

*significant at the 0.05 probability level
**significant at the 0.01 probability level

The relationships among foliar ratings and NDVI measurements were positively and significantly correlated in all trials and nurseries in both years (Table 10). Using NDVI as a replacement for subjective foliar ratings would likely improve analytical capabilities, especially if these screening methods became more automated with high-throughput phenotyping. Root stem stain ratings were frequently negatively correlated with other measures of FOV4 HPR. Problem with this method are the labor requirements to rate plots and the potential for mischaracterization in populations that are highly

segregating like we would expect to see in progeny row lines or lines derived without multiple generations of re-selection.

**Table 11. Analysis of variance of responses of five lines advanced to elite strains for responses to FOV4 at Fabens, TX, in 2019, 2020, and 2022.**

| | | Mean Squares | | |
|---|---|---|---|---|
| **Source** | **df** | **Survival** | **Rating** | **Root Stain** |
| **Year** | 2 | 4,161.9** | 3.2* | 7.5** |
| **Genotype** | 4 | 128.3 | 0.9 | 0.4 |
| **Year X Genotype** | 8 | 164.7 | 1.4 | 0.7 |
| **Error** | 35 | 175.0 | 0.9 | 0.8 |

*significant at the 0.05 probability level
**significant at the 0.01 probability level

In 2022, five lines had advanced into the elite strain cohort. Data from these lines were analyzed in a factorial design across the three years (Table 11). Results from this analysis indicate that each growing season inflicted an increasing level of FOV4 disease pressure upon the lines. There was no significant year X genotype interaction detected for any of the three screening methods. Since the overwhelming amount of variation was from the year effects, differences among genotypes were not observed with data pooled across years. If we had included all the original lines from the preliminary strain trial that included highly susceptible lines as well as susceptible check cultivars, we likely would have measured enough variation attributed to the genotype effect to declare significant differences among genotypes across years. However, from a practical standpoint when managing a breeding program with finite resources, inferior lines need to be removed on a yearly basis to conserve resources. Moreover, we likely achieved a narrow, albeit improved, level of HPR to FOV4 among these five candidate lines, which

also contributed to our inability to detect differences among them in our analysis of variance.

Upon examination of these five lines across three years (Table 12), we can see the trend is towards lower survivability and poorer foliage ratings. Some of these responses are likely attributed to increasing FOV4 inoculum in the field, but it is important to consider other factors.

**Table 12. FOV4 disease responses of five elite strain lines for 2019, 2020, and 2021 at Fabens, TX.**

| Year | Designation | Pedigree | survival | NDVI | rating | productivity | root stain |
|---|---|---|---|---|---|---|---|
| | | | (%) | (%) | (1-5)[a] | (1-5)[b] | (0-5)[c] |
| **19** | 16-SHS-32 | 08 WZ-75/07 V-45 | 95.0 | 7.3 | 4.0 | - | 1.0 |
| **20** | 16-SHS-32 | 08 WZ-75/07 V-45 | 80.9 | 26.7 | 4.0 | 4.0 | 2.5 |
| **22** | 16-SHS-32 | 08 WZ-75/07 V-45 | 53.9 | - | 2.5 | 4.0 | 1.9 |
| **19** | 16-SHU-38 | 11323/11333 | 88.1 | 6.7 | 3.7 | - | 1.1 |
| **20** | 16-SHU-38 | 11323/11333 | 74.1 | 23.0 | 3.3 | 3.7 | 2.5 |
| **22** | 16-SHU-38 | 11323/11333 | 54.7 | - | 2.3 | 3.3 | 1.5 |
| **19** | 17SHJ_48 | 10 WA-07/[(08WZ-51/08WZ-39)F1] | 94.0 | 7.3 | 4.0 | - | 1.3 |
| **20** | 17SHJ_48 | 10 WA-07/[(08WZ-51/08WZ-39)F1] | 74.1 | 28.7 | 4.7 | 4.3 | 2.1 |
| **22** | 17SHJ_48 | 10 WA-07/[(08WZ-51/08WZ-39)F1] | 64.9 | - | 2.8 | 2.3 | 3.2 |
| **19** | 17SHO_26 | 09 SIUP 120/11 HA-27 | 93.8 | 8.6 | 4.3 | - | 1.1 |
| **20** | 17SHO_26 | 09 SIUP 120/11 HA-27 | 79.6 | 25.3 | 3.3 | 4.7 | 1.8 |
| **22** | 17SHO_26 | 09 SIUP 120/11 HA-27 | 61.2 | - | 2.0 | 3.0 | 2.3 |
| **19** | 17SHK_74 | 11 HA-27/09 WJ-37 | 98.2 | 7.0 | 4.0 | - | 0.9 |
| **20** | 17SHK_74 | 11 HA-27/09 WJ-37 | 70.2 | 19.3 | 3.0 | 3.3 | 2.3 |
| **22** | 17SHK_74 | 11 HA-27/09 WJ-37 | 78.6 | - | 2.8 | 3.5 | 2.6 |

[a] 1 = high susceptibility, poor stands, and chlorotic foliage; 5= a healthy stand with no vegetative chlorosis or wilting.
[b] 1= a poor preforming plant with no bolls; 5 = a healthy high yielding plant
[c] 0 = a healthy root with no visible discoloration; 5 = a dead and rotten root with severe staining.

This field was cultivated in continuous cotton during this period. A cotton monoculture can negatively affect soil health and increase soil-borne pathogens aside from just FOV4 (Bullock, 1992; Constable et al., 1992; Hague et al., 2002). Root stem ratings appeared to be worse in 2020 and 2021 than in 2019 for all lines. It is for these reasons that pooling data derived from field screenings across years and testing locations is probably ineffectual. Instead, breeders should only consider results on a location-by-location or year-by-year basis and make their advancement decisions based on comparisons to known check genotypes and/or competing lines within the trial or nursery.

### 4.2 2021 Disease Screening and Fiber Quality

Field trials at Fabens was not possible in 2021 due to poor quality planting seed. When screening for FOV4 resistance, especially in the production systems used in the El Paso region, high-quality seed is essential to achieve full healthy stands. Therefore, it was impossible to distinguish poor stands resulting from low-quality seed versus poor-stands due to FOV4.

In 2021, breeding efforts for the FOV4 program were maintained at College Station with the objectives of generating high-quality seeds, selecting lines with enhanced fiber quality and yield potential, and creating additional genetic diversity within the program. The mean values for fiber traits for each of the breeding groups were fairly similar across groups (Table 13). One exception would be lint percent, which appears to be slightly lower among the EST group than earlier cohorts. By examining

fiber qualities and lint percent values across breeding groups, we can see the value of including yield components and fiber quality as selection criteria for FOV4 breeding programs because these qualities appear to be segregating independently from HPR for FOV4 within the CIL germplasm pool.

**Table 12. Means of fiber traits of FOV4 breeding lines grown at College Station, TX in 2021.**

| Population | # of lines | Lint % | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|---|
| | | | Unit | mm | % | kNm/kg | % |
| EST | 5 | 37.8 | 4.6 | 28.9 | 84.6 | 325 | 6.1 |
| AST | 9 | 41.1 | 4.4 | 28.6 | 83.2 | 313 | 6.1 |
| PST | 17 | 39.6 | 4.5 | 28.8 | 83.1 | 313 | 5.9 |
| PR | 90 | 41.0 | 4.4 | 28.7 | 83.3 | 308 | 5.9 |
| **Nursery Average** | - | 40.6 | 4.4 | 28.7 | 83.3 | 310 | 5.9 |

### 4.3 2022 Disease Screening and Fiber Quality

**Table 13 Average disease screening results of populations grown in Fabens, TX, 2022.**

| Population | # Lines | Early Rating | Survival | Late Rating | Productivity | Root Stem Stain |
|---|---|---|---|---|---|---|
| | | (1-5)[a] | (%) | (1-5)[a] | (1-5)[b] | (0-5)[c] |
| **EST** | 5 | 2.5 | 11.6 | 3.1 | 3.2 | 2.3 |
| **AST** | 9 | 2.9 | 22.2 | 3.6 | 3.8 | 2.3 |
| **PST** | 17 | 2.7 | 21.6 | 3.2 | 3.1 | 2.4 |
| **PSO** | 22 | 2.4 | 21.2 | 2.9 | 3.0 | 2.6 |
| **PR** | 96 | - | 17.6 | 3.5 | 3.2 | 2.4 |
| **F3** | 9 | 2.4 | 15.1 | 2.6 | 3.0 | 2.5 |

[a] 1 = high susceptibility, poor stands, and chlorotic foliage; 5= a healthy stand with no vegetative chlorosis or wilting.
[b] 1= a poor preforming plant with no bolls; 5 = a healthy high yielding plant
[c] 0 = a healthy root with no visible discoloration; 5 = a dead and rotten root with severe staining.

When assessing the breeding value across a cotton breeding program using a pedigree breeding scheme, it would be expected to see an ‘arc’ in performance with the

most advanced lines having a slightly lower performance than lines that are 1-2 generations behind them as well as a lesser performance in unselected lines that are likely derived from many of the same lines in the most advanced stages via re-selection or as parents used in hybridizations for novel populations. We observed this type of arc in our FOV4 breeding program in 2022 (Table 14). Progeny rows and $F_3$ populations tended to be more susceptible on average to FOV4 than AST, PST and PSO lines that went through at least one evaluation cycle in an FOV4 infested field. It was also encouraging to see HPR to FOV4 was generally better in the AST and PST groups in comparison to the EST group.

We observed this same type of arc in the CIL FOV4 breeding program for lint percent and fiber quality traits (Table 15). Individual plant selections had among the lowest mean values for all these parameters. This was likely the result of using parents for new population development strictly on the basis of FOV4 resistance as well as the integration of international and exotic breeding lines, which were used in an effort to broaden genetic diversity in the germplasm pool. Therefore, $F_3$ populations were expected to have many individuals with low-quality fiber and lint percent. On the other hand, we would have preferred to have strain lines with higher quality fiber. In the standard CIL breeding program, our benchmark for advancement is generally around 39% for lint percent and 31 mm for fiber length as an example.

**Table 14. Cotton fiber quality as measured by HVI from 2022 populations in College Station, TX.**

| Population | # of lines | Lint % | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|---|
| | | | Unit | mm | % | kNm/kg | % |
| EST | 5 | 36.9 | 4.7 | 28.3 | 82.9 | 334 | 5.9 |
| AST | 9 | 38.6 | 4.7 | 27.9 | 82.7 | 323 | 5.9 |
| PST | 17 | 37.1 | 4.6 | 27.7 | 82.5 | 318 | 5.7 |
| PSO | 22 | 37.6 | 4.6 | 27.2 | 82.0 | 284 | 5.7 |
| PR | 99 | 37.0 | 4.4 | 27.4 | 81.8 | 295 | 5.7 |
| IPS | 322 | 35.9 | 4.4 | 26.9 | 81.5 | 279 | 5.6 |
| Tamcot 73 | - | 38.0 | 4.9 | 29.2 | 83.4 | 330 | 6.0 |
| **Nursery Average** | 474 | 36.4 | 4.4 | 27.2 | 81.6 | 29.1 | 5.6 |

In 2022, five strain lines and two check cultivars were included in the Elite Strain Trial (EST) (Table 16). Each entry was replicated four times. The initial germination rate was low with just barely more than ¼ of all seed planted emerging. This was disappointing because our seed quality used in this trial had a tested germination rate of more than 90%. We believe that emergence issues other than FOV4 probably contributed to the low germination rate. The survival rate was calculated from the total number of seed planted to the final seedling stand that was rated three weeks after the irrigation used to initiate germination. For instance, 2/3 of the seeds of DP 1646B2XF that emerged were probably able to survive the first wave of FOV4 infection (18% germination and 12% survival).

The poor stands resulted in a negative interaction with the rating systems which are partially dependent upon intra-plant competition (Table 16). This issue complicated the analysis of variance to discern differences among lines for survivability, and foliage ratings (Table 17). Root stem stains were significantly different among lines with only ‘17 SHJ_48’, ’17 SHK_74’, and DP 1646B2XF being worse than the resistant check,

FM 2334GLT (Table 16). This suggests that three of the candidate lines may have resistance similar to FM 2334GLT.

**Table 15. FOV4 disease response of elite strains at Fabens, TX in 2022.**

| Source | Germination | Survival | Early rating | Late rating | Productivity | Root stem stain | Seed Index |
|---|---|---|---|---|---|---|---|
| | (%) | (%) | (1-5)[a] | (1-5)[a] | (1-5)[b] | (0-5)[c] | g |
| 16SHS_32 | 32.5 | 18.0 | 2.5 | 3.3 | 4.0 | 1.9 | 8.3 |
| 16SHU_38 | 31.0 | 18.0 | 2.3 | 2.3 | 3.3 | 1.5 | 8.6 |
| 17SHJ_48 | 31.5 | 21.5 | 2.8 | 3.0 | 2.3 | 3.2 | 8.1 |
| 17SHO_26 | 22.5 | 13.5 | 2.0 | 2.8 | 3.0 | 2.3 | 8.9 |
| 17SHK_74 | 28.5 | 22.0 | 2.8 | 4.3 | 3.5 | 2.6 | 8.0 |
| DP1646B2XF | 18.0 | 12.0 | 1.5 | 1.8 | 3.0 | 3.1 | 6.6 |
| FM2334GLT | 24.0 | 19.5 | 2.5 | 3.8 | 4.3 | 1.1 | 9.3 |
| **Mean** | 26.9 | 17.8 | 2.3 | 3.0 | 3.3 | 2.2 | 8.3 |
| **LSD (0.05)** | n.s. | n.s | n.s | n.s | 0.7 | 1.4 | 0.1 |
| **CV.,%** | 39.2 | 55.0 | 29.0 | 37.0 | 2.3 | 41.0 | 1.1 |

[a] 1 = high susceptibility, poor stands, and chlorotic foliage; 5= a healthy stand with no vegetative chlorosis or wilting.
[b] 1= a poor preforming plant with no bolls; 5 = a healthy high yielding plant
[c] 0 = a healthy root with no visible discoloration; 5 = a dead and rotten root with severe staining.

**Table 16 Mean squares of the elite strain trial at Fabens, TX, 2022.**

| Source | df | Mean Squares | | | | | |
|---|---|---|---|---|---|---|---|
| | | Germination % | Survival % | Early foliage rating | Late foliage rating | Productivity | Root stem stain |
| Genotypes | 6 | 119.2 | 57.6 | 0.81 | 2.92 | 1.81** | 2.49* |
| Rep | 3 | 115.4 | 69.1 | 0.42 | 0.67 | 0.42 | 1.31 |
| Error | 18 | 110.9 | 96.5 | 0.45 | 1.25 | 0.22 | 0.86 |

*significant at the 0.05 probability level
** significant at the 0.01 probability level

The EST was planted in an area of the field believed to contain high levels of inoculum. No significant differences were observed between replications unlike trials in previous years. This is likely because the entire trial was conducted in a smaller area that gave us greater local control compared to past years with more entries in trials. Genotypes that performed worse than DP1646 B2XF should be removed from the program and considered susceptible.

**Table 17 Pearson correlation of disease screening methodologies for the FOV4 elite strain trial at Fabens, TX, 2022.**

| | Germination | Survival | Early Rating | Late Rating | Productivity | Root Stain |
|---|---|---|---|---|---|---|
| **Survival** | 0.88** | - | - | - | - | - |
| **Early Rating** | 0.82** | 0.85** | | | | |
| **Late Rating** | 0.51** | 0.57** | 0.66** | - | - | - |
| **Productivity** | 0.24 | 0.32 | 0.27 | 0.42* | | |
| **Root Stain** | -0.36 | -0.40* | -0.21 | -0.31 | -0.43* | |
| **Seed Index** | 0.17 | 0.15 | 0.30 | 0.32 | 0.35 | -0.51 |

*significant at the 0.05 probability level
** significant at the 0.01 probability level

Germination, survival, and foliar ratings were interrelated (Table 18). This is expected since the early rating is conducted at the same time as the survival count. A highly significant correlation between germination and survival might suggest once a stand is established the seedlings have a decent chance at survival. It also appears that lines with a higher degree of root staining were less productive. Seed index is not significantly correlated with any of the screening methods indicating it is not a good indirect selection method for FOV4 resistance.

An elite strain yield trial was conducted in 2022 to ensure the most advanced material in the program was competitive against industry standards for yield and fiber quality (Table 19). P1646B2XF is well suited to growing conditions in College Station, Texas, while the other commercial variety FM2334GLT is better suited for West Texas. These checks were included in the FOV4 disease screening trial at Fabens, Texas.

The five EST candidate lines all exhibited lint percents considered too low for commercial varieties which need to be close if not above 40%. Lint yields among these lines were acceptable given the low lint percent, but still not at a commercially

acceptable level. Fiber quality for the most part was good to excellent, which suggests that FOV4 HPR can be improved simultaneously with fiber quality.

**Table 18. Lint yield and fiber quality of the elite strain (EST) yield trial at College Station, TX, in 2022.**

| Genotype | Lint | Lint % | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|---|
| | kg $Ha^{-1}$ | % | unit | mm | % | kNm/kg | % |
| **DP1646B2XF** | 1,758a | 39.9 | 4.7 | 31.7 | 83.6 | 317 | 6.7 |
| **16SHS_32** | 1,075ab | 34.7 | 4.7 | 31.2 | 83.3 | 324 | 5.9 |
| **17SHJ_48** | 933b | 32.6 | 5.0 | 32.8 | 84.8 | 371 | 6.5 |
| **16SHU_38** | 877b | 32.1 | 4.6 | 31.7 | 84.2 | 343 | 6.2 |
| **17SHK_74** | 852b | 34.4 | 4.7 | 29.0 | 82.9 | 317 | 5.9 |
| **17SHO_26** | 799b | 31.4 | 4.3 | 30.2 | 83.7 | 370 | 5.4 |
| **FM2334GLT** | 764b | 38.8 | 4.8 | 30.4 | 84.0 | 346 | 5.5 |
| **Mean** | 1,008 | 34.8 | 4.7 | 31.0 | 83.8 | 341 | 6.0 |
| **CV., %** | 17.7 | 9.4 | 4.6 | 4.0 | 0.7 | 6.8 | 8.2 |

**Table 19. FOV4 disease response of the advanced strain trial (AST) at Fabens, TX, 2022.**

| Source | Germination (%) | Survival (%) | Early rating (1-5)[a] | Late rating (1-5) | Prod. rating (1-5)[b] | Root stain (0-5)[c] | Seed Index g |
|---|---|---|---|---|---|---|---|
| 14 E-12-FOV-05 | 37.0 | 19.5 | 2.8 | 3.8 | 3.3 | 2.6 | 6.6 |
| 16-SHU-11-FOV-02 | 25.5 | 17.5 | 2.3 | 3.3 | 4.0 | 2.7 | 8.4 |
| 16-SHU-11-FOV-04 | 47.5 | 28.5 | 3.3 | 4.5 | 3.8 | 2.2 | 8.4 |
| 16-SHU-11-FOV-05 | 34.0 | 24.0 | 3.0 | 3.5 | 3.5 | 2.2 | 7.8 |
| 16-SHU-27-FOV-05 | 23.0 | 11.5 | 2.0 | 2.5 | 3.5 | 2.4 | 6.5 |
| 16-SHU-27-FOV-06 | 31.5 | 22.5 | 2.8 | 3.5 | 4.0 | 2.5 | 7.8 |
| 16-SHU-27-FOV-09 | 28.5 | 21.5 | 3.0 | 3.8 | 3.8 | 2.2 | 7.7 |
| 16-SHU-38-FOV-5 | 38.5 | 24.5 | 3.3 | 3.8 | 4.0 | 2.3 | 8.1 |
| 16-SHU-38-FOV-14 | 39.0 | 30.0 | 3.5 | 4.3 | 4.8 | 1.9 | 8.6 |
| DP1646B2XF | 20.0 | 17.5 | 2.3 | 3.0 | 4.0 | 3.4 | 6.6 |
| FM2334GLT | 28.0 | 22.5 | 3.0 | 3.8 | 4.0 | 1.6 | 9.3 |
| **Mean** | 32.0 | 21.8 | 2.8 | 3.6 | 3.9 | 2.4 | 7.8 |
| **LSD** | 5.9 | 9.7 | 0.8 | n.s. | n.s. | n.s. | 0.2 |
| **CV., %** | 24.0 | 18.0 | 20.0 | 27.0 | 20.0 | 36.0 | 1.3 |

[a] 1 = high susceptibility, poor stands, and chlorotic foliage; 5= a healthy stand with no vegetative chlorosis or wilting.
[b] 1= a poor preforming plant with no bolls; 5 = a healthy high yielding plant
[c] 0 = a healthy root with no visible discoloration; 5 = a dead and rotten root with severe staining.

In the AST tested at Fabens, TX, in 2022 ’16-SHU-38-FOV-14’ had the best HPR for FOV4 of any line tested in replicated trials. It rated at the top or near the top for germination, survivability, and the early foliage rating. What was encouraging and insightful about this particular line is that it was a reselection from ’16-SHU-38’ from the 2022 EST cohort. This suggests that there are transgressive segregants within elite populations that exceed the parental performance for HPR.

**Table 20. Mean squares for FOV4 disease screening methods in the advanced strain trial (AST) at Fabens, TX, 2022.**

| Source | df | Mean Squares | | | | | |
|---|---|---|---|---|---|---|---|
| | | Germination | Survival % | Early Foliage Rating | Late Foliage Rating | Productivity | Root Stem Stain |
| Genotype | 10 | 262.5* | 109.5* | 0.91* | 1.21 | 0.62 | 0.78 |
| Rep | 3 | 88.3 | 45.2 | 0.06 | 0.52 | 1.00 | 0.97 |
| Error | 30 | 69.4 | 45.2 | 0.31 | 0.97 | 0.60 | 0.71 |

*significant at the 0.05 probability level
** significant at the 0.01 probability level

Interestingly, no significant differences were observed among replications (Table 21), which suggests that FOV4 inoculum levels within the testing area for this trial were evenly distributed at likely a high density. These types of conditions reduce the likelihood of lines escaping from disease pressure. Many of the plots had poor stands created by low germination rates. It was noticed during the collection of root stem stain ratings, that plants that were in a low plant population density took advantage of the extra row space to grow and reproduce profusely. While these same plants appeared to be highly productive, it was common to see them with root stem stained. This staining was likely the result of the prolific rooting behavior which would increase the chances of those root systems encountering FOV4 spores in the soil. Therefore, the poor stands probably contributed to an overabundance of staining in the root stems in most AST plots.

**Table 21. Pearson rank correlation of disease screening methods in the advanced strain trial (AST) at Fabens, TX, 2022.**

| | Germination | Survival | Early Rating | Late Rating | Productivity | Root Stem Stain |
|---|---|---|---|---|---|---|
| **Survival** | 0.77** | | | | | |
| **Early Rating** | 0.68** | 0.80** | | | | |
| **Late Rating** | 0.66** | 0.76** | 0.67** | | | |
| **Productivity** | 0.07 | 0.27 | 0.17 | 0.37* | | |
| **Root Stain** | -0.19 | -0.40** | -0.33* | -0.43** | -0.37* | |
| **Seed Index** | 0.27 | 0.43** | 0.43** | 0.33* | 0.27 | -0.36* |

*significant at the 0.05 probability level
** significant at the 0.01 probability level

All the early-season ratings appeared to be inter-related (Table 22). Root staining tended to be worse in plots with low plant populations. It is unknown if the increase in root staining may be attributed to a greater susceptibility to FOV4 or if the increased root systems per plant would have increased the exposure and incidence of FOV4 infections. The correlations related to seed index are interesting because we previously though it would play the largest role in germination. Germination is one of the only methods in this trial seed index was not highly correlated with.

**Table 22. Fiber quality and lint percent values from advanced strain seed increase plots at College Station, TX, 2022.**

| Source | Lint % | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|
| | | Unit | mm | % | kNm/kg | % |
| 14 E-12-FOV-05 | 43.5 | 4.2 | 26.5 | 80.7 | 284 | 6.7 |
| 16-SHU-11-FOV-02 | 38.7 | 4.2 | 28.3 | 82.5 | 324 | 5.5 |
| 16-SHU-11-FOV-04 | 37.0 | 4.2 | 29.1 | 83.4 | 345 | 5.4 |
| 16-SHU-11-FOV-05 | 41.3 | 4.7 | 26.8 | 81.3 | 317 | 5.6 |
| 16-SHU-27-FOV-05 | 39.6 | 4.8 | 29.3 | 84.3 | 331 | 5.9 |
| 16-SHU-27-FOV-06 | 36.8 | 4.9 | 26.9 | 83.2 | 315 | 6.1 |
| 16-SHU-27-FOV-09 | 38.5 | 4.8 | 27.6 | 82.4 | 322 | 5.7 |
| 16-SHU-38-FOV-5 | 35.5 | 4.8 | 29.0 | 83.5 | 336 | 6.3 |
| 16-SHU-38-FOV-14 | 36.1 | 5.1 | 28.3 | 82.8 | 334 | 6.6 |
| Tamcot73 | 38.0 | 4.9 | 29.2 | 83.4 | 330 | 6.0 |
| mean | 38.6 | 4.7 | 28.0 | 82.7 | 323 | 6.0 |

All breeding lines tested in the FOV4 site in El Paso County were also maintained in a non-replicated nursery in a non-FOV4 field near College Station, TX, in 2022. While the primary purpose of this maintenance nursery was to rogue off-types and plants containing adventitious presence of commercial genetically engineered (GE) traits, as well as generate high-quality planting seed for the next growing season; it also provided us with an opportunity to check fiber quality and lint percent. Within the AST group ’14-E-12-FOV-05’ had a high lint percent value, 43.5%, but relatively poor fiber qualities. This was not surprising since this line was re-selected from ’14-E-12’, which is a finished germplasm line with high lint yield and lint percent potential, but modest fiber quality. Such findings provide evidence that HPR for FOV4 could be obtained through

re-selection of lines without losing yield and fiber quality potential found in the original breeding population.

Most plots in the PST disease trial at Fabens suffered from poor emergence which then translated into low overall survivability, which in turn impacted foliage ratings and productivity (Table 24). The only response in this trial that exhibited differences among lines was root stem staining.

**Table 23. FOV4 disease screening performance of the preliminary strain trial (PST) at Fabens, TX, 2022.**

| source | Germination | Survival | Early Rating | Late Rating | Productivity | Root Stain | Seed Index |
|---|---|---|---|---|---|---|---|
| | (%) | (%) | (1-5)[a] | (1-5)[a] | (1-5)[b] | (0-5)[c] | g |
| 206-20 | 38.0 | 26.7 | 3.3 | 4.0 | 3.7 | 2.3 | 8.6 |
| 233-02 | 24.7 | 18.7 | 2.7 | 4.0 | 3.7 | 2.0 | 8.7 |
| 233-09 | 19.3 | 14.0 | 2.3 | 2.7 | 2.7 | 2.5 | 7.6 |
| 233-11 | 28.7 | 22.0 | 2.7 | 3.3 | 3.3 | 1.8 | 7.4 |
| 233-12 | 25.3 | 18.0 | 2.7 | 3.3 | 3.0 | 2.2 | 7.9 |
| 238-02 | 46.0 | 36.7 | 3.0 | 3.3 | 3.0 | 1.3 | 8.7 |
| 238-03 | 44.7 | 25.3 | 3.0 | 3.0 | 2.7 | 1.7 | 9.1 |
| 209-14 | 32.0 | 26.0 | 2.7 | 3.7 | 3.7 | 2.0 | 7.8 |
| 209-16 | 36.7 | 27.3 | 3.0 | 3.7 | 4.0 | 1.9 | 8.1 |
| 209-23 | 27.3 | 19.3 | 2.3 | 3.7 | 3.7 | 3.5 | 8.0 |
| BB-04 | 46.7 | 18.7 | 2.3 | 2.7 | 2.3 | 3.2 | 8.7 |
| 17 MM-51-59-09 | 28.7 | 16.7 | 2.3 | 2.0 | 2.0 | 2.9 | 8.7 |
| 17 NN-31-39-07 | 26.7 | 18.7 | 2.3 | 2.7 | 2.7 | 3.1 | 7.4 |
| 17 PP-31-39-02 | 29.3 | 20.4 | 2.7 | 2.7 | 2.3 | 2.9 | 10.0 |
| 17 PP-41-49-16 | 28.7 | 11.3 | 2.7 | 3.0 | 3.0 | 1.7 | 9.0 |
| 17 QQ-31-39-02 | 28.7 | 23.3 | 2.3 | 3.0 | 3.0 | 2.9 | 8.1 |
| 17 QQ-41-49-11 | 33.3 | 23.3 | 3.0 | 3.7 | 3.3 | 2.1 | 8.5 |
| DP1646B2XF | 19.3 | 12.7 | 2.3 | 2.7 | 2.7 | 2.6 | 6.6 |
| FM2334GLT | 32.0 | 28.0 | 2.3 | 4.7 | 4.7 | 0.9 | 9.3 |
| | | | | | | | |
| Mean | 31.4 | 21.4 | 2.6 | 3.3 | 3.1 | 2.3 | 8.3 |
| LSD | n.s | n.s | n.s | n.s | n.s | 1.3 | n.s |
| CV | 25.4 | 28.6 | 12.4 | 19.6 | 21.3 | 29.9 | 9.5 |

[a] 1 = high susceptibility, poor stands, and chlorotic foliage; 5= a healthy stand with no vegetative chlorosis or wilting.
[b] 1= a poor preforming plant with no bolls; 5 = a healthy high yielding plant
[c] 0 = a healthy root with no visible discoloration; 5 = a dead and rotten root with severe staining.

**Table 24. Mean squares for FOV4 disease screening methods in the preliminary strain trial (PST) at Fabens, TX, 2022.**

| Source | df | Mean Squares | | | | | |
|---|---|---|---|---|---|---|---|
| | | Germination | Survival % | Early Foliage Rating | Late Foliage Rating | Productivity | Root Stem Stain |
| Genotype | 18 | 190.7 | 112.9 | 0.29 | 1.22 | 1.30 | 1.38 |
| Rep | 2 | 819.4** | 625.3** | 3.32** | 5.28** | 1.96 | 1.59 |
| Error | 36 | 139.4 | 96.1 | 0.20 | 0.98 | 0.69 | 0.69 |

*significant at the 0.05 probability level
** significant at the 0.01 probability level

Many of the PST entries produced similar results to the susceptible check, DP1646B2XF and should be removed from the program. 2022 was the first year this material was evaluated in a replicated trial so lines showing susceptibility to FOV4 were expected. Unlike the EST and AST in 2022, the PST contained significant differences among replications for germination, survivability, and foliage ratings. This is likely due to being a larger trial allowing more diverse inoculum levels and other aberrant soil conditions to occur within the trial area. An important takeaway from this occurrence is that trial sizes and/or designs should be such that the testing area is limited in scope and number and spacing of check cultivars are critical to assess disease pressure within a given block and trial.

**Table 25. Pearson rank correlation of FOV4 disease screening methods in the preliminary strain trial (PST) at Fabens, TX, 2022.**

| | Germination | Survival | Early rating | Late rating | Productivity | Root Stem stain |
|---|---|---|---|---|---|---|
| **Survival** | 0.84** | | | | | |
| **Early rating** | 0.65** | 0.64** | | | | |
| **Late rating** | 0.43** | 0.56** | 0.49** | | | |
| **Productivity** | 0.36** | 0.53** | 0.40** | 0.94** | | |
| **Root stain** | -0.29* | -0.36** | -0.34* | -0.44** | -0.45** | |
| **Seed index** | 0.28* | 0.16 | 0.15 | 0.09 | 0.01 | -0.18 |

*significant at the 0.05 probability level
** significant at the 0.01 probability level

Poor germination in the PST at Fabens likely affected all other later measurements for FOV4 resistance (Table 26). Seed index showed no relationship to other traits except a modest positive correlation to germination. Root stem stains were negatively correlated to all other traits suggesting that infection as detected by the xylem stains was also detracting from early season stand establishment, mid-season appearances, and final productivity.

In the non-replicated maintenance plots for the preliminary strains, most lines had lint percent and fiber qualities that were at or below the standards expected from commercial cultivars. For these lines to contribute to developing commercialized cultivars they will either have to be re-selected for higher yield potential and fiber quality or used as donor parents for FOV4 resistance alleles in new populations.

**Table 26. Fiber quality and lint percent of the preliminary strain seed increase nursery (PST) at College Station, TX, 2022.**

| Source | Lint % | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|
| | | Unit | mm | % | kNm/kg | % |
| 206-20 | 36.1 | 5.0 | 29.6 | 84.3 | 362 | 6.2 |
| 233-02 | 37.7 | 4.5 | 30.7 | 83.3 | 331 | 5.6 |
| 233-09 | 39.3 | 4.5 | 27.0 | 83.7 | 313 | 5.9 |
| 233-11 | 39.9 | 4.5 | 28.0 | 82.5 | 330 | 5.4 |
| 233-12 | 38.0 | 4.6 | 27.2 | 81.2 | 289 | 5.7 |
| 238-02 | 34.3 | 4.8 | 28.6 | 84.9 | 391 | 5.3 |
| 238-03 | 35.0 | 4.5 | 28.4 | 82.6 | 348 | 6.3 |
| 209-14 | 39.2 | 4.9 | 26.5 | 81.7 | 289 | 6.0 |
| 209-16 | 38.9 | 5.0 | 25.8 | 83.6 | 312 | 5.8 |
| 209-23 | 38.8 | 4.5 | 26.3 | 82.1 | 285 | 5.6 |
| BB-04 | 36.8 | 4.6 | 27.0 | 83.0 | 275 | 5.6 |
| 17 MM-51-59-09 | 31.5 | 4.5 | 27.6 | 81.5 | 313 | 6.0 |
| 17 NN-31-39-07 | 36.6 | 4.2 | 27.9 | 80.1 | 298 | 5.3 |
| 17 PP-31-39-02 | 38.5 | 4.7 | 26.7 | 82.4 | 372 | 5.7 |
| 17 PP-41-49-16 | 36.5 | 4.3 | 27.7 | 81.8 | 297 | 5.3 |
| 17 QQ-31-39-02 | 38.8 | 4.5 | 28.9 | 82.2 | 287 | 5.0 |
| 17 QQ-41-49-11 | 34.9 | 4.3 | 27.6 | 81.5 | 317 | 5.6 |
| Tamcot73 | 38.0 | 4.9 | 29.2 | 83.4 | 330 | 6.0 |
| Mean | 37.1 | 4.6 | 27.7 | 82.5 | 318 | 5.7 |

The average performance of the preliminary strain observation nursery was similar to the PST (Table 15). Complete results from the PSO nursery can be found in Table 37 in the appendix. ‘21 CS SH F5 L-25’ and ‘21 CS SH F5 N-26’ were both noted for their high levels of HPR to FOV4 (Table 27). These lines should be elevated into replicated trials to confirm high levels of resistance.

**Table 27. Disease screening evaluation of the highest performing preliminary strain observation lines at Fabens, TX, in 2022.**

| PSO Line | Survival | Early rating | Late rating | Productivity | Root stem stain | Seed Index |
|---|---|---|---|---|---|---|
| | (%) | (1-5)[a] | (1-5)[a] | (1-5)[b] | (0-5)[c] | g |
| **21 CS SH F5 L-25** | 22 | 3 | 5 | 5 | 3.0 | 8.6 |
| **21 CS SH F5 N-26** | 38 | 3 | 5 | 5 | 0.4 | 7.2 |

[a] 1 = high susceptibility, poor stands, and chlorotic foliage; 5= a healthy stand with no vegetative chlorosis or wilting.
[b] 1= a poor preforming plant with no bolls; 5 = a healthy high yielding plant
[c] 0 = a healthy root with no visible discoloration; 5 = a dead and rotten root with severe staining.

’21 CS SH F5 L-25’ averaged a root stain score of 3, indicating infection of FOV4 but still maintained high productivity. This is a classic example of disease tolerance (Medzhitov et al., 2012). On the other hand, ‘21 CS SH F5 L-25’ presented an average root stain score of 0.4, which is likely the result of disease resistance. We still need to confirm if this occurred because of avoidance (escape) or due to a resistance mechanism. It is important to note that 21 CS SH F5 L-25 was in a plot between two other heavily infested and low performing plots so the likelihood of escape was minimal. The fiber quality and lint percent of 21 CS SH F5 N-26 also appeared to surpass the potential of 21 CS SH F5 L-25 (Table 28).

**Table 28. Fiber quality and lint percent of two selected lines from the preliminary strain observation group at College Station, TX, 2022.**

| Source | Lint % | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|
| | | unit | mm | % | kNm/kg | % |
| **21 CS SH F5 L-25** | 37.0 | 4.6 | 27.1 | 81.2 | 269 | 5.3 |
| **21 CS SH F5 N-26** | 38.3 | 4.6 | 30.9 | 84.7 | 332 | 6.0 |
| **Tamcot73** | 38.0 | 4.9 | 29.2 | 83.4 | 330 | 6.0 |

On average, the single replication progeny row entries produced moderate resistance results (Table 29). This is expected since this was the first year these lines were grown in the infested nursery and selections have not been made yet. If selections were to be made based on end of season foliage ratings and productivity ratings, the average performance of the trial would surpass all the other trials. If all but the best 31 entries were discarded, the end of season ratings would both be above a 4.

**Table 29. Disease screening results of the progeny row trial before and after selections were made at Fabens, TX, in 2022.**

| Progeny Row Trial | Germination | Survival | Late Rating | Productivity | Root stain |
|---|---|---|---|---|---|
| | (%) | (%) | (1-5)[a] | (1-5)[b] | (0-5)[c] |
| **Before Selections** | **44.6** | **17.6** | **3.5** | **3.2** | **2.4** |
| **After Selections** | **54.8** | **18.2** | **4.6** | **4.3** | **2.1** |

[a] 1 = high susceptibility, poor stands, and chlorotic foliage; 5= a healthy stand with no vegetative chlorosis or wilting.
[b] 1= a poor preforming plant with no bolls; 5 = a healthy high yielding plant.
[c] 0 = a healthy root with no visible discoloration; 5 = a dead and rotten root with severe staining

These lines were planted in a section of the field with lower known inoculum levels so they should be advanced to a PSO trial and eventually a replicated trial to confirm higher levels of resistance. The results from a Pearson Rank Correlation test were inconsistent with results from other trials. Seed index was once again not significantly correlated with any other screening method.

**Table 30. Disease screening results of genotypes in the F3 population in Fabens, TX, in 2022.**

| Source | Germination (%) | Survival (%) | Early Rating (1-5)[a] | Late Rating (1-5)[a] | Productivity (1-5)[b] | Root stain (0-5)[c] |
|---|---|---|---|---|---|---|
| **19277** | 48 | 16 | 2 | 2 | 3 | 3.4 |
| **19278** | 30 | 12 | 3 | 1 | 4 | 1.7 |
| **19280** | 32 | 18 | 2 | 3 | 4 | 1.4 |
| **19281** | 16 | 4 | 3 | 3 | 2 | 3.4 |
| **19282** | 44 | 18 | 2 | 3 | 3 | 3.0 |
| **19283** | 20 | 8 | 2 | 3 | 2 | 2.2 |
| **19284** | 26 | 16 | 3 | 3 | 3 | 1.8 |
| **19286** | 56 | 26 | 3 | 3 | 4 | 3.2 |
| **19287** | 36 | 18 | 2 | 2 | 2 | 2.5 |
| **Mean** | 34.2 | 15.1 | 2.4 | 2.6 | 3.0 | 2.5 |

[a] 1 = high susceptibility, poor stands, and chlorotic foliage; 5= a healthy stand with no vegetative chlorosis or wilting.
[b] 1= a poor preforming plant with no bolls; 5 = a healthy high yielding plant.
[c] 0 = a healthy root with no visible discoloration; 5 = a dead and rotten root with severe staining

On average, the $F_3$ lines screened in Fabens was one of the lowest performing cohorts. This was the first generation of material to be screened in Fabens developed from the first generation of moderately resistant parents. While ratings on average were poor, a few populations showed low severity of root stem stains while still maintaining fair to good foliage ratings (Table 30). None of these lines will be placed in replicated trials because individual plant selections will be made from many of these populations. Since IPSs cannot be made in Fabens, selections will be made in College Station based on fiber quality and lint percent.

**Table 31. Fiber quality and lint percent data of F3 populations at College Station, TX, 2022.**

| Population | Lint % | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|
| | | Unit | mm | % | kNm/kg | % |
| | | | **Mean Value of Each Population** | | | |
| 19277 | 39.8 | 4.6 | 28.0 | 82.6 | 287 | 5.4 |
| 19278 | 38.1 | 4.3 | 27.1 | 81.3 | 261 | 5.6 |
| 19280 | 36.0 | 4.8 | 26.9 | 81.3 | 284 | 5.6 |
| 19281 | 34.5 | 4.3 | 26.1 | 81.7 | 271 | 5.5 |
| 19282 | 36.3 | 4.5 | 26.5 | 82.9 | 289 | 5.7 |
| 19283 | 33.0 | 4.1 | 29.7 | 82.4 | 341 | 5.8 |
| 19284 | 35.8 | 4.6 | 27.3 | 80.8 | 289 | 5.5 |
| 19286 | 35.7 | 4.7 | 25.0 | 79.8 | 235 | 5.3 |
| 19287 | 33.5 | 3.9 | 25.6 | 80.3 | 251 | 5.6 |
| | | | **Median Value of Each Population** | | | |
| 19277 | 39.9 | 4.6 | 27.9 | 82.5 | 282 | 5.4 |
| 19278 | 37.6 | 4.3 | 27.2 | 81.1 | 263 | 5.6 |
| 19280 | 35.1 | 4.9 | 27.3 | 81.6 | 289 | 5.6 |
| 19281 | 35.2 | 4.3 | 26.2 | 81.9 | 271 | 5.5 |
| 19282 | 36.2 | 4.2 | 26.5 | 83.2 | 286 | 5.6 |
| 19283 | 33.0 | 4.1 | 29.4 | 82.4 | 347 | 5.9 |
| 19284 | 36.4 | 4.6 | 27.1 | 80.6 | 287 | 5.5 |
| 19286 | 35.2 | 4.6 | 24.9 | 79.8 | 233 | 5.3 |
| 19287 | 33.7 | 3.8 | 25.5 | 80.0 | 244 | 5.6 |
| | | | **Maximum Value in Each Population** | | | |
| 19277 | 48.0 | 6.2 | 30.7 | 85.1 | 345 | 6.3 |
| 19278 | 42.6 | 5.1 | 28.7 | 83.8 | 290 | 6.7 |
| 19280 | 43.4 | 6.2 | 30.1 | 84.8 | 345 | 6.6 |
| 19281 | 44.5 | 6.0 | 28.9 | 83.3 | 320 | 6.3 |
| 19282 | 41.8 | 6.6 | 28.8 | 84.9 | 320 | 7.0 |
| 19283 | 40.4 | 5.6 | 33.8 | 85.6 | 415 | 7.2 |
| 19284 | 45.6 | 5.7 | 30.8 | 85.8 | 361 | 6.8 |
| 19286 | 41.8 | 5.9 | 29.2 | 84.8 | 326 | 5.8 |
| 19287 | 41.0 | 5.1 | 29.6 | 84.0 | 318 | 6.5 |

While the data from the F3 created for FOV4 resistance is from a non-replicated nursery, we can infer about the potential of individuals from each population. Based on these inferences, breeders must decide on different approaches to improve HPR, yield, and fiber quality simultaneously. One approach would be to select many plants from populations that probably have the highest levels of FOV4 HPR such as ‘19278’, ‘19280', and ‘19284’. From these IPSs, we would hope to find plants with lint percent and fiber qualities better than the average of the population mean. Another approach would be to select plants for tolerance to HPR as suggested by a positive relationship between the productivity rating and root stem staining. These types of plants are likely to have a high yield potential, but fiber quality would still need to serve as an important selection criterion. A third approach would be to take numerous IPSs from lines with the best lint percent and fiber quality such as ‘192777’ and ‘19283’ and then search for lines with high levels of HPR. As molecular tools become available for FOV4, the decision about which strategy will become easier. If functional markers associated with QTLs for FOV4 resistance can be identified, creating populations with combinations of alleles for resistance and for high yield and fiber quality potential become more likely.

While average lint percent of the populations were low, each population contained IPSs with lint percents over 40, a benchmark in the program. The variation within each population will allow for selections to be planted as progeny rows. Maintaining variability is key for a breeding program. After selections are made, this generation could be the first to contain moderate resistance while maintaining decent fiber quality.

### 4.4 2022 Incremental Root Stain Testing

For the first time in this project, incremental root stain measurements were collected in attempt to understand how the pathogen moves through a susceptible and resistant plant. A susceptible check, DP1646B2FX, a resistant check FM2334GLT and a promising AST line '16-SHU-38-FOV-14' were included in this test. This trial included four replications of the three lines. Initial horizontal cuts were made until the highest point of vascular staining was identified with 0 representing the initial cut at the first cotyledonal node. These results were compared to the other disease screening methods.

**Table 32. Analysis of variance of FOV4 disease screening methods of genotypes at Fabens, TX, 2022.**

| | Mean Squares | | | | | | |
|---|---|---|---|---|---|---|---|
| **Source** | Germination | Survival | Early Rating | Late Rating | Productivity | Root Stain score | Root Stain Increment |
| **Genotype** | 364.0 | 158.3 | 1.58 | 1.58 | 0.75 | 3.36* | 9,762** |
| **Rep** | 44.9 | 51.6 | 0.31 | 1.11 | 0.31 | 0.44 | 161 |
| **Error** | 116.9 | 57.9 | 0.47 | 1.03 | 0.31 | 0.46 | 577 |

*significant at the 0.05 probability level
** significant at the 0.01 probability level

**Table 33. FOV4 disease reaction of three selected genotypes at Fabens, TX, in 2022.**

| **Designation** | **Germination** | **Survival** | **Early Rating** | **Late Rating** | **Prod.** | **Root Stain** | **Increment Root Stain** | **Seed Index** |
|---|---|---|---|---|---|---|---|---|
| | (%) | (%) | (1-5)[a] | (1-5)[a] | (1-5)[b] | (0-5)[c] | (mm) | (g) |
| **DP1646B2XF** | 20 | 17.5 | 2.3 | 3.00 | 4.00 | 3.35 | 83.0 | 6.6 |
| **FM2334GLT** | 28 | 22.5 | 3.0 | 3.75 | 4.00 | 1.63 | 2.0 | 9.3 |
| **16-SHU-38-FOV-14** | 39 | 30.0 | 3.5 | 4.25 | 4.75 | 1.95 | -6.5 | 8.6 |

[a] 1 = high susceptibility, poor stands, and chlorotic foliage; 5= a healthy stand with no vegetative chlorosis or wilting.
[b] 1= a poor preforming plant with no bolls; 5 = a healthy high yielding plant
[c] 0 = a healthy root with no visible discoloration; 5 = a dead and rotten root with severe staining.

No significant differences between replications were identified (Table 32). The differences between the incremental root stain ratings showed high significance as well as a significant difference in root stain scores. However, in this trial there were not

significant differences between genotypes for survival, stand ratings, or productivity. disease screening. This is most likely due to the small trial size, since it only included three lines. If this experiment were to be continued in the future, more lines should be included. DP1646B2XF was the lowest performing line across all screening methods except it had a decent productivity rating (Table 33). On average, staining was found in the stem 83mm above the first cotyledon node. This was significantly different from the other lines. The AST line ’16-SHU-38-FOV-14’ outperformed FM2334GLT in all the disease screening methods and was the only line in the test to have an average root stain measurement below the cotyledonal node. These measurements could suggest the method of resistance in the host is related to preventing the spread of the pathogen through the stem instead of preventing the pathogen from entering the plant all together. Future research could analyze roots throughout the season to understand if resistance comes from delay of infection or slowing the progression of the pathogen traveling through the vascular system.

# CHAPTER V

# CONCLUSIONS

## 5.1 Disease Screening Methods

The first objective of this project was to improve screening methods used to measure FOV4 resistance. Correlation analyses suggest that screening methods can be affected by inoculum levels, soil and water conditions of the field, and weather. Ratings and survival counts can be obfuscated by other agronomic issues related to seedling health and vigor aside from FOV4. Our findings suggest that seed quality and early-season emergence are essential to obtain reliable results of host plant resistance to FOV4. While our screening methods improved since the start of the project, variable field conditions from year to year compromises the reliability of results and creates challenges when interpreting data. For this reason, we recommend breeders make selection decisions based on a year-by-year or location-by-location basis by comparing genotypes to known checks within the trial. Root stains are an important diagnostic symptom of FOV4, but they do not always correlate well with above-ground symptoms. In early years of screening, root stem stain ratings and foliar ratings were not always discriminating enough to identify HPR. With our findings, it is still unknown if the level of root staining correlates with the level of HPR or simply correlates to the inoculum density the plant was grown in. Measuring incremental root stem staining could provide insight into better understanding the plant's resistance mechanism. Seed index was not a useful tool even as an indirect selection method because we observed almost no

relationship between seed index and any of the other traits within our screening protocols.

## 5.2 Improving FOV4 Resistance

The second objective of this project was to improve levels of FOV4 germplasm resistance in the Cotton Improvement Lab breeding program at Texas A&M University. FOV4 resistance has proven to be difficult to improve because disease pressure in a field nursery varies across years and can be affected by other agronomic factors.

The most advanced lines in the FOV4 resistance program have a moderate degree of FOV4 resistance and tolerance; however, they are not commercially competitive as varieties because of low lint yield potential. These lines were selected exclusively for FOV4 resistance during the early stages of the program. Initially, we observed only a handful of breeding lines with putative FOV4 resistance or tolerance. These lines became the basis of additional populations through hybridization and re-selection. On average, the most successful cohort were those in the advanced strain trial. These lines were created from re-selections and had been through one cycle of field screening. While we observed several potentially promising lines in more preliminary breeding cohorts, none of those lines were screened in the field until 2022. We suspect highest levels of FOV4 resistance are probably within the newest lines in the program, but that will need to be confirmed with additional field screening.

### 5.3 Improving Fiber Quality and Yield Potential

The third objective of this study was the simultaneous improvement of fiber quality and lint yield during the selection process for FOV4 resistance. The 2022 F3 population was the first to be developed by parents that showed potential FOV4 resistance. Hybridizations to establish this population also included exotic breeding lines with intent to increase diversity. This choice in parents resulted in a population with low mean values for fiber traits. All other populations came from parents selected for lint yield and fiber quality. As selections were made based on disease screening results in Fabens, TX, fiber quality began to decline. This was an expected outcome but also a challenge the program will need to overcome in the future. The most advanced lines in 2022 produced lower lint percents but had fiber quality potential similar to original CIL breeding populations. This suggests that it is possible to improve HPR and fiber quality simultaneously. The individual plant selections from the 2022 F3 population show potential in restoring some of the lost lint yield and fiber quality. While they were developed for resistance, the large variation of fiber quality within each line allows for selections that will increase overall lint percent and other fiber traits of the population. Maintaining variation in the early generations is essential moving forward to ensure our resistant lines maintain fiber traits competitive with commercial varieties.

# REFERENCES


Abdel-Raheem, A., & Bird, L. S. (1968). Resistance and susceptibility of Cotton strains to Verticillium and Fusarium wilts as influenced by soil texture and organic matter levels. In *Beltwide Cotton Production Research Conferences Proc. 1968. pp* (pp. 167-169).

Abdel-Raheem, A., H. Elassbli, Y. Zhu, V. Kuraparthy, L. Hinze, D. Stelly, T. Wedegaertner, and J. Zhang. 2019. A genome-wide association study uncovers consistent quantitative trait loci for resistance to verticillium wilt and fusarium wilt race 4 in the US upland cotton. Theoretical and Applied Genetics 133(2): 563–577.

Allen, S. J., & Lonergan, P. A. (2000). Control strategies for Fusarium wilt of cotton in Australia. In *2000 Proceedings Beltwide Cotton Conferences, San Antonio, USA, 4-8 January, 2000: Volume 1*. (pp. 136-138). National Cotton Council.

Armstrong GM, Armstrong JK (1960) American, Egyptian, and Indian cotton-wilt fusaria: their pathogenicity and relationship to other wilt fusaria. US Dep Agric Tech Bull 1219:1–19

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

Armstrong, J. K., & Armstrong, G. M. (1958). A race of the cotton wilt Fusarium causing wilt of Yelredo soybean and flue-cured tobacco. *Plant Dis. Rep*, *42*, 147-151.

Baker, K. F., & Smith, S. H. (1966). Dynamics of seed transmission of plant pathogens. *Annual Review of Phytopathology*, *4*(1), 311-332.

Beckman, C. H. (1987). *The nature of wilt diseases of plants*. APS press.

Bell, A. A., Gu, A., Olvey, J., Wagner, T. A., Tashpulatov, J. J., Prom, S., Quintana, J., Nichols, R. L., & Liu, J. (2019). Detection and characterization of fusarium oxysporum f. sp.. vasinfectum VCG0114 (race 4) isolates of diverse geographic origins. Plant Disease, 103(8), 1998–2009. https://doi.org/10.1094/pdis-09-18-1624-re

Bennett RS, Colyer PD (2010) Dry heat and hot water treatments for disinfesting cottonseed of Fusarium oxysporum f. sp. vasinfectum. Plant Dis 94:1469–1475

Bennett RS, Hutmacher RB, Davis RM (2008) Seed transmission of Fusarium oxysporum f. sp. vasinfectum race 4 in California. J Cotton Sci 12:160–164

Blaise, D., & Kranthi, K. R. (2019). Cotton production in India. *Cotton Production*, 193-215.

Blasingame, D., and Patel, M. V. 2022. Cotton disease loss estimate committee report. Pages 259-262 in: Proc. Beltwide Cotton Conf. National Cotton Council of America, Memphis, TN.

Bullock, D. G. (1992). Crop rotation. *Critical reviews in plant sciences*, *11*(4), 309-326.

Charudattan, R., and Kalyanasudaram, R. 1966. Carrier hosts of F. vasinfectum Atk. Phytopathol. Z. 55:239-242.

Chen, Q., Ji, X., & Sun, W. (1985). Identification of races of cotton wilt Fusarium in China. Scientia Agricultura Sinica, 6, 1–6.

Constable, G. A., Rochester, I. J., & Daniells, I. G. (1992). Cotton yield and nitrogen requirement is modified by crop rotation and tillage method. *Soil and Tillage Research*, *23*(1-2), 41-59.

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

Duczek, L. J., & Piening, L. J. (1982). Effect of seeding depth, seeding date and seed size on common root rot of spring barley. *Canadian Journal of Plant Science*, *62*(4), 885-891.

Elliot, J. A. (1923). Cotton wilt: a seed borne disease. *Journal of Agricultural Research.*, *23*, 387-393.

Fahmy, T. (1927). The Fusarium disease (wilt) of cotton and its control. Phytopathology, 17, 749–767.

Gao, H., Beckman, C. H., & Mueller, W. C. (1995). The nature of tolerance to Fusarium oxysporum f. sp. lycopersici in polygenically field-resistant marglobe tomato plants. *Physiological and molecular plant pathology*, *46*(5), 401-412.

Garber, R. H., and Paxman, G. A. 1963. Fusarium wilt of cotton in California. Plant Dis. Rep. 47:398-400.

Geisseler, D., & Horwath, W. (2016). *Cotton production in California*. Retrieved February 15, 2023, from https://apps1.cdfa.ca.gov/FertilizerResearch/docs/Cotton_Production_CA.pdf

Ghobrial, E. (1976). Effect of seed size in controlling root rot diseases on barley. *Agricultural research review*.

Gilbert, W.W. 1921. Cotton diseases and their control. U. S. Dep. Agric. Farmers' Bull. 1187:1-32.

Gordon, T. R. (2017). Fusarium oxysporum and the Fusarium wilt syndrome. *Annual review of phytopathology*, *55*, 23-39.

Groves, F. E., Bourland, F. M., & Jones, D. C. (2016). Relationships of yield component variables to yield and fiber quality parameters. *Journal of Cotton Science*, *20*(4), 320-329.

Hague, S. S., Overstreet, C., & Van Santen, E. (2002). Crop rotation effects on nematode populations. *Making Conservation Tillage Conventional: Building a Future on 25 Years of Research*, 156.

Halpern, H. C., Bell, A. A., Wagner, T. A., Liu, J., Nichols, R. L., Olvey, J., ... & Brewer, M. T. (2018). First report of Fusarium wilt of cotton caused by Fusarium oxysporum f. sp. vasinfectum race 4 in Texas, USA. *Plant disease*, *102*(2), 446-446.

Hillocks, R. J. (1992). Fusarium wilt. *Cotton diseases.*, 127-160.

Holdeman, Q. L., & Graham, T. W. (1954). Effect of the sting nematode on expression of fusarium wilt in cotton. *Phytopathology*, *44*(12), 683-685.

Holmes EA, Bennett RS, Spurgeon DW, Colyer PD, Davis RM (2009) New genotypes of Fusarium oxysporum f. sp. vasinfectum from the southeastern United States. Plant Dis 93:1298–1304

Kappelman, A. J., Jr. 1971. Inheritance of resistance to Fusarium wilt in cotton. Crop Sci. 11:672-674.

Kappelman AJ (1980) Long-term progress made by cotton breeders in developing Fusarium wilt resistant germplasm. Crop Sci 20:613–615

Katsantonis, D., Hillocks, R. J., & Gowen, S. (2003). Comparative effect of root-knot nematode on severity of Verticillium and Fusarium wilt in cotton. *Phytoparasitica*, *31*(2), 154-162.

Kim, Y., Hutmacher, R. B., & Davis, R. M. (2005). Characterization of California isolates of Fusarium oxysporum f. sp. vasinfectum. Plant Disease, 89, 366–372. doi:10.1094/PD-89-0366.

Kochman, J. K. (1995). Fusarimn wilt in cotton—A new record in Australia. *Australasian Plant Pathology*, *24*(1), 74-74.

Kochman JK, Swan L, Moore NY, Bentley S, O'Niell WT, Mitchell A,Obst NR, Lehane J, Gulino LL, Salmond G (2002) The Fusarium threat - are we making the progress? 11th Australian Cotton Conference Proceedings. Australian Cotton Growers ResearchAssociation, Brisbane, pp 643–652

Lakey, C. (2021). *Breeding for Resistance to Fusarium oxysporum vasinfectum- Race 4* (thesis).

Lawrence K, Hatchett TB, Nightingale S (2007) Evaluation of Avicta alone and in combination with seed treatment fungicides for protection of cotton from Fusarium wilt, 2006. Plant Dis Manag Rep 1:N013

Lievens, B., Houterman, P. M., & Rep, M. (2009). Effector gene screening allows unambiguous identification of Fusarium oxysporum f. sp. lycopersici races and discrimination from other formae speciales. *FEMS microbiology letters*, *300*(2), 201-215.

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

Main, C. L., Barber, L. T., Boman, R. K., Chapman, K., Dodds, D. M., Duncan, S., ... & Bronson, K. F. (2013). Effects of nitrogen and planting seed size on cotton growth, development, and yield. *Agronomy Journal*, *105*(6), 1853-1859.

Medzhitov, R., Schneider, D. S., & Soares, M. P. (2012). Disease tolerance as a defense strategy. *Science*, *335*(6071), 936-941.

Michell, R. E., & Powell, W. M. (1972). Influence of Pratylenchus brachyurus on the incidence of Fusarium wilt in cotton. *Phytopathology*.

Minton, N. A., & Minton, E. B. (1966). Effect of root knot and sting nematodes on expression of fusarium wilt of cotton in 3 soils. *Phytopathology*, *56*(3), 319.

Minton, E. B., & Supak, J. R. (1980). Effects of Seed Density on Stand, Verticillium Wilt, and Seed and Fiber Characters of Cotton 1. *Crop Science*, *20*(3), 345-347.

Mundkur, B. B. (1936, June). Resistance of American cottons to Fusarium wilt in India. In *Proceedings/Indian Academy of Sciences* (Vol. 3, No. 6, pp. 498-501). Springer India.

Neal, D.C. 1928. Cotton diseases in the Mississippi and their control. Miss. Agric. Exp. Stn. Bull. 248:1-30.

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

Nirenberg, H. I., Ibrahim, G., and Michail, S. H. 1994. Race identity of three isolates of Fusarium oxysporum Schlect. f. sp. vasinfectum (Atk.) Snyd. & Hans. From Egypt and the Sudan. Z. Pflanzenkr. Pflanzenschutz 101:594-597.

Okungbowa, F. I., & Shittu, H. O. (2012). Fusarium wilts: An overview. *Environ. Res. J*, *6*(2), 83-102.

Piccinni, G., Shriver, J. M., & Rush, C. M. (2001). Relationship among seed size, planting date, and common root rot in hard red winter wheat. *Plant disease*, *85*(9), 973-976.

Pitt, J. I., Hocking, A. D., Bhudhasamai, K., Miscamble, B. F., Wheeler, K. A., & Tanboon-Ek, P. (1994). The normal mycoflora of commodities from Thailand. 2. Beans, rice, small grains and other commodities. *International Journal of Food Microbiology*, *23*(1), 35-53.

Presley, J. T. (1972). Cotton Diseases of the U.S.A. In *Cotton* (pp. 72–74). essay, Ciba-Geigy.

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

Rathinavel, K., Priyadharshini, C., & Kavitha, H. (2021). Influence of seed reserves on seedling vigour, seed cotton productivity and fiber properties of upland cotton (Gossypium hirsutum). *Journal of Cotton Research and Development*, *35*(1), 1-11.

Rodriguez-Galvez, E., & Mendgen, K. (1995). The infection process of Fusarium oxysporum in cotton root tips. *Protoplasma*, *189*, 61-72.

Sanogo, S., & Zhang, J. (2016). Resistance sources, resistance screening techniques and disease management for Fusarium wilt in cotton. *Euphytica*, *207*, 255-271.

Shepherd, R. L. (1982). Genetic Resistance and its Residual Effects for Control of the Root-Knot Nematode-Fusarium Wilt Complex in Cotton 1. *Crop Science*, *22*(6), 1151-1155.

Shepherd, R. L. (1974). Transgressive Segregation for Root-Knot Nematode Resistance in Cotton 1. *Crop Science*, *14*(6), 872-875.

Sivan A, Chet I (2008) Biological control of Fusarium spp. in cotton, wheat and muskmelon by Trichoderma harzianum. J Phytopathol 116:39–47

Skovgaard, K., Nirenberg, H. I., O'Donnell, K., & Rosendahl, S. (2001). Evolution of Fusarium oxysporum f. sp. vasinfectum races inferred from multigene genealogies. *Phytopathology*, *91*(12), 1231-1237.

Smith, A. L., and Dick, J. B. 1960. Inheri-tance of resistance to Fusarium wilt in upland and Sea Island cottons as complicated by nematodes under field conditions. Phytopa-thology 50:44-48.

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

Snider, J. L., Collins, G. D., Whitaker, J., Chapman, K. D., & Horn, P. (2016). The impact of seed size and chemical composition on seedling vigor, yield, and fiber quality of cotton in five production environments. *Field crops research*, *193*, 186-195.

Steinkellner, S.,Mammerler, R.and Vierheilig, H. (2008). Germination of Fusarium oxysporum in root exudates from tomato plants challenged with different Fusarium oxysporum strains. European Journal of Plant Pathology 122:395-401.

Taubenhaus, J. J., Ezekiel, W. N., & Killough, D. T. (1928). Relation of Cotton Root Rot and Fusarium Wilt to the Acidity and Alkalinity of the Soil. *Texas FARMER Collection*.

Ulloa, M., Hutmacher, R. B., Davis, R. M., Wright, S. D., Percy, R., & Marsh, B. (2006). Breeding for Fusarium wilt race 4 resistance in cotton under field and greenhouse conditions.

Ulloa, M., R.B. Hutmacher, T.L. Schramm, M.L. Ellis, R. Nichols, P.A. Roberts, and S.D. Wright. 2020. Sources, selection and breeding of fusarium wilt (fusarium oxysporum f. sp. vasinfectum) race 4 (FOV4) resistance in Upland (gossypium hirsutum L.) cotton. Euphytica 216(7).

USDA ERS - Cotton Sector at a GlanceAvailable at https://www.ers.usda.gov/topics/crops/cotton-wool/cotton-sector-at-a-glance/#:~:text=Currently%2C%20India%20is%20the%20world's,percent%20of%20the%20world%20total. (Verified 1 May 2022).

Wagner, T. A., Duke, S. E., Davie, S. M., Magill, C., & Liu, J. (2022). Interaction of Fusarium Wilt Race 4 with Root-Knot Nematode Increases Disease Severity in Cotton. *Plant Disease*, *106*(10), 2558-2562.Wang, B., Dale, M. L., & Kochman, J. K. (1999). Studies on a pathogenicity assay for screening cotton germplasms for resistance to Fusarium oxysporum f. sp. vasinfectum in the glasshouse. *Australian Journal of Experimental Agriculture*, *39*(8), 967-974

Walter, S., Nicholson, P., & Doohan, F. M. (2010). Action and reaction of host and pathogen during Fusarium head blight disease. *New Phytologist*, *185*(1), 54-66.

Wang B, Dale ML, Kochman JK, Obst NR (1999a) Effects of plant residue, soil characteristics, cotton cultivars and other crops on fusarium wilt of cotton in Australia. Aust J Exp Agric 39:203–209

Zhang J, Howell CR, Starr JL (1996) Suppression of Fusarium colonization of cotton roots and Fusarium wilt by seed treatments with Gliocladium virens and Bacillus subtilis. Biocontrol Sci Technol 2:175–188

Zhu, Y., A. Abdelraheem, T.A. Wheeler, J.K. Dever, T. Wedegaertner, K.D. Hake, and J. Zhang. 2021. Interactions between cotton genotypes and fusarium wilt race 4 isolates from Texas and resistance evaluation in Cotton. Crop Science 61(3): 1809–1825.

Zhu, Y., A. Abdelraheem, P. Lujan, J. Idowu, P. Sullivan, R. Nichols, T. Wedegaertner, and J. Zhang. 2021. Detection and characterization of fusarium wilt (*fusarium oxysporum* f. sp.. *vasinfectum*) race 4 causing fusarium wilt of cotton seedlings in New Mexico

APPENDIX A

**Table 32. Coefficient of variations for disease screening methods of trials grown at Fabens, TX, in 2019 and 2020**

| Trial | # Lines | Survival | Foliar rating | Early NDVI | Mid-season NDVI | Root Stem Stain | Productivity |
|---|---|---|---|---|---|---|---|
| | | (%) | (%) | (%) | (%) | (%) | (%) |
| **2019** | | | | | | | |
| PST | 60 | 16.0 | 31.8 | 21.3 | 33.2 | 55.2 | - |
| PR | 30 | 14.0 | 43.6 | 25.4 | 48.8 | 57.4 | - |
| **2020** | | | | | | | |
| AST | 16 | 31.1 | 32.6 | - | 42.0 | 36.5 | 29.1 |
| PST | 26 | 24.9 | 34.3 | - | 37.4 | 40.3 | - |
| PR | 114 | 43.4 | 55.1 | - | 54.0 | | - |

**Table 33 Mean disease screening ratings of genotypes in the preliminary strain trial in Fabens, TX, in 2019**

| Designation | Pedigree | May NDVI | survival | rating | July NDVI | root stain |
|---|---|---|---|---|---|---|
| | | (%) | (%) | (1-5)* | (%) | (0-5)** |
| 16-SHT-02 | 08 WZ-46/07 WD-57 | 8 | **93.6** | **3.3** | **48.0** | **1.1** |
| 16-SHT-31 | Acala Royale/08 WZ-83 | 7 | 81.9 | 3.3 | 46.0 | |
| 17 SHK-74 | 11 HA-27/09 WJ-37 | 7 | 98.2 | 4.0 | 53.0 | 0.9 |
| 17 SHK-76 | 11 HA-27/09 WJ-37 | 6 | 90.0 | 3.0 | 42.0 | |
| 17 SHK-79 | 11 HA-27/09 WJ-37 | 8 | 85.4 | 4.0 | 49.0 | 2.0 |
| 17 SHK-86 | 11 HA-27/09 WJ-37 | 9 | 95.4 | 4.7 | 63.0 | 0.9 |
| 17 SHM-79 | 07 SIUP 148/11 HA-14 | 6 | 84.7 | 3.3 | 48.0 | 1.1 |
| 17 SHO-30 | 09 SIUP 120/11 HA-27 | 6 | 82.1 | 3.3 | 38.0 | |
| 17 WSH-12 | 06WE-14x(06WE-14 x 06WE-14/GH18-3) | 7 | 84.9 | 3.0 | 43.0 | |
| 15 EE-56 | 10F4-IP-V-27-05/08 WZ-52 | 7 | 81.6 | 3.0 | 44.0 | |
| 13 T-38 | 8009/F1/8008/F1 | 7 | 86.0 | 2.7 | 44.0 | |
| 13 V-57 | 6513/F2/8041/F1 | 6 | 72.9 | 2.3 | 39.0 | |
| 14 B-72 | 05 A-46 / GB-0696 | 6 | 74.7 | 2.3 | 33.0 | |
| 14 C-41 | 07 X-26 / DP 5415 | 7 | 83.8 | 3.0 | 43.0 | |
| 14 C-81 | 07 X-26 / LA 887 | 6 | 81.9 | 3.0 | 42.0 | |
| 14 G-56 | 09 PP-03-2 / 9913 | 6 | 63.6 | 1.7 | 32.0 | |
| 15 EE-20 | 07WC-13/2010-V-13-03 | 7 | 88.9 | 2.7 | 46.0 | |
| 15 EE-52 | BRS 269/08 WZ-51 | 7 | 89.4 | 2.7 | 43.0 | 3.0 |
| 15 FF-17 | 10F4-IP-V-27-05/08 WZ-52 | 8 | 81.4 | 3.3 | 49.0 | |
| 15 MM-26 | 06WE624/Tamcot22 | 6 | 74.9 | 2.0 | 36.0 | |
| 15 MM-32 | 06WE624/Tamcot22 | 7 | 73.7 | 3.0 | 45.0 | |
| 15 NN-04 | 06WE624/03B18233 | 7 | 83.9 | 2.3 | 32.0 | |
| 15GG-14 | CNPA 809/WK 11 | 7 | 88.3 | 3.3 | 44.0 | |
| 15GG-24 | CNPA 809/TAMCOT 22 | 7 | 95.3 | 3.7 | 58.0 | 0.9 |
| 15GG-44 | CNPA 158/TAM B-139-17 | 7 | 86.7 | 2.7 | 47.0 | |
| 16-SHS-29 | 08 WZ-75/07 V-45 | 6 | 77.4 | 3.3 | 43.0 | |
| 16-SHS-32 | 08 WZ-75/07 V-45 | 7 | 95.0 | 4.0 | 53.0 | 1.0 |
| 16-SHS-56 | 08 WZ-91/07 V-45 | 8 | 95.0 | 3.7 | 49.0 | |
| 16-SHT-28 | SI Samrong 60/08 WZ-83 | 8 | 89.1 | 3.0 | 54.0 | |
| 16-SHT-55 | CIANO Alamos 92/08 WZ-91 | 5 | 78.9 | 2.0 | 29.0 | |
| 16-SHU-11 | 08 WZ-87/08 WZ-83 | 7 | 92.1 | 4.0 | 53.0 | 1.3 |
| 16-SHU-24 | DPL 51/08 WZ-87 | 7 | 97.0 | 3.7 | 53.0 | |
| 16-SHU-27 | Hyperformer HY007/08 WZ-87 | 7 | 93.5 | 3.7 | 60.0 | 1.5 |
| 16-SHU-36 | CIANO_Tajimaroa92/08WZ-87 | 6 | 87.3 | 3.3 | 49.0 | |
| 16-SHU-38 | 11323/11333 | 7 | 88.1 | 3.7 | 53.0 | 1.1 |
| 16-SHU-43 | 11323/11333 | 7 | 89.1 | 4.0 | 55.0 | 1.1 |
| 16-SHV-12 | 11322/11328 | 10 | 96.2 | 4.3 | 57.0 | 0.9 |
| 16-SHV-19 | 09WJ-37/11328 | 7 | 83.4 | 3.3 | 42.0 | |
| 16-SHV-22 | 09WJ-37/11328 | 7 | 78.8 | 3.3 | 47.0 | |
| 16-SHV-36 | 10WD-08/11328 | 7 | 91.6 | 3.7 | 53.0 | |
| 16-SHV-38 | 10WD-08/11328 | 7 | 76.0 | 3.0 | 42.0 | |

**Table 36.2 Mean disease screening performance of pedigrees included in progeny rows in Fabens, TX, in 2019**

| Level | May NDVI | Survival | Rating | July NDVI | Root Stain |
|---|---|---|---|---|---|
| | | % | (1-5)* | | (0-5)** |
| 08 WY-26/0 ISH HQPSO-23 | 4.5 | 73.1 | 1.0 | 10.0 | |
| 08 WZ-46/07 V-45 | 6.0 | 84.0 | 2.2 | 21.4 | 1.8 |
| 08 WZ-83/07 V-45 | 7.0 | 72.0 | 3.0 | 24.0 | |
| 08 WZ-91/07 V-45 | 5.6 | 76.5 | 2.6 | 28.4 | 2.0 |
| 09WJ-37/11328 | 3.5 | 72.7 | 1.3 | 14.8 | |
| 8009/F1/8008/F1 | 4.8 | 68.6 | 1.5 | 13.5 | |
| 11323/11333 | 6.0 | 84.7 | 3.0 | 37.8 | 12.0 |
| **mean** | 5.2 | 76.7 | 2 | 21.0 | 1.6 |
| **LSD** | 2.2 | NS | 1.8 | 11.0 | NS |
| **CV** | 14.9 | 14.1 | 31.1 | 35 | 63.7 |

*1 represents high susceptibility, poor stands, and chlorotic foliage. 5 represents a healthy stand with no vegetative chlorosis or wilting

**0 represents a healthy root with no visible discoloration. 5 represents a dead and rotten root with sever staining

**Table 34. Disease screening results of the advanced strain trial at Fabens, TX, 2020**

| Designation | NDVI | Rating | Survival | Productivity | Root Stain |
|---|---|---|---|---|---|
| | | (1-5) | (%) | (1-5) | (0-5) |
| 14 E-12 | 21.0 | 3.0 | 69 | 3.3 | 3.1 |
| 15 GG-24 | 17.3 | 3.3 | 53 | 4.0 | 2.9 |
| 16 SHS-32 | 26.7 | 4.0 | 81 | 4.0 | 2.5 |
| 16 SHU-27 | 20.0 | 3.3 | 67 | 3.0 | 2.4 |
| 16 SHU-38 | 23.0 | 3.3 | 74 | 3.7 | 2.5 |
| 16 SHU-43 | 17.3 | 3.0 | 54 | 3.3 | 3.2 |
| 16-SHT-02 | 24.7 | 4.0 | 78 | 4.0 | 2.5 |
| 16-SHU-11 | 21.7 | 4.0 | 73 | 4.0 | 2.7 |
| 16-SHV-12 | 21.3 | 3.0 | 73 | 3.3 | 2.7 |
| 17 SHJ-48 | 28.7 | 4.7 | 74 | 4.3 | 2.1 |
| 17 SHK-74 | 19.3 | 3.0 | 70 | 3.3 | 2.3 |
| 17 SHK-86 | 19.0 | 3.0 | 74 | 3.3 | 3.5 |
| 17 SHM-79 | 30.7 | 4.3 | 68 | 3.7 | 2.3 |
| 17 SHO-26 | 25.3 | 3.3 | 80 | 4.7 | 1.8 |
| DP 357 | 6.7 | 1.0 | 22 | 1.0 | 2.9 |
| FM 2334GLT | 31.7 | 4.0 | 96 | 3.7 | 1.7 |
| **Mean** | 22.2 | 3.4 | 69 | 3.5 | 2.6 |
| **LSD** | 9.5 | 1.2 | 20.2 | 1.4 | N.S. |
| **CV** | 25.7 | 21.1 | 18.4 | 23.4 | 30.8 |

1 represents high susceptibility, poor stands, and chlorotic foliage. 5 represents a healthy stand with no vegetative chlorosis or wilting

**0 represents a healthy root with no visible discoloration. 5 represents a dead and rotten root with sever staining

***1 represents a poor preforming plant with no bolls. 5 represents a healthy high yielding plant

**Table 35. Fiber quality of the elite strain trial at College Station, TX, in 2021**

| Designation | Lint % | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|
| | | Unit | mm | % | kNm/kg | % |
| 16 SHS-32 | 40.3 | 4.7 | 29.0 | 85.1 | 321 | 6.0 |
| 16 SHU-38 | 39.1 | 4.5 | 30.5 | 85.8 | 331 | 6.1 |
| 17 SHJ-48 | 39.4 | 4.5 | 29.7 | 84.1 | 341 | 6.7 |
| 17 SHO-26 | 35.3 | 4.7 | 27.7 | 83.9 | 340 | 5.6 |
| 17 SHK-74 | 34.7 | 4.9 | 27.7 | 83.9 | 293 | 6.3 |
| | | | | | | |
| **mean** | 37.8 | 4.6 | 28.9 | 84.6 | 325 | 6.1 |

**Table 36. Fiber quality of the advanced strain trial at College Station, TX, in 2021**

| Designation | Lint% | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|
| | | Unit | mm | % | kNm/kg | % |
| 14 E-12-FOV-05 | 44.5 | 4.2 | 27.9 | 81.9 | 312 | 6.7 |
| 16-SHU-11-FOV-02 | 40.5 | 3.9 | 29.7 | 82.6 | 305 | 5.7 |
| 16-SHU-11-FOV-04 | 39.3 | 4.3 | 28.4 | 83.4 | 320 | 5.7 |
| 16-SHU-11-FOV-05 | 41.4 | 4.3 | 27.7 | 83.6 | 338 | 5.9 |
| 16-SHU-27-FOV-05 | 45.1 | 4.2 | 28.4 | 84.1 | 303 | 5.7 |
| 16-SHU-27-FOV-06 | 40.1 | 4.6 | 29.0 | 84.7 | 311 | 5.9 |
| 16-SHU-27-FOV-09 | 42.0 | 4.6 | 27.4 | 81.3 | 306 | 6.1 |
| 16-SHU-38-FOV-5 | 39.1 | 4.5 | 29.5 | 83.8 | 307 | 6.3 |
| 16-SHU-38-FOV-14 | 37.4 | 4.8 | 29.2 | 83.4 | 314 | 6.5 |
| | | | | | | |
| **Averages** | 41.1 | 4.4 | 28.6 | 83.2 | 313 | 6.1 |

**Table 37. Fiber quality of the preliminary strain trial at College Station, TX, in 2022**

| Designation | Lint% | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|
| | | Unit | mm | % | kNm/kg | % |
| 206-20 | 37.4 | 4.5 | 29.7 | 84.8 | 328 | 6.7 |
| 233-02 | 38.1 | 4.6 | 31.0 | 85.1 | 338 | 5.6 |
| 233-09 | 41.7 | 5.2 | 26.9 | 84.4 | 317 | 6.3 |
| 233-11 | 42.7 | 4.9 | 29.0 | 83.7 | 315 | 6.2 |
| 233-12 | 41.4 | 4.8 | 28.7 | 81.6 | 287 | 5.4 |
| 238-02 | 37.4 | 4.3 | 27.4 | 84.6 | 355 | 5.3 |
| 238-03 | 34.3 | 4.1 | 30.7 | 82.3 | 368 | 6.2 |
| 209-14 | 40.3 | 4.5 | 28.2 | 83.9 | 295 | 6.1 |
| 209-16 | 41.4 | 5.1 | 26.7 | 82.7 | 313 | 6.3 |
| 209-23 | 42.0 | 4.5 | 27.4 | 82.4 | 317 | 6.2 |
| BB-04 | 40.3 | 4.6 | 27.7 | 82.1 | 275 | 6.2 |
| 17 MM-51-59-09 | 35.4 | 4.6 | 29.5 | 82.9 | 298 | 5.9 |
| 17 NN-31-39-07 | 42.0 | 4.4 | 26.9 | 79.6 | 255 | 5.6 |
| 17 PP-31-39-02 | 41.5 | 4.0 | 29.7 | 84.0 | 348 | 5.7 |
| 17 PP-41-49-16 | 38.7 | 3.9 | 30.2 | 81.8 | 285 | 5.6 |
| 17 QQ-31-39-02 | 41.9 | 4.8 | 29.0 | 84.2 | 305 | 5.3 |
| 17 QQ-41-49-11 | 36.1 | 4.3 | 30.2 | 82.5 | 323 | 5.6 |
| | | | | | | |
| **mean** | 39.6 | 4.5 | 28.8 | 83.1 | 313 | 5.9 |

**Table 38 Disease screening results of genotypes in the preliminary strain observation trial in Fabens, TX, in 2022**

| Source | Germination | Survival | Early Rating | Late Rating | Productivity | Root Stain | Seed Index |
|---|---|---|---|---|---|---|---|
| | (%) | (%) | (1-5)* | (1-5)* | (1-5)** | (0-5)*** | (G) |
| 21CSSHF5K-21 | 34 | 14 | 2 | 2 | 3 | 2.8 | 7.0 |
| 21 CS SH F5 K-22 | 50 | 34 | 3 | 4 | 3 | 3.4 | 7.6 |
| 21 CS SH F5 K-23 | 32 | 26 | 2 | 3 | 4 | 2.8 | 8.3 |
| 21 CS SH F5 K-24 | 36 | 12 | 2 | 3 | 3 | 2.4 | 7.7 |
| 21 CS SH F5 K-25 | 40 | 14 | 2 | 3 | 3 | 2.2 | 8.9 |
| 21 CS SH F5 L-21 | 46 | 22 | 2 | 3 | 2 | 2.2 | 8.2 |
| 21 CS SH F5 L-22 | 44 | 14 | 2 | 1 | 2 | 2.7 | 7.3 |
| 21 CS SH F5 L-23 | 34 | 12 | 2 | 1 | 2 | 4.5 | 9.2 |
| 21 CS SH F5 L-24 | 34 | 24 | 2 | 3 | 2 | 1.4 | 7.9 |
| 21 CS SH F5 L-25 | 42 | 22 | 3 | 5 | 5 | 3.0 | 8.6 |
| 21 CS SH F5 L-26 | 32 | 20 | 3 | 2 | 3 | 3.2 | 8.5 |
| 21 CS SH F5 M-21 | 30 | 10 | 2 | 3 | 2 | 2.6 | 7.5 |
| 21 CS SH F5 M-22 | 48 | 22 | 3 | 3 | 2 | 2.2 | 7.4 |
| 21 CS SH F5 M-24 | 46 | 18 | 2 | 3 | 3 | 1.8 | 7.4 |
| 21 CS SH F5 M-25 | 38 | 30 | 3 | 4 | 4 | 1.2 | 7.3 |
| 21 CS SH F5 M-26 | 44 | 34 | 3 | 4 | 5 | 2.2 | 7.5 |
| 21 CS SH F5 N-21 | 36 | 18 | 3 | 2 | 2 | 2 | 6.3 |
| 21 CS SH F5 N-22 | 38 | 28 | 3 | 3 | 4 | 3.6 | 6.9 |
| 21 CS SH F5 N-23 | 28 | 16 | 2 | 3 | 2 | 2.8 | 6.9 |
| 21 CS SH F5 N-24 | 32 | 24 | 2 | 3 | 3 | 3 | 6.1 |
| 21 CS SH F5 N-25 | 22 | 14 | 2 | 1 | 2 | 4 | 6.4 |
| 21 CS SH F5 N-26 | 42 | 38 | 3 | 5 | 5 | 0.4 | 7.2 |
| | | | | | | | |
| **Mean** | 37.6 | 21.2 | 2.4 | 2.9 | 3.0 | 2.6 | 7.6 |

*1 represents high susceptibility, poor stands, and chlorotic foliage. 5 represents a healthy stand with no vegetative chlorosis or wilting

** 1 represents a plant with no bolls and 5 represents a healthy plant with a large number of bolls

***0 represents a healthy root with no visible discoloration. 5 represents a dead and rotten root with sever staining

**Table 39. Fiber quality of the preliminary strain observation at College Station, TX, in 2022**

| Source | Lint% | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|
| | | Unit | mm | % | kNm/kg | % |
| 21CSSHF5K-21 | 39.9 | 4.8 | 25.4 | 81.0 | 244 | 6.8 |
| 21 CS SH F5 K-22 | 39.1 | 4.3 | 27.2 | 82.8 | 272 | 6.1 |
| 21 CS SH F5 K-23 | 39.2 | 4.4 | 28.4 | 83.1 | 289 | 6.1 |
| 21 CS SH F5 K-24 | 37.9 | 4.4 | 27.5 | 83.7 | 296 | 5.7 |
| 21 CS SH F5 K-25 | 35.7 | 4.2 | 27.8 | 82.5 | 276 | 6.1 |
| 21 CS SH F5 L-21 | 39.3 | 4.8 | 25.8 | 82.5 | 303 | 5.3 |
| 21 CS SH F5 L-22 | 39.6 | 4.8 | 26.0 | 81.4 | 289 | 5.5 |
| 21 CS SH F5 L-23 | 37.1 | 4.5 | 26.5 | 81.6 | 263 | 5.1 |
| 21 CS SH F5 L-24 | 40.4 | 5.3 | 23.5 | 78.4 | 252 | 5.3 |
| 21 CS SH F5 L-25 | 37.0 | 4.6 | 27.1 | 81.2 | 269 | 5.3 |
| 21 CS SH F5 L-26 | 38.9 | 4.4 | 26.5 | 80.3 | 256 | 5.4 |
| 21 CS SH F5 M-21 | 38.0 | 4.3 | 26.2 | 81.8 | 253 | 5.0 |
| 21 CS SH F5 M-22 | 39.6 | 4.4 | 25.9 | 81.4 | 264 | 5.4 |
| 21 CS SH F5 M-24 | 41.8 | 4.4 | 28.5 | 83.7 | 297 | 5.5 |
| 21 CS SH F5 M-25 | 42.2 | 5.0 | 27.6 | 82.5 | 298 | 6.1 |
| 21 CS SH F5 M-26 | 41.9 | 4.8 | 27.1 | 81.8 | 301 | 5.8 |
| 21 CS SH F5 N-21 | 42.5 | 4.7 | 28.1 | 81.5 | 276 | 5.8 |
| 21 CS SH F5 N-22 | 40.6 | 4.4 | 28.4 | 82.3 | 339 | 4.9 |
| 21 CS SH F5 N-23 | 39.6 | 4.4 | 26.6 | 81.3 | 294 | 5.0 |
| 21 CS SH F5 N-24 | 40.7 | 4.2 | 27.6 | 83.1 | 272 | 5.8 |
| 21 CS SH F5 N-25 | 42.2 | 5.1 | 28.9 | 81.7 | 322 | 6.5 |
| 21 CS SH F5 N-26 | 38.3 | 4.6 | 30.9 | 84.7 | 332 | 6.0 |
| | | | | | | |
| mean | 39.6 | 4.6 | 27.2 | 82.0 | 284 | 5.7 |

**Table 40 Coefficient of variation of fiber quality data as measured by HVI of F3 populations in College Station, TX in 2022.**

| Population | Lint % | Micronaire | Length | Uniformity | Strength | Elongation |
|---|---|---|---|---|---|---|
| | % | % | % | % | % | % |
| **19277** | 8.7 | 9.7 | 3.0 | 1.4 | 7.1 | 6.2 |
| **19278** | 7.2 | 11.3 | 2.6 | 1.5 | 6.6 | 6.7 |
| **19280** | 11.0 | 11.0 | 5.6 | 2.5 | 13.8 | 6.5 |
| **19281** | 11.1 | 16.0 | 4.5 | 1.3 | 8.7 | 7.0 |
| **19282** | 6.6 | 15.7 | 3.0 | 1.4 | 5.5 | 7.7 |
| **19283** | 12.0 | 14.0 | 6.2 | 2.0 | 10.4 | 8.8 |
| **19284** | 12.8 | 11.6 | 4.8 | 2.9 | 12.5 | 10.4 |
| **19286** | 7.5 | 11.5 | 4.9 | 2.5 | 13.7 | 4.5 |
| **19287** | 12.2 | 16.6 | 5.5 | 2.0 | 11.3 | 5.7 |