Analyzing Populations of Cotton Seedling Disease and Evaluating Seed Treatment Efficacy

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DEDICATION

I would like to dedicate this research to several individuals. First and foremost, my parents Melissa and Neill Pate. Thank you for your constant support and encouragement, I would not be where I am today without your love and guidance. To my grandmother, Jo Barker, thank you for your constant interest in my research and for your love. Karen Gardener, thank you for all the work you do behind the scenes to help keep my life running smoothly. You are an integral part of my success. In graduate school it is so important to have a support group behind you that constantly listens, supports, and loves you unconditionally. Alyssa Counce, McKayla Cunningham, and Kaitlyn Melton have been my best friends since my undergraduate years at UT Martin, and they continue to be the rocks of my support system. To Dawson Kerns, there are not enough words to express how thankful I am that I have had you by my side since day 1 of graduate school. Your patience, companionship, humor, and kind words are appreciated. I also would not have been able to maintain my sanity without the affection of my cat, Bentley. There is no doubt that he contributed greatly to my project with his statistical advising sessions and by serving as my study buddy/therapist. Most importantly, I would like to dedicate this research to my Nannie, Faye Phelps. She was my biggest fan, and made me feel like I could do anything in the world. She taught me what it meant to be Christ-centered, selfless, and loving. Without her influence, I would not be the person I am today.

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ABSTRACT

Cotton seedling diseases are continuously associated with causing yield loss across the United States. To provide Tennessee cotton growers, and cotton growers across the southeastern portion of the cotton belt, with more accurate pathogen identification of soilborne diseases known to cause cotton seedling diseases, this project was carried out in conjunction with the National Cotton Seed Treatment (NCST) program. The aim was to identify populations of pathogens implicated in cotton seedling disease and evaluate seed treatment efficacy. Over the past 24 years, the NCST Treatment program has been conducted with the purpose of analyzing populations of soilborne pathogens known to cause cotton seedling diseases across the United States cotton belt. However, this data has only been collected using morphological identification for the species *Thielaviopsis basicola*, and *Rhizoctonia solani,* and identification to the genus level for species of *Pythium* and *Fusarium*. Several *Fusarium* and *Pythium* species can be difficult to identify with morphological features alone. Therefore, to generate a more accurate data set, Enzyme-Linked Immunosorbent Assay (ELISA) was used to identify *Pythium* and *Rhizoctonia solani.* Regarding pathogen populations in each field trial location, populations from 2018 were greater than those recovered in 2019. However, it can be argued that the 2019 data is more accurate due to the implementation of pathogen identification lab guides, new selective media, and ELISA identification in 2019. In addition to quantification of pathogen populations, the NCST trial analyzes the seed

treatment efficacy against seedling diseases caused by these pathogens. Although sites and seed treatments varied between the 2018 and 2019 growing season, the control fungicide treatment, which controlled all pathogens of interest, increased stand over the nontreated check each year. In addition, locations in Alabama, Arkansas, Tennessee, and Texas had a significant overall stand response in both years.

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INTRODUCTION

Cotton

Cotton is a critical component in the textile industry, accounting for roughly 25% of the world's fiber use (USDA-ERS 2019). The United States plays a significant role in the world's cotton industry, as the third largest producer and the top exporter of cotton; the U.S. accounts for one-third of the world's raw cotton trade. Within the U.S., the cotton industry is responsible for creating and maintaining more than $21 billion annually in terms of products and services, and generates over 125,000 jobs (USDA-ERS a 2019).

In the United States, cotton planting begins as early as February and typically extends through June (National Cotton Council 2013). The 17 states that produce cotton are known as the Cotton Belt. These states include: Texas, California, Arizona, Mississippi, Louisiana, Alabama, Arkansas, Georgia, Missouri, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Virginia, Kansas, and Florida. Out of 17 states, Texas produces 45% of total U.S. cotton annually (USDA-ERS b 2019).

Cotton is a climate dependent crop, and can be grown in a variety of soils. Cotton thrives in areas where summer temperatures are between 68-86°F, and other environmental conditions create a long hot growing season (Patterson1967). Unfortunately, these environmental factors are also favorable for several soil-borne pathogens (Chaudhry 2003).

Cotton Production in Tennessee

Tennessee cotton growers typically produce around 400,000 acres of cotton (USDA-NASS 2019). Across the state, there are 23 counties that grow cotton, making it the third largest cash crop in Tennessee. Planting typically occurs between April 20 and May 10, but the crop may be planted as late as the first week of June because producers must consider soil temperature and moisture, and the five-day forecast when selecting planting dates. Earlier planting dates can result in greater levels of disease and cold stress, while later planting dates can push the crop into periods of greater insect pressure (Main b 2012).

Selecting a variety that is best suited to the region's soil and environmental factors is very important and cotton variety trials are performed across the state each year. These results are made available to growers to aid them in varietal selection (Raper et al. 2019; Raper et al. 2020).

Although there are steps that can be taken in regards to pest management during variety selection (such as planting varieties with value added technologies), there are also management decisions that need to made during the season. It is important to scout the crop for pests and diseases and keep up-to-date on information provided by Extension on pest and insect development in the immediate area throughout the growing season.

Insect and disease pressure are highly dependent on weather conditions. Several species of pathogens and insects are commonly associated with cotton production in Tennessee. During 2019, target spot, leaf spot complex, bacterial

blight, and cotton seedling diseases were observed in Tennessee (Kelly 2019). Cotton pests observed during 2019 included plant bugs, bollworms, and stinkbugs (Stewart 2019).

Although cotton is a perennial plant, the crop is managed commercially as an annual (Ritchie et al. 2007). This is achieved by applying plant growth regulators (PGRs), that suppress plant growth (Main 2012), which maximizes the amount of lint that can be harvested from each crop (Ritchie et al. 2007). When making input decisions, producers often consider growing degree days to estimate the growth date of the plant. Growing degree days are calculated by taking the average of the maximum temperature and the minimum temperature (typically using degrees Fahrenheit), and subtracting 60 from that number.

$$DD_{60} = (°F_{MAX} + °F_{MIN})/2 - 6$$

Sixty is subtracted from the original average because it is the lowest temperature at which cotton can grow (Main a 2012).

Cotton Seedling Germination

A consistent plant stand is key in producing a profitable cotton crop. Although cotton can compensate for some stand loss, low populations that are not uniform will restrict yields. To achieve an overall vigorous crop, it is important to plant high quality seed as well as ensure that field conditions are optimal for cotton seedling development. Cotton seed is usually purchased pre-treated with

fungicide and insecticides seed treatments to mitigate any detrimental pest damage early in seedling development (Kelly et al. 2018). There are a variety of treatments that can be applied to seed, and treatment decisions should be influenced by field history, pathogen presence, and environmental factors (Kelly et al. 2019).

Field Conditions

There are several factors to consider in terms of what field conditions are best for developing strong stands. Plant growth is unlikely in field conditions that are not conducive to seed germination. Most importantly, growers should consider physical factors, planting factors, and post-planting factors (Wanjura,1972).

Soil conditions which play a role in hypocotyl elongation and the emergence of the seedling include temperature, moisture, and physical barriers. The best temperature range for seedling development is 68-86°F. This temperature range promotes rapid elongation of the hypocotyl. If soil moisture is decreasing, the radical will grow rapidly, which changes the ratio of radicle to hypocotyl regions (Wanjura 1986).

Planting factors are also important in seedling development. Seeds should be planted into soil environments that allow seeds to germinate and grow. This means the soil should be properly prepared before planting. In addition to preparing the seedbed, it is imperative that an appropriate seed rate and depth is

selected for the planter being used. Seed depth is extremely important (Wanjura 1986). The depth of planting directly influences the energy and time needed by the seed to develop and emerge. Seeds planted at a moderate depth (1.5 cm) seem to be more vigorous than seeds plated at deeper depths (2.7 cm) (Dalianis 1982). In Tennessee, cotton is planted at a depth of 1.3 to 2.5 cm.

Factors such as soil crusting and low temperatures, which occur after planting can also greatly impact seedling emergence. Although these factors occur after planting there are steps that can be taken to reduce the impact of these occurrences. When preparing the seed bed for planting, it is important that the final structure of the seed bed can mitigate runoff water from rainfall, and soil temperatures are monitored (Wanjura 1986).

Seedling Development

The structure of a mature seed plays a huge role in water imbibition. The rounded end of a cotton seed is referred to as the chalaza and the pointed end is the micropyle. Water is taken up through the chalaza. Once water enters the seed, tissue swells, which in turn prompt cell growth. The radicle breaks through the micropyle, and rotates downward to grow into the soil. The radicle will eventually become the taproot, which is the main root that takes in water and nutrients from soil. The hypocotyl becomes longer and forms an arch shape. The arch moves towards the soil surface. Once there, the hypocotyl straightens to

pull the cotyledons through the surface. The cotyledons usually appear above the soil surface 4 to 14 days after planting (Ritchie et al. 2007) (Main 2012).

Soilborne Pathogens

Soilborne plant pathogens survive in soil via specialized structures or as saprophytes of decaying plant material. The type of specialized structure varies among pathogens. For example, *Rhizoctonia solani* overwinters via sclerotia and hyphae (Hyakumachi 1982, Lockwood 1988), *Thielaviopsis basicola* and *Fusarium* spp. overwinter via chlamydospores (Biddle 2007, Nyvall 1970), and *Pythium* spp. overwinter as thick-walled oospores (Schwartz 2011). Overall, overwintering structures are inactive in soil (Toussoun 1970). They can persist for long periods of time with allocation of very few resources (Lockwood 1988). Germination of survival structures are stimulated by exudates released from germinating seeds and roots (Buxton 1962). Infection potential is heavily influenced by the pathogen's interaction with environmental conditions. Each pathogen has a specific life cycle that requires optimal soil temperature, air temperature, precipitation factors, and other soil conditions to thrive (Toussoun 1970). In addition to environmental factors, exposure to susceptible host plants, plant stress, and inoculum density play a role in infection potential (Lockwood 1988, Baker 1971).

Methods of Dispersal

Plant pathogens are introduced to the soil in a multitude of ways. Usually this occurs through water movement, cultural practices such as soil cultivation, wind, and infected seed. Pathogens can build up their inoculum potential over time by producing specialized structures that allow them to survive in soil for long periods of time. It is also advantageous for survival of pathogens to be dispersed over time, which may enable them to contact and infect more plants, which will happen in the presence of alternative hosts (Baker 1965).

Spores and survival structures have a very small range of mobility within the soil. However, some pathogens such as oomycetes may form zoospores that allow them to move small distances in moist soil to locate an infection site (Zentmyer 1961). Most of the ‘movement’ of soilborne pathogens is facilitated through contaminated field equipment, transportation of infected plant debris, and infected seed (Issac 1957), by air, and water (Baker 1965).

Rhizoctonia solani

Ecology of Pathogen

Although sclerotia may be formed by some isolates of *Rhizoctonia solani*, morphological identification of this fungus is usually based on vegetative structures. Hyaline hyphae of *R. solani* emerge and become dark brown with age (Ajayi-Oyetunde and Bradley 2018). The hyphae of this fungus are multicellular and septate, and hyphal branching emerges from the dolipore/parenthosome

septum (Ajayi-Oyetunde and Bradley 2018), which can be identified by observing the characteristic swelling around the point of branching (Orlovich and Ashford 1994).

Infection Cycle/Symptoms

Rhizoctonia solani has been detected in all stages of seedling disease. However, this pathogen is most associated with post-emergence damping-off in cotton seedlings (Rothrock 1996), and is known to cause death of the seedling 50 to 60 days after planting. If the seedling survives infection, it will bear circular reddish-brown lesions on the stem in the hypocotyl region. The wound-like appearance of these lesions on the hypocotyl of the seedling has contributed to the common name of the disease, which is sore shin (Khedri and Heydari et al. 2014). When making isolations, *R. solani* can be baited from the soil using flat wooden toothpicks. After the hyphae have adhered to the toothpicks, the toothpicks are placed onto Terry Spurlock medium to encourage mycelial growth (Spurlock et al. 2016).

Rhizoctonia solani can have a negative impact on plant yield due to the stunting in growth that it causes on both a physical and physiological level (Heydari and Pessarakli 2010). Infection of the cotton seedling is achieved through hyphal growth of the fungi. Hyphae grow at right angles across the epidermal cells of the seedling. After aligning themselves in the grooves of connecting epidermal cells, two lateral hyphae are formed, which results in t-

shaped branching after termination of growth. The tips of the t-shaped hyphae germinate to produce lateral hyphae that are parallel to the first hyphae formed. This pattern of hyphal growth results in the formation of an infection cushion (Armentrout et al. 1987). Hyphal infection cushions can adhere to the epidermal cells via mucilaginous material (Armentrout et al. 1987), and hyphal tips on the bottom the infection cushion can form infection pegs that penetrate the cuticle of the plant and the epidermal cell walls. Once penetration of the epidermal cell wall has occurred, the fungus can proceed to the intercellular spaces, and the lumen. The pathogen can colonize all cells, except the xylem, both intracellularly and intercellularly (Khadga et al. 1963). It should be noted that in some isolates of *R. solani*, the fungus achieved infection only by hyphal penetration, without the production of pegs (Weinhold and Motta 1973). As well as an isolate that only grew over the surface of the plant without the production of infection cushions, and achieved infection via hyphal tip penetration (Sneh et al. 1989).

Infection of the seedling occurs quickly. Hyphal growth can be observed at 10 to 12 hours after inoculation. At 15 hours, branching of hyphae begins, and the infection cushion begins to be formed. Finally, in as little as 24 hours, discoloration of the hypocotyl can be observed because of cuticle penetration by the infection pegs, and lesions can be seen around 30 hours after inoculation (Armentrout et al. 1987).

Growth Conditions

Although *R. solani* is often prevalent wherever cotton is grown, it is difficult to determine the exact role that environmental factors play in disease severity (Rothrock 1996). *Rhizoctonia solani* seems to thrive best in soil conditions that are also optimal for cotton seedling development (~68 to 86°F); however, isolation frequencies cannot be correlated with soil temperature or soil moisture levels (Johnson et al. 1969). But, cotton seedlings that are continually exposed to cold soil temperatures can become predisposed to disease caused by *R. solani* (McCarter et al. 1971).

Pythium spp.

Ecology of Pathogen

Unlike other soilborne pathogens associated with cotton seedling disease, *Pythium* is classified as an oomycete. Even though *Pythium* is not a fungal organism, oomycetes are fungal-like in a variety of ways. In culture, *Pythium* can be identified using morphological structures that can include: non-septate hyaline hyphae, sporangia, oogonia, antheridia, oospores, and motile-biflagellate zoospores (McLeod 2009). It is important to note that septations are observable, but only when septa are walling off reproductive structures from the hypha to which they are attached (Deacon 2006). In the laboratory, use of selective media can be very useful for prompting growth of *Pythium* spp. Pimaricin-Ampicillin-

Rifampicin-PCNB (P_5ARP) agar is a good selective medium option for this purpose.

Infection Cycle/Symptoms

Pythium survives in the soil as oospores, which are thick-walled overwintering structures. Oospores of *Pythium* exhibit constitutive dormancy, which leads to a low level of germination. Therefore, not all oospores germinate at once. This allows the ungerminated oospores to remain in soil as inoculum for infection of seedlings in cases where conditions for survival of germinated oospores becomes impossible (Martin et al. 1999). Oospores will germinate in the presence of chemical root and seed exudates from a host plant. As a resultof germination, oospores will initiate infection by production of hyphal growth, or the formation of sporangia that release diploid motile zoospores. Dissemination of zoospores is achieved by breakdown of the sporangial tip, or the formation of an exit tube (Deacon 2006).

Once the motile zoospore has recognized and attached to host tissue, it forms a germ tube that penetrates susceptible tissues (Donaldson et al. 1993). If sporangia or oospores contact host tissue, they can also form penetrating germ tubes that initiate infection (Martin and Looper 1999). Infection rates among *Pythium* species varies, but germination of the propagule, infection via germ tube, and colonization by the pathogen occur rather quickly among most *Pythium* interactions with cotton seeds and seedlings (Nelson 1988, Nelson 1991).

Seedlings infected by *Pythium* can exhibit both preemergence and postemergence damping off (Martin et al. 1999). Reduced vigor and stunting of the seedlings may also be observed in seedlings that have no evidence of necrosis at the site of infection (Larkin et al. 1995). When examining the roots, *Pythium* is known to eliminate feeder roots, as well as root hairs (Martin et al. 1999).

Growth Conditions

Soil moisture content is an integral factor in development of *Pythium* spp. *Pythium* develops better in soils that contain at least 60% of moisture holding capacity (Rothrock 1943). Increased moisture is influential in the ability of the motile zoospores to move through the soil and germinate indirectly. As soil moisture increases, the amount of oxygen decreases. As oxygen decreases, it is converted to carbon dioxide, which leads to an anaerobic soil environment. Increased carbon dioxide levels stifle the activity of most microorganisms in the soil (Martin et al.1999). Because *Pythium* is tolerant to increased levels of carbon dioxide, it can thrive in such soil conditions without having to compete with other microorganisms (Mitchell et al. 1973). Optimum soil temperatures vary between different species of *Pythium* (Martin et al. 1999), and temperature influence on *Pythium* spp. is highly variable among temperatures associated with the host plant's optimal temperature range. In the case of pathogenic *Pythium* spp. on cotton seedlings, infection can occur via direct or indirect germination in the

temperature range of cotton seed germination, which is 68 to 86°F (Martin et al. 1999).

Thielaviopsis basicola

Ecology of Pathogen

Hyphae of *Thielaviopsis basicola* are hyaline and septated. In culture, the morphological structures of *T. basicola* include chlamydospores, that occur individually or are produced endogenously in conidia. Chlamydospores become dark brown with age (Horst 2013). For isolations of *T. basicola*, TB-CEN selective medium is useful (Rothrock 1992). In addition to selective medium, *T. basicola* can be baited from soil with carrot slices (Yarwood 1946). Although synonomyous with *T. basicola*, *Chalara elegans* is more commonly asscocitaed with mycological terms. When referring to the *Chalara elegans* more emphasis is placed on the development of hyaline endoconida formed than the pigmented clamydospores (Punja and Sun 2000). Recently, *T. basicola* and *C. elegans* have undergone a change in nomenclature that encompasses both terms for the fungus. *T. basicola* is now referred to as *Berkeleyomyces basicola* (Wingfield et al. 2018).

Infection Cycle/Symptoms

Thielaviopsis basicola overwinters in soil as thick-walled chlamydospores, which serve as the resting stage of the fungus. The chlamydospores germinate in the presence of cotton seedlings and wet soil conditions (Rothrock 1992). The

spores will infect the cortical root tissue, which causes dark discoloration of the roots as well as the hypocotyl. The infection propagules do not penetrate deeper than the cortical tissue, and a true characteristic of black root rot is the slippage of the out root when touched. Along with discoloration, the infection also delays seedling growth and maturation. Seedling vigor is decreased also. Initial infection of cotton seedling by *T. basicola* can occur from 10 to 14 days after planting (Pereg 2014).

Growth Conditions

To further elaborate on optimum soil temperatures for germination of this pathogen, *T. basicola* thrives in soil conditions that are cool, and wet, with temperatures below 75.2°F. When these conditions occur early in the season, the severity of black root rot can increase (Olsen et al. 2001). If the infection is not severe, the infected cortical tissue is shed, and secondary root growth begins as temperatures increase throughout the growing season (Pereg 2014).

Fusarium spp.

Ecology of Pathogen

Morphological features of *Fusarium* spp. include septated hyaline mycelia, macroconidia, microconidia, and chlamydospores. Most *Fusarium* spp. produce macroconidia on sporodochia (Nucci and Anaissie 2009). Chlamydospores may be observed within the hyphae (Leslie and Summerell 2008). In culture, *Fusarium* can produce several different colors ranging from white, light purple,

pink, and grey (Nucci and Anaissie 2009). Both pathogenic and nonpathogenic *Fusarium spp.* can be found in abundance in soil, and can be isolated using a variety of selective media. However, for species that are pathogenic to cotton, *F. oxysporum* and *F. solani* (Palmateer et al. 2004), the use of Nash and Snyder medium, Czapek-Dox agar, and malachite green agar are sufficient for isolation (Zhang et al. 1996, Leslie and Summerell 2008).

Infection Cycle/Symptoms

In the presence of a suitable host, spores of *Fusarium* germinate to penetrate the epidermis and cortical cells of the seedling (Bloomberg 1973). After penetration, the fungus proceeds to grow throughout the cortical cells and eventually infect the root endodermis and vascular tissue (Bloomberg 1976, James et al. 1991). The severity of symptom development is dependent on the virulence of the strain of the pathogen (Bloomberg 1971).

Symptoms of *Fusarium* infection within cotton seedlings are often confused with other symptoms of cotton seedling diseases caused by *Pythium* spp. *Rhizoctonia solani*, and *Thielaviopsis basicola*. Infected seedlings exhibit wilted cotyledons. If seedling death occurs, uneven stands will develop in the field, which can be another indicator of pathogen presence. When examining the vascular tissue, browning within the hypocotyl can help in differentiating *Fusarium* spp. from other cotton diseases (Davis et al. 2006).

Growth Conditions

Conducive soil conditions vary among species of *Fusarium* (Davis et al. 2006). However, *Fusarium* can be found in soils that are optimum for cotton production. *Fusarium* may remain in the soil up to 10 years in the absence of cotton (Smith et al. 2001), which classifies the fungus as a true soil inhabitant (Davis et al. 2006).

Pathogen Detection Methods

Selective Media

Although there are general growth media such as potato dextrose agar (PDA) and water agar (WA) that can be used for the general promotion of fungal growth (Choi et al. 1999). There are several types of selective media that can aid in culturing specific genera and even species of fungi. Selective media are developed to contain the appropriate nutrients to support the culture of specific pathogens, and often contain antibiotics to inhibit growth of unwanted bacteria (Taso 1970).

Enzyme-Linked Immunosorbent Assays in Plant Pathology

Although enzyme-linked immunosorbent assays (ELISAs) originally gained popularity for use in medical and forensic diagnostics, plant pathologists quickly realized their potential for pathogen detection. The majority of early ELISAs were developed for detection of viral plant pathogens (Shcherbakova 2007). However, attempts were soon made to adapt ELISAs for detection of

other types of plant pathogens. Now, ELISAs are one of the most widely used serological assays in plant pathology (Reddy et al. 1988). The appeal of ELISAs are that they are easy to run due to the small amounts of reagents required, multiple tests can be run at the same time, and results can be automated (Clausen 1997).

Double Antibody Sandwich ELISA

The double antibody sandwich (DAS) is the most common ELISA used when testing plant material for pathogen presence (Shcherbakova 2007). A DAS ELISA is classified as a heterogeneous assay that is used to detect macromolecules in contrast to basic ELISAs that are homogenous and/or detect micromolecules. Heterogeneous assays differ from homogenous assays in that they require an extra step that involves separation of bound and free labels (Clark 1981).

DAS-ELISAs are unique in that they involve trapping antibodies in a solid phase. During this entire process, the pathogen is immobilized by a specific antibody. The antibody reacts with the antigen produced by the pathogen, and the unreacted antibody is removed. After the excess has been removed, a substrate is added to produce a color change that allows quantification of pathogen contained per well (Clark 1981).

Management Options: Seed Treatments

Although crop rotation can be effective in controlling cotton seedling diseases, it is not often utilized by cotton producers because growers do not practice prolonged crop rotations. This had led to a heavy reliance on seed fungicide treatments. Fungicide seed treatments are sorted into groups by the Fungicide Resistance Action Committee (FRAC). Some of the most used seed treatment chemicals fall under FRAC codes 3, 4, 7, and 11. FRAC codes refer to fungicide mode of action. For example, FRAC code 7 includes succinate-dehydrogenase inhibitor (SDHI) fungicides, which affect the complex 2 cell respiration. The following are not the only seed treatments utilized to manage cotton seedling diseases, but these serve as examples of treatment options.

Two triazole fungicides (FRAC code 3), myclobutanil and triadimenol, have both been shown to exhibit some efficacy against black root rot (*T. basicola*) (Toksoz 2009, Rothrock et al. 2009). In addition to *T. basicola*, newer seed treatments also aim to manage other seedling pathogens (Toksoz 2009, Rothrock et al. 2009, Kelly 2018). When targeting *Pythium* spp., seeds are usually treated with varying rates of metalaxyl (FRAC code 4). However, mefenoxam (FRAC code 4) is also associated with management of *Pythium* spp. As a preventative measure for sore shin (*R. solani*) control, seed treatments containing penflufen (FRAC code 7), trifloxystrobin (FRAC code 11), and myclobutanil (FRAC code 3) can be utilized (Kelly 2018). In addition to the

respective active ingredients listed above, there are other seed treatment options that are available for pathogen management in cotton.

CHAPTER I
EVALUATION OF SEED TREATEMENT EFFICACY

Abstract

Each year the fungicide nominations submitted for the National Cotton Seed Treatment (NCST) program are tested in multiple locations across the U.S. cotton belt. In 2018, there were 11 total fungicide treatments that were included on the cooperator distribution list; whereas in 2019 there were a total of 15 treatments. For both years, the first four seed treatments served as control treatments. Treatment 4 was a base fungicide treatment [Allegiance (0.75 fl. oz/cwt), Evergol Prime (0.32 fl. oz/cwt), Spera (1.85 fl. oz/cwt), and Proline (0.16 fl. oz/cwt)] that increased stand the most over the non-treated check for both 2018 and 2019. Although treatment response in a specific field site varied slightly between years, at some sites (AL, AR, TN, and TX1), the difference was always significant. Overall, location and the effect of treatment x location significantly affected stand counts across both years, which is likely due to the influence of disease pressure and environmental factors on stand establishment. It should also be noted that stand count percentages across all locations showed that all nominated treatments from both years provided at least an equal amount of efficacy in all locations, and greater efficacy than the control treatment in some locations.

Introduction

Cotton is a critical component of the textile industry, and accounts for roughly 25% of the world's fiber use. The United States plays a huge role in the world's cotton industry as the top exporter, and the third largest global producer of cotton. Nationally, the cotton industry is responsible for creating and maintaining more than $21 billion annually in terms of products and services. This in turn generates over 125,000 jobs within the cotton industry (USDA-ERS 2019).

Over the last 5 years, seedling diseases have ranked among the top 5 diseases that are responsible for yield loss. In 2018 alone, it is estimated that 162,000 bales of cotton were lost to seedling disease, which is equivalent to 48.6 million dollars lost (Lawrence et al. 2019). This staggering loss is what makes cotton seedling disease not only important at the state level, but on a national level as well.

There are four pathogens that have been consistently associated with causing seedling diseases in cotton in the U.S. These include: *Thielaviopsis basicola*, *Rhizoctonia solani*, *Fusarium* spp., and *Pythium* spp. (Horst 2013). Twenty-five years ago, the NCST program was introduced to analyze the impact of seed treatments on cotton seedling diseases across the U.S. Cotton Belt (Guyer 2018).

Seed treatments are a common method for combating seedling diseases in cotton (Kelly et al. 2018). Each year, a call for nominations is sent out to

company representatives to submit their nominations for seed treatments. Once company nominations are received, the NSCT coordinator treats all seed and distributes to each cooperator. A master list of treatments is also released to each cooperator. Every cooperator is responsible for deploying each treatmentin each of their field trial locations. Having multiple cooperators from several different states yields a good representation of seed treatment efficacy across the Cotton Belt. The objective of this study was to analyze seed treatment efficacy based on seedling emergence data from field trials across the U.S. Cotton Belt.

Materials and Methods

Experimental Design

During the 2018 growing season, there were 12 locations that participated in the NCST program, which ranged across 8 states (Figure 1). For the 2019 growing season, there were also 12 locations; however, these locations ranged across 9 different states which gave broader representation on seedling disease presence in the southeastern portion of the U.S. Cotton Belt (Figure 2).

A randomized complete block design was used for each field trial setup. Plots were 6 m (20 ft) in length or greater, and the planting rate ranged from 9.8 to 16.4 seed per meter (3 to 5 seeds per foot). Replications between locations ranged from three to four, and each cooperator was responsible for reporting stand count data about 30 days after planting, for each treatment across all reps

within a location (Tables 1 and 2). Stand count data for each treatment within a location was converted to percent emergence with the following equation:

$$Percent\ emergence = (\frac{A_{average}}{T_{total}}) \times 100$$

Where $A_{average}$ = the average number of emerged plants across all reps per treatment, and T_{total} = the total number of seeds planted for the plot length.

Treatment Factors

The cotton cultivar DP 1522 B2XF was used in 2018 and 2019 in this program. Each year, a call for nominations is sent to industry representatives for fungicide seed treatment nominations to be tested in the NCST program. For the 2018 growing season, there were 11 total treatments tested across all locations (Table 1); and in 2019, there were 15 seed treatments tested (Table 2). During both years, the first four treatments on the nomination list were control treatments. Control treatments, with rate applied, were as follows: 1 = Non-treated check, 2 = Allegiance FL (1.5 fl. oz/cwt), 3 = Evergol Prime (0.64 fl. oz/cwt), and 4 = Allegiance (0.75 fl. oz/cwt), Evergol Prime (0.32 fl. oz/cwt), Spera (1.85 fl. oz/cwt), and Proline (0.16 fl. oz/cwt). Fungicides were mixed with water at a total slurry rate of 30 fl. oz/cwt., and Gaucho 600 was applied at a rate of 12.8 fl. oz/cwt to all seed, including treatment 1, which did not receive fungicide treatment. The purpose of applying Gaucho 600 to all treatments was

to mitigate the effects of insect damage that may occur during the duration of each trial.

Seed Germination Evaluation

The seed germination rate for each treatment was tested independently at the West Tennessee AgResearch and Education Center. For each treatment, 4 replications of 100 seedlings were tested by wrapping each replication in moist paper towels. All seeds were incubated at 30°C for 3 days. After 3 days, the number of seeds that failed to germinate was recorded. Germination was defined as the radical extending beyond the length of the seed. Seed germination rates were determined by taking the average number of seeds that failed to germinate per treatment across all replications. That average was then subtracted from 100 to yield a percent germination per treatment.

Data Analysis

Seed treatment efficacy was analyzed using a GLIMMIX-mixed model feature with a concurrent Tukey HSD means separation test to determine significant differences between treatments in JMP 14 Pro (SAS Institute Corp. Cary, NC). When evaluating treatment effect across all locations, an alpha level

of 0.05 was used to determine significance. However, for evaluation of treatment effects on individual sites, an alpha level of 0.1 was utilized.

Estimated pathogen pressure for *R. solani* and *Pythium* spp. was calculated for each location using percent emergence data, which was calculated with the following equation:

$$Estimated\ pathogen\ pressure = [\frac{(A_{control} - T_{control})}{A_{control}}] \times 100$$

Where: $A_{control}$ = emergence data from treatment #4 for each location, which has efficacy across all major seedling disease pathogens, $T_{control}$ = emergence data from a targeted control for each location, i.e. from treatment #2 (controls *Pythium* spp., an oomycete) or #3 (controls *R. solani,* a true fungus). Hence, using data from treatment #2 for $T_{control}$ yielded the estimated pathogen pressure for true fungi, since treatment #2 controls *Pythium* spp. Similarly, using data from treatment #3 for $T_{control}$ estimated pathogen pressure for *Pythium* spp. because treatment #3 controlled *R. solani*.

Results and Discussion

For the 2018 growing season, all locations exhibited a significant ($P \leq 0.0001$) percent emergence response to treatment, location, and location x treatment interaction. This significant response indicates that plant stand response to treatment was dependent on abiotic/biotic factors and the aggressiveness of pathogens present in each field trial location. In regards to treatment efficacy associated with a specific site, of 12 locations, 5 sites yielded a significant plant stand response to treatment. Sites with significant responses included AL, AR, LA2, TN, and TX1 (Figure 3). When estimating pathogen pressure, *Pythium* spp. had an average pressure of 8.83%. Whereas, *R. solani* had an estimated pressure of 12.58% across all locations. While true fungi could be contributing to a reduction in percent emergence data in treatment #2 and #3, it is assumed that both treatments would be affected similarly by true fungi, hence, the difference between the emergence data for those treatments is assumed to be from *R. solani* and *Pythium* spp., respectively. Unfortunately, an accurate estimation of reduction in stand establishment from *T. basicola* and *Fusarium* spp. is unattainable at this time due to the lack of fungicides that would solely control each pathogen. Based on seedling emergence response to treatment #2, another explanation of low stand emergence in some locations could be due to fungicide resistance. Metalaxyl has been a frequently used active ingredient when combatting *Pythium* spp., and although the base treatment includes metalaxyl, it still provided adequate control in all field trial locations.

There are no other seed treatments labeled in cotton for control of *Pythium* spp. that are not in the same FRAC group. Therefore, it may be advantageous to test for insensitivity within FRAC group 4.

For all locations included in the 2019 growing season, there was also a significant ($P \leq 0.001$) plant stand response to treatment, location, and location x treatment interaction, which again signifies that treatment response was dependent on abiotic/biotic factors and specific pathogens present at each location. When looking at treatment response by site, there were six locations that were significant, including AL, AR, LA1, TN, TX1, and TX2 (Table 4). Seed germination rates associated with this trial year were all above 90%, except for treatment #8, which had a 50% germination rate when tested in the lab. Although treatment #8 had low seed germination, stand counts were similar to other fungicide treatments across all locations with an average of 66%, which was an increase in stand over the non-treated check. This suggests an error in the germination testing procedure in the lab. Estimated pathogen pressure between *Pythium* spp. and *R. solani* was similar to results in 2018, where *R. solani* exhibited a greater disease pressure calculation (15.41) than *Pythium* spp. (4.60).

For both years, locations AL, AR, TN, and TX2 resulted in significant treatment responses. Although not all trials were in the exact same location within each state, they are representative of the prominent cotton growing regions in each state. Also, all control treatments increased stand over the non-

treated check. Among those control treatments, the base fungicide treatment (Treatment #4) increased stand the most, which was to be expected. When looking at stand response to treatment #2, stand counts were lower than treatment #3 in some locations, which indicates that treatment #2 did not control as evenly across all locations when compared to treatment #3. Additionally, this may have contributed to *R. solani* having a greater pressure than *Pythium* spp. When only treatment #2 was applied to seed, seedlings may have suffered because they were not protected against *R. solani* and other true fungi. This was also the case in 2019, which was expected considering that *R. solani* exhibited higher disease pressure than *Pythium* spp. In addition to the control treatments increasing stand over the non-treated check, all nominated treatments included in the 2018 and 2019 growing seasons increased stand over the non-treated check at both locations.

CHAPTER II
DETERMINING THE PRESENCE OF COTTON SEEDLING DISEASES AND AN EVALUATION OF DETECTION METHODS

Abstract

The National Cotton Seed Treatment (NCST) program evaluates fungicide seed treatment efficacy against cotton seedling diseases and provides data on the presence and incidence of *Thielaviopsis basicola*, *Rhizoctonia solani, Pythium* spp. and *Fusarium* spp. using selective media and ELISA. Information on the growth stage of seedlings at the time of disease assessment, and disease ratings on hypocotyl and roots of seedlings are recorded. Data from 2018 and 2019 across 12 locations were analyzed. Seedling development across all locations at the time of sampling averaged 6 nodes in 2018 and 3 nodes in 2019. Across all locations, in 2018, hypocotyl disease indices averaged 2.0 and root disease indices averaged 3.8, using a 1=best to 5 or 6=worst scale, respectively; and in 2019 averaged 2.1 and 3.9, respectively. In 2018, *Pythium* spp., *Fusarium* spp., and *R. solani* were isolated from seedlings from all 12 locations and *T. basicola* was isolated from seedlings at 11 of 12 locations. Whereas in the 2019 growing season, *Fusarium* spp. and *R. solani* were recovered from all locations, *T. basicola* was isolated from seedlings at 10 of 12 locations, and *Pythium* spp. were isolated form every location except MS2. Isolation frequencies determined with ELISA were lower than those from selective media, but estimated pathogen pressure was not significantly correlated to either detection method. However, selective media had a positive relationship (positive r value) with disease pressure, and ELISA results had a negative relationship to estimated disease pressures. In 2019, *R. solani* was detected in 5 of the 9 sites with ELISA. The

isolation frequencies of *Pythium* spp. with ELISA in 2019 yielded detection from 6 of 12 sites and isolation frequencies ranged from 5 to 35%, compared to selective media isolation frequencies of 7 to 67%. In terms of pathogenicity of *Pythium* spp., while TX1 had one of the lowest isolation frequencies of *Pythium* spp., the two isolates tested from that location had the greatest pathogenicity. Pathogenicity of *Pythium* spp. ranged from 1 to 3.7, averaging 2.3 across all isolates tested. Overall, isolation frequencies varied between 2018 and 2019; however, it could be argued that isolation frequencies in 2019 were more reliable due to the use of additional pathogen identification materials, selective media, and additional detection methods. Although ELISA and selective media are both known identification techniques, based on this study, selective media continues to provide greater isolation frequencies that are more congruent with estimated disease pressures.

Introduction

Soilborne pathogens cause disease of cotton seedlings (Toussoun 1970). More specifically, four soilborne pathogens are the primary cause of cotton seedling diseases in the southeastern cotton belt of the U.S., including *Thielaviopsis basicola*, *Rhizoctonia solani*, *Pythium* spp., and *Fusarium* spp. (Horst 2013). There are several research techniques commonly used to better understand and detect these pathogens. A couple of these methods include morphological and genetic identification.

Soilborne pathogens survive in the soil as propagules, which can be dispersed in several ways (Toussoun 1970). Propagules can be moved via water (Renfro 1959), tillage (Campbell 1962), and air (Gregory 1961). Some types of soilborne pathogens can also form motile zoospores that allow them to move along the water film contained within the soil to find an infection site. This is the case with oomycetes, such as *Pythium* spp. (Zentmyer 1961).

Propagules begin to germinate in the presence of root and seed exudates (Buxton 1962). In the case of *T. basicola*, *R. solani*, *Pythium* spp., and *Fusarium* spp., a very specific set of exudates and nutrients are needed for pathogenesis to occur (Toussoun1970). In the presence of those exudates and nutrients, these pathogens accomplish infection in different ways.

Rhizoctonia solani, utilizes hyphae as its infective propagule, which form infection cushions (Armentrout and Downer 1987). *Pythium* spp. use a combination of hyphae and motile zoospores to find an infection site where a

germ tube is produced to penetrate the root tissue (Donaldson et aI. 1993). *Thielaviopsis basicola* infects seedlings via chlamydospores and hyphal growth (Pereg 2014.). Finally, *Fusarium* spp. also infects the tissue of the host plant via hyphae often from germinated chlamydospores, and macro- and microspores (Nucci and Anaissie 2009). Pathogenesis of any of these pathogens is highly dependent on environmental factors, and these four generally prefer cool, wet soil conditions (Rothrock,1992).

For detection of these pathogens, selective media is an extremely common method used in a laboratory. *Rhizoctonia solani* is usually baited from soil and placed onto Terry Spurlock selective medium (Spurlock and Rothrock et al. 2016). There are several selective media available for *Fusarium*; however, Malachite Green Agar (MGA) (Zhang et al., 1996) is selective for pathogenic species of *Fusarium*, and was the most applicable to this study. *T. basicola* Carrot Etridiazole Nystatin (TB-CEN) agar is a selective medium that contains fresh carrot juice that can be used to recover *T. basicola* from plant materials. Whereas, *Pythium* spp. are commonly isolated using the selective medium P_5ARP (Jeffers 1986). Double Antibody Sandwich Enzyme Linked Immuno-Sorbent Assays (DAS ELISAs) have also been used to detect fungal pathogens from plant tissues, and can be used as further confirmation of pathogen presence (Scherbakova 2007). If high isolation frequencies are recovered in comparison to stand response from a certain location, pathogenicity assays may be needed to determine pathogenicity of recovered isolates. Therefore, the objectives of this

study were to analyze pathogen populations of cotton seedling diseases across the U.S. Cotton Belt, improve and compare identification techniques, and evaluate the pathogenicity of *Pythium* isolates.

Materials and Methods

Sample Collection

Each NCST cooperator was responsible for collecting 100 random cotton seedlings form each location from the non-treated check plots at 27 to 41 days after planting. The 100 seedlings were shipped overnight to the West Tennessee AgResearch and Education Center Field Crops Pathology Lab to be processed for evaluation. Cooperators also collected 10 soil cores from each non-treated check plot, and included those cores in their shipment. All material received was shipped in Styrofoam coolers containing ice packs, to maintain viability of the samples. Samples were processed within 48 hours of receipt, and most samples were processed on the same day that they were received; samples not immediately processed, were refrigerated until processed.

Seedling Processing

Seedlings were rinsed in running tap water for 20 minutes to remove soil or debris that could be mistaken for symptoms or signs of disease. After rinsing, seedlings were blotted dry with paper towels and 50 seedlings were randomly

selected to be rated for symptoms of seedling disease. Of those 50, 5 were selected at random to determine the number of nodes.

Disease Severity Rating

Each seedling, in the subset of 50 seedlings for disease severity ratings, was cut at the cotyledon scar to remove excess plant material to make visual ratings of root and hypocotyl damage more accessible and efficient. Both the hypocotyl and root regions of each seedling were evaluated using separate disease rating scales. The rating scale for the hypocotyl region was a 1 to 5 scale: where 1 = no symptoms, 2 = a few pinpoint lesions and diffuse color areas, 3 = distinct necrotic lesions, 4 = girdling lesion, and 5 = dead seedling. Whereas, the rating scale for the root region was a 1 to 6 scale, where 1 = no symptoms, 2 = 1-10% of root system discolored, 3 = 11-25% of root system discolored, 4 = 26-50% of root system discolored, 5 = 51-75% of root system discolored, and 6 = >75% of root system discolored.

Seedling Plating

Seedling roots were plated onto a variety of selective media for the 2018 and 2019 growing seasons, with slight variations in the plating protocol. In 2018, the original 100 seedlings received from each location were divided into two groups of 50 after being rinsed in tap water as described previously. The first group was plated onto P_5ARP (Jeffers 1986), a selective medium for *Pythium* spp. The second group was surfaced sterilized for 60 seconds via submersion in

a 10% bleach solution. After removal from bleach solution, roots were dipped into sterile water, and blotted dry with sterile paper towels before being plated onto water agar. The water agar was amended with rifampicin, ampicillin, and danitol. Isolations were taken from any fungal growth on the water agar for morphological identification. After 3 to 5 days, roots were removed from water agar and placed onto TB-CEN agar (Specht 1985) to isolate *T. basicola*. All plates were incubated at 78°F in the dark to allow for optimum mycelial growth. P_5ARP and TB-CEN plates were incubated for 1 to 2 weeks. Water agar plates were incubated for 3 to 5 days before isolations were made. After incubation, growth was analyzed under a compound microscope for morphological identification.

In 2019, some plating methods were either eliminated or revised. Although 100 seedlings were still received from each location's non-treated check plots, the seedlings were split into 5 groups of 20. If cooperators had sent seedlings in separate bags based on replication, then an equal number of seedlings from each replication was included in each group. The first group of 20 seedlings were plated onto P_5ARP for *Pythium* spp., the second group onto MGA for *Fusarium* spp. (Castellá 1997), the third onto TB-CEN for *T. basicola*, and the remaining 2 groups were stored at -80°C for ELISA testing. Both P_5ARP and TB-CEN were incubated in the dark at 78°F for 1 to 2 weeks to allow for mycelial growth and sporulation of reproductive structures to aid in morphological identification. Seedlings plated onto MGA were also incubated in the dark at 18°C. However, pathogen growth was evident within 3 to 5 days. Seedlings that were originally

plated onto MGA were split into 2 groups and plated onto P_5ARP and TB-CEN to increase the sample size of seedlings associated with identification of *Pythium* spp. and *T. basicola*, respectively. Additional plating and re-use of seedlings was done to increase screening for *Pythium* spp. and *T. basicola; Fusarium* spp. was consistently found in almost all MGA plates after 3 to 5 days. All isolation frequencies were determined by microscopy to confirm identification based on morphological features.

Soil Evaluation

In addition to the seedlings received from each location, cooperators were responsible for collecting 10 soil cores from their non-treated check plots. Upon arrival, soil cores were screened to evenly mix samples and remove debris. The soil screen was sterilized between samples with 10% bleach or 70% ethanol). In 2018, soil populations of *Pythium* spp. and *T. basicola* were detected by diluting 30 g (wet weight) of soil in 0.2% water agar to a total volume of 250 ml and shaking on a Wrist action shaker for 20 minutes. *Pythium* spp. were quantified with the spread-plate method, where the soil solution was spread over the selective medium P5ARP using a plastic soil spreader. Populations of *T. basicola* were quantified using the pour-plate method, where the soil solution is incorporated into the modified selective medium TB-CEN before pouring into plates. Quantification via soil techniques were removed from the 2019 protocol.

Rhizoctonia solani was recovered from the soil in both 2018 and 2019. Sieved soil was used to fill a 4” plastic pot to the fill line located below the headspace. Pots were placed onto a solid plastic tray filled with water to saturate the soil in the pot. Pots were drained overnight and nine sterile, flat toothpicks were inserted vertically into the soil in an evenly distributed square pattern. Toothpicks remained in the soil for 48 hours. After removal, toothpicks were placed onto Terry Spurlock medium, which is selective for *R. solani* (Spurlock et al. 2016). Each plate contained three toothpicks, which resulted in a total of three plates per location. Plates were incubated in the dark for 48 hours at 80°F, and isolations were transferred to potato dextrose agar for morphological identification later. The soil isolation frequency conversion equation for *R. solani* was as follows:

$$\frac{Percent\ isolation\ of\ propagules}{100\ cm^3} = \frac{P_{total}}{1.389}$$

Where P_{total} = toothpick positions exhibiting hyphal growth. The total was divided by 1.389, which is the conversion factor of the number of positions represented by the toothpick length to soil volume while accounting for the area of nine toothpicks per location.

ELISA Processing and Testing

Approximately 20 seedlings from each location were evaluated with ELISA. Two different kits were utilized to test for the presence of *R. solani* and *Pythium* spp. Initially, all kits were purchased from Neogen Corporation

(Scotland, UK); however, Neogen discontinued their ELISA kits for *R. solani* and *Pythium* spp. before this study was completed. Therefore, all remaining kits were purchased from LOEWE (Sauerlach, Germany). Upon confirmation that the extraction buffer from Neogen could be used to process plant material for the LOEWE kits, all seedlings were processed using the same extraction buffer and tested according to the corresponding protocol provided in each kit using 96-well plates.

Each plate contained samples from two locations. Also, within each plate, there were two replications of a known positive sample, a known negative sample, a positive kit control, empty wells, and buffer only wells. These checks were used to further confirm and quantify the light absorbance results from running the completed test through a spectrophotometer.

Based on the values yielded from each run, each individual value was adjusted by subtracting the value of the average of the four uncoated wells that were included in each test plate. Using the average computed between each replication, if the average across both reps of one seedling was greater than or equal to 2 times the average of the negative controls it was considered positive (Sutula 1986).

Pathogenicity Assays

Pathogenicity testing for *Pythium* spp. recovered from cotton seedlings was adapted from the methodology used in corn and soybean as described by B.Q. Zhnag and X.B. Yang (2000). Isolations were taken from each P_5ARP plate that was confirmed to contain *Pythium* spp. Isolates were placed on filter paper and stored in a freezer (-20°C) until tested. At the time of testing, two isolates from each location were selected at random.

To begin the assay, two replications of each isolate (stored on hole punched filter paper) were placed in the center of a plate containing 1% water agar and allowed to sit at room temperature for 7 days to revive the isolate, and allow mycelial growth. After 7 days, 10 black cottonseeds, which had been sterilized via immersion in a 1% bleach solution, were placed onto the outer edge in a circular pattern on each plate. Seeds were also placed onto 2 plates containing only 1% water agar to serve as the controls. All plates were deposited into an incubator set at 78.8°F for 7 to 8 days to accommodate the optimal sporulation temperature of *Pythium* spp. Plates were removed from the low temperature incubator and incubated again at room temperature for 2 days to allow for additional mycelial growth.

At the conclusion of each assay, seedling germination was evaluated to compare the aggressiveness/pathogenicity of each isolate. To accomplish this, a scale of 0 to 4 was used, where: 0 = seed germinated without visible infection, 1 = germinated with light discoloration on roots, 2 = germinated with short severely

discolored roots, 3 = died after germination, and 4 = died before germination. Germination was defined as those seedlings with a radicle that had extended beyond the length of the seed.

Data Analysis

Data for percent stand, disease ratings for hypocotyl and root, pathogen isolation frequency, and soil populations over locations was analyzed for significance with JMP 14 Pro (SAS Institute Corp. Cary, NC). To determine if there was statistical difference between the isolation frequencies recovered from selective media and ELISA, a nonparametric Wilcoxon Test was used to account for the lack of normality, and for its resistance to outliers. When comparing the relationship of the two types of isolation frequencies in relation to pathogen pressure, isolation frequencies were rank-transformed and used in a nonparametric test. All tests were performed at an alpha level of 0.05. Using ranked isolation percentages to account for outliers, multivariate correlations were performed to determine the relationship between pathogen pressure effect and isolation techniques at an alpha level of 0.05.

Results and Discussion

For the 2018 growing season, seedling development across all locations ranged from 2.2 to 10.0 nodes. Hypocotyl disease indices ranged from 1.4 to 2.7, averaging 2.0 across all locations. Root disease indices ranged from 2.0 to 5.0, averaging 3.8 across all locations. *Pythium* spp. were isolated from seedlings from all 12 locations with isolation frequencies ranging from 20 to 100%, averaging 80% (Table 5). *Thielaviopsis basicola* was isolated from seedlings at 11 of the 12 locations and was isolated from more than 70% of the seedlings at 7 of the 12 locations (Table 5). *Fusarium* spp. were isolated from seedlings at all 12 locations with isolation frequencies ranging from 16 to 90%, averaging 53% (Table 5). *Rhizoctonia solani* was detection in 7 of the 7 soils assayed, and ranged from 2.2 to 15.8 propagules/100 cm^3 of soil (Table 5). From other soils assayed in 2018, *T. basicola* was recovered at 7.13 CFU/g across all locations and *Pythium* spp. was recovered at 197 CFU/g across all locations (Table 5).

For the 2019 growing season, seedling development across all locations ranged from 1.6 to 6.0 nodes. Hypocotyl disease indices ranged from 1.7 to 2.8, averaging 2.1 across all locations. Root disease indices ranged from 2.5 to 5.3, averaging 3.9 across all locations. *Thielaviopsis basicola* was isolated from seedlings at 10 of the 12 locations with isolation frequencies ranging from 20 to 100%, averaging 48% (Table 6). *Fusarium* and *Pythium* spp. were recovered from all locations and averaged over 90% isolation frequency and 26% frequency, respectively (Table 6). *Rhizoctonia solani* was recovered from soil

screened from all 12 sites, ranging from 27.4 to 33.1 propagules/100 cm^3 of soil, with an average of 26.8 across all locations (Table 6). Soil dilutions for *T. basicola* and *Pythium* spp. were removed from the 2019 protocol.

Although *Pythium* spp. had the greatest isolation frequencies in 2018 and the lowest in 2019 based on selective medium, this could be due to the development and implementation of pathogen identification guides, which led to more accurate identification of oomycetes in 2019. Whereas the increase in *Fusarium* spp. recovered in 2019 was most likely due to the implementation of the selective medium, Malachite Green Agar. When comparing *Fusarium* spp. isolation frequencies between 2018 and 2019, the high recovery of *Fusarium* spp. but lack of effect on stand emergence suggests that many isolates or species of *Fusarium* recovered are nonpathogenic. This prompts the implementation of a pathogenicity assay for *Fusarium* spp. in future studies. While all isolates of *Pythium* spp. evaluated were pathogenic, their pathogenicity ranged from 1.0 to 3.7, averaging 2.3 (Figure 3). Additionally, while TX1 had one of the lowest isolation frequencies of *Pythium* spp. (13%) (Table 4), the two isolates tested from that location had the greatest pathogenicity (2.85 and 3.65) (Figure 3).

Using a Nonparametric Wilcoxon's Test, there was a significant difference between isolation techniques over both years and pathogens (*Pythium* spp. and *R. solani*) at P = 0.05. While *Pythium* spp. had greater isolation frequencies in 2018, *R. solani* had greater isolation frequencies in 2019, and selective medium

resulted in greater isolation frequencies than ELISA for both *Pythium* spp. and *R. solani*. Additional investigation is needed to understand the lack of congruency between the *Pythium* spp. detection from selective medium and ELISA, where selective medium identified *Pythium* spp. at 11 locations with isolation frequencies ranging from 7 to 67%, ELISA detected *Pythium* spp. only at 6 locations with isolation frequencies ranging from 5 to 35%. Whereas, in 2019, *R. solani* was recovered on selective medium for at every location at 12.2 to 33.1 propagules/ 100 cm^3. In contrast, ELISA only detected *R. solani* in 6 locations with a wide range of isolation frequencies (5 to 80%; Table 4).

Using ranked isolation percentages to account for outliers, multivariate correlations were performed to determine the relationship between estimated pathogen pressure effect and isolation techniques. When analyzing *R. solani* isolation frequencies for 2018 from PDA and soil baiting, the response to pathogen pressure was non-significant. In addition, the number of isolations on PDA exhibited a negative relationship to pathogen pressure at (r=-0.16, P=0.73). Similarly, soil baiting was also negatively related to pathogen pressure (r=-0.14, P=0.76) Similarly, in 2019, isolation percentages for ELISA detection techniques used for *R. solani* (r=-0.24, P=0.53) and *Pythium* spp. (r=-0.26, P=0.40) were both negatively correlated with pathogen pressure. Although there was no significant response when looking at selective media for both pathogens in 2019, in relation to pathogen pressure, there was a positive relationship between isolation frequency and pathogen pressure: *Pythium* spp. (r=0.38, P=0.22), and

R. solani (r=0.23, P=0.38). For *R. solani*, this could be due to the difference between isolations recovered from seedlings and soil. For *Pythium* spp., use of the original seedlings that were assessed on P_5ARP with ELISA kits may yield more congruent isolation frequencies between the detection methods. Another option would be to save isolates form each selective medium plate and use ELISA to confirmed they contained *Pythium* spp. or *R. solani*. During preliminary calibration of each ELISA kit used in this study, we determined that mycelium retrieved from each pathogen did in fact give a positive test result.

When analyzing correlations between number of nodes, stand percentage, plant disease ratings, isolation frequencies, and soil pathogen isolation frequencies for 2018 and 2019, the Pearson product moment correlation method revealed the following. For 2018, the number of nodes was negatively correlated with both the isolation frequency of *T. basicola* and *Fusarium* spp., -0.69 (P=0.0269) and -0.73 (P=0.0172), respectively. This can be expected because larger, more vigorous seedlings will usually have less seedling disease. However, the number of nodes were positively correlated with seedling isolation frequency of *Pythium* spp. from water agar plating, 0.71 (P=0.0224), indicating possible errors in *Pythium* spp. identification and/or identification of nonpathogenic *Pythium* spp. As expected, the hypocotyl and root disease ratings were positively correlated, 0.76 (P=0.0101). There was a trend for *Pythium* spp. population frequencies from the selective medium (P_5ARP) to be associated with

an increase in root and hypocotyl disease indices, 0.55 (P=0.0795) and 0.56 (P=0.0924), respectively.

In 2019, larger seedlings exhibited less root discoloration (number of nodes was negatively correlated with root disease rating index, -0.60 P=0.04). Sites with greater root discoloration also yielded greater isolation frequencies of *Pythium* spp. There was an expected trend for stand establishment in non-treated checks to increase with node count, 0.57 (P=0.05). Using ELISA detection, root index correlated with *Pythium* spp. isolation, 0.64 (P=0.03). However, isolation frequencies of *Pythium* spp. and *R. solani* were positively correlated, 0.70 (P=0.04), which may indicate co-infection at some locations where both pathogens were present. When considering isolation frequencies from selective media, *Pythium* spp. and *Fusarium* spp. were negatively correlated, -0.70 (P=0.01), which could indicate competition between these pathogens in colonization of seedlings.

In conclusion, isolation frequencies were recovered in 2018 through a combination of plating onto selective media, general growth media, soil baiting, and soil dilution plating. However, in 2019 changes were made to make the overall process more efficient and reliable. With implementation of new selective media and enhanced identification literature in the lab in 2019, isolation frequencies of pathogens differed greatly from 2018. It could be argued that because of these additions, the morphological data obtained in 2019 is more reliable than that recovered in 2018. ELISA was used for detection of *R. solani*

and *Pythium* spp. in 2019 in an effort to add innovative techniques to the protocol, and the comparison to selective media indicated that selective media continued to provide greater isolation frequencies and more congruent relationships with estimated disease pressure. However, with the implementation of new preparation techniques for ELISA, more consistent data could be recovered in the future. Finally, when conducting pathogenicity assays for *Pythium* isolates for each location in 2019, it was revealed that Texas had the most pathogenic isolates overall even though those locations had the lowest *Pythium* isolation frequencies. This illustrates the importance of including not only pathogen detection, quantification, and virulence; but also environmental factors when understanding seedling disease risk at specific locations. These data can be further utilized to better understand seedling disease impact on cotton and development of risk models.

CONCLUSION

In summary, across 2018 and 2019, all locations exhibited a significant (P≤0.0001) plant emergence response to treatment, location, and location x treatment interaction. This significant response indicates that plant emergence response to treatment was dependent on abiotic/biotic factors and the aggressiveness of pathogens present at each field trial location. Also, across both years, *Pythium* spp. exhibited less pressure than *R. solani* across all locations. Although isolation frequencies for each pathogen varied between years, *R. solan*i was always present at high levels. In 2019, *R. solani* had high isolation frequencies among locations, which suggests that this pathogen has the most potential to affect stand establishment. When looking at treatment response, the standard base fungicide treatment always increased stand over the non-treated check. In addition to this, all nominated seed treatments for both years provided equal control to the base treatment, which was statistically significantly greater in some locations across both years. For example, in 2018, treatment #5 provided the greatest stand increase over the base treatment. Whereas in 2019, treatment #14 provided the greatest stand increase over the non-treated check than any seed treatment tested. Therefore, the standard base treatment continues to exhibit sufficient control. In terms of resistance management, which is important because there are new formulations continuously being tested to provide multiple control options. The importance of this can further be supported by looking at the stand response to treatment #2 in

both years, which may suggest potential resistance to metalaxyl. Currently, there are no other seed treatments labeled in cotton for control of *Pythium* spp. that are not in the same FRAC group. Therefore, it may be advantageous to test for insensitivity within FRAC group 4. For pathogen recovery from each location, isolation frequencies varied between 2018 and 2019. However, it can be argued that the isolation frequency from selective media was more accurate in 2019 than 2018 due to the implementation of new selective media and distribution of pathogen identification aids in the lab. Although, this could be due to a possible variance between years. When comparing detection techniques in 2019, selective media yielded greater isolation frequencies than ELISA. In contrast, neither detection method had a significant response to pathogen pressure for *Pythium* or *R. solani*. However, for both pathogens, selective media had a positive relationship when compared to ELISA that exhibited a negative relationship. Finally, when testing the pathogenicity of *Pythium* spp. isolates from each field trial location, both locations in Texas had the most pathogenic isolates of *Pythium*. These pathogenicity assays were insightful because they helped rate the aggressiveness of each isolate tested, and identified which locations had the most pathogenic isolates. Additionally, pathogenicity testing was important at locations that exhibited similar stand emergence results, but differed in isolation frequencies. Overall, this study produced multi-dimensional data that can be analyzed in a variety of ways in the future to help aid in management recommendations and risk modeling.

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APPENDIX

Table 1: Treatments included in the 2018 NCST Program

TRT #	Common or registered name[1]	Formulation	A.I. (%)	Rate (oz/cwt)	Target pathogen
1	Gaucho 600[2]	F	Imidacloprid	12.8	No target
2	ALLEGIANCE FL	F	Metalaxyl (28.35)	1.5	*Pythium*
3	EVERGOL PRIME	F	Penflufen (22.7)	0.64	*R. solani*
4	ALLEGIANCE FL	F	Metalaxyl (28.35)	0.75	*Pythium*
	EVERGOL PRIME	F	Penflufen (22.7)	0.33	*R. solani*
	SPERA 240FS	F	Myclobutanil (22.37)	1.8	*R. solani, T. basicola*
	PROLINE 480 SC	F	Prothioconazole (41.0)	0.16	*R. solani, Fusarium*
	SP102000026368 (Bayer)	F		0.16	
5	ALLEGIANCE FL	F	Metalaxyl (28.35)	0.75	*Pythium*
	EVERGOL PRIME	F	Penflufen (22.7)	0.33	*R. solani*
	SPERA 240FS	F	Myclobutanil (22.37)	1.8	*R. solani, T. basicola*
	PROLINE 480 SC	F	Prothioconazole (41.0)	0.32	*R. solani, Fusarium*
	SP102000026368 (Bayer)	F		0.32	
6	EVERGOL XTEND	F	Penflufen (14.26) Trifloxystrobin (14.26)	1	*R. solani*

Table 1 Continued

8	Base fungicide (Albaugh LLC)	F	Myclobutanil (63.34) Metalaxyl (30.25) Fludioxonil (3.78)	2.15	*R. solani, T. basicola, Pythium, Fusarium*
9	Premium fungicide (Albaugh LLC)	F	Myclobutanil (63.34), Metalaxyl (30.25), Fludioxonil (3.78)	5	*R. solani, T. basicola, Pythium, Fusarium*
10	METALAXYL 4.0 ST	EC	Metalaxyl (44.08)	0.5	*Pythium*
	MAXIM 4 FS	F	Fludioxonil (40.3)	0.08	*R. solani*
	SYSTHANE WSP	WP	Myclobutanil (40)	0.84	R. solani
	VIBRANCE CST	F	Mefenoxam(6.71) Azoxystrobin (6.71) Sedaxane (3.13) Fludioxonil (1.12)	3.06	*Pythium, R. solani, Fusarium*
11	METALAXYL 4.0 ST	EC	Metalaxyl (44.08)	0.5	*Pythium*
	MAXIM 4 FS	F	Fludioxonil (40.3)	0.08	*R. solani, Fusarium*
	SYSTHANE WSP	WP	Myclobutanil (40)	0.84	*R. solani, T. basicola*
	VIBRANCE CST	F	Mefenoxam (6.71), Azoxystrobin (6.71), Sedaxane (3.13), Fludioxonil (1.12)	4.08	*Pythium, R. solani, Fusarium*

[1] - Registered chemical name, all capital letters

[2] - All treatments included GAUCHO 600, Flowable, Imidacloprid (48.7%), 16 oz/cwt

Table 2: Treatments included in the 2019 NCST Program

Trt #	Common or registered name[1]	Formulation	A.I. (%)	Rate oz/cwt	Target Pathogen
1	Gaucho 600[2]	F	Imidacloprid (47.8)	12.8	No target
2	ALLEGIANCE FL	F	Metalaxyl (28.35)	1.5	*Pythium*
3	EVERGOL PRIME	F	Penflufen (22.7)	0.64	*R. solani*
4	SPERA 240FS	F	Myclobutanil (22.37)	1.85	*R. solani, T. basicola*
	PROLINE 480 SC	F	Prothioconazole (41.0)	0.16	*R. solani, Fusarium*
	EVERGOL PRIME	F	Penflufen (22.7)	0.32	*R. solani*
	ALLEGIANCE FL	F	Metalaxyl (28.35)	0.75	*Pythium*
5	Albaugh Premium fungicide	F	Myclobutanil (63.34), Metalaxyl (30.25), Fludioxonil (3.78)	5	*Pythium, R. solani, T. basicola, Fusarium*
6	BAS500 F	F	Pyraclostrobin (90.2)	1.54	
	BAS700 F	F	Fluxapyroxad	0.94	
	ALLEGIANCE FL	F	Metalaxyl (28.35)	0.75	*Pythium*
	SPERA 240FS	F	Myclobutanil (22.37)	1.96	*R. solani, T. basicola*
7	BAS500 F	F	Pyraclostrobin (90.2)	3.07	
	BAS700 F	F	Fluxapyroxad	0.94	
	ALLEGIANCE FL	F	Metalaxyl (28.35)	0.75	*Pythium*
	SPERA 240FS	F	Myclobutanil (22.37)	1.96	*R. solani, T. basicola*
8	BAS500 F	F	Pyraclostrobin (90.2)	1.54	
	BAS700 F	F	Fluxapyroxad	0.94	
	ALLEGIANCE FL	F	Metalaxyl (28.35)	0.75	*Pythium*
	SPERA 240FS	F	Myclobutanil (22.37)	1.96	*R. solani, T. basicola*
	COPeO PRIME	F	Fluopyram (48.4)	5.97	*Nematodes*

Table 2 Continued

9	APRON XL	F	Mefenoxam (33.3)	0.5	*Pythium*
	MAXIM 4FS	F	Fludioxonil (40.3)	0.08	*R. solani, Fusarium*
	RALLY 40WSP	WP	Myclobutanil (40)	0.84	*T. basicola*
	VIBRANCE CST	F	Mefenoxam (6.71), Azoxystrobin (6.71), Sedaxane (3.13), Fludioxonil (1.12)	3.06	*Pythium, R. solani, Fusarium*
10	APRON XL	F	Mefenoxam (33.3)	0.5	*Pythium*
	MAXIM 4FS	F	Fludioxonil (40.3)	0.08	*R. solani, Fusarium*
	RALLY 40WSP	WP	Myclobutanil (40)	0.84	*T. basicola*
	VIBRANCE CST	F	Mefenoxam (6.71), Azoxystrobin (6.71), Sedaxane (3.13), Fludioxonil (1.12)	4.08	*Pythium, R. solani, Fusarium*
11	KABINA ST	F	Penthiopyrad (40)	0.87	*R. solani*
	SPERA 240FS	F	Myclobutanil (22.37)	1.8	*R. solani, T. basicola*
	ALLEGIANCE FL	F	Metalaxyl (28.35)	1.5	*Pythium*
	MAXIM 4FS	F	Fludioxonil (40.3)	0.16	*R. solani, Fusarium*
12	ALLEGIANCE FL	F	Metalaxyl (28.35)	0.75	*Pythium*
	EVERGOL PRIME	F	Penflufen (22.7)	0.33	*R. solani*
	FLUOXASTROBIN FS480	F	Fluoxastrobin (40.3)	0.38	*Pythium, R. solani*
	PROLINE 480 SC	F	Prothioconazole (41.0)	0.16	*R. solani, Fusarium*
	SPERA 240FS	F	Myclobutani(22.37)	1.8	*R. solani, T. basicola*
13	ALLEGIANCE FL	F	Metalaxyl (28.35)	0.75	*Pythium*
	EVERGOL PRIME	F	Penflufen (22.7)	0.64	*R. solani*
	FLUOXASTROBIN FS480	F	Fluoxastrobin (40.3)	0.38	*Pythium, R. solani*
	PROLINE 480 SC	F	Prothioconazole (41.0)	0.16	*R. solani, Fusarium*
	SPERA 240FS	F	Myclobutanil (22.37)	1.8	*R. solani, T. basicola*

Table 2 Continued

14	ALLEGIANCE FL	F	Metalaxyl (28.35)	0.75	*Pythium*
	EVERGOL PRIME	F	Penflufen (22.7)	0.33	*R. solani*
	FLUOXASTROBIN FS480	F	Fluoxastrobin (40.3)	0.38	*Pythium, R. solani*
	PFL+TFS FS308 (EVERGOL XTEND)	F	Penflufen (14.26), Trifloxystrobin (14.26)	1	*R. solani*
	PROLINE 480 SC	F	Prothioconazole (41.0)	0.16	*R. solani, Fusarium*
	SPERA 240FS	F	Myclobutanil (22.37)	1.8	*R. solani, T. basicola*
15	ALLEGIANCE FL	F	Metalaxyl (28.35)	0.75	*Pythium*
	EVERGOL ENERGY	F	Prothioconazole (07.18), Penflufen (3.59), Metalaxyl (5.74)	1	*R. solani*
	EVERGOL PRIME	F	Penflufen (22.7)	0.33	*R. solani*
	FLUOXASTROBIN FS480	F	Floxacrine (40.3)	0.38	*Pythium, R. solani*
	PROLINE 480 SC	F	Prothioconazole (41.0)	0.16	*R.solani, Fusarium*
	SPERA 240FS	F	Myclobutanil (22.37)	1.8	*R.solani, T. basicola*

[1] - Registered chemical name, all capital letters

[2] - All treatments included GAUCHO 600, Flowable, Imidacloprid (48.7%), 16 oz/cwt

Table 3: 2018 Percent Emergence Data

	Percent Emergence[2]												
Trt[1]	AL	AR	LA1	LA2	MS2	OK	TN	TX1	TX2	TX3	VA	MS1	AVG
1	49A	13B	47	60B	71	25	50BC	51C	55	50	75	79	52C
2	56A	19B	39	64AB	77	64	50C	57BC	61	51	68	86	58B
3	53A	44A	43	60B	77	61	53BC	56BC	66	59	75	83	61AB
4	60A	51A	46	75A	76	56	59ABC	62ABC	62	57	73	84	63A
5	57A	48A	51	65AB	74	67	61ABC	66AB	66	56	71	83	64A
6	55A	49A	34	70AB	75	40	61ABC	66AB	63	57	75	83	61AB
7	52A	44A	49	72A	76	41	58ABC	64AB	68	57	73	81	61AB
8	48A	49A	50	69AB	78	62	59ABC	63AB	63	57	76	81	63A
9	50A	45A	50	66AB	78	50	66AB	60ABC	65	53	78	85	62AB
10	54A	52A	48	70AB	74	57	62ABC	68A	66	55	73	81	63A
11	53A	48A	44	64AB	77	55	73A	66AB	63	50	74	85	63A
Avg	53	42	45	67	76	53	59	62	64	55	74	83	61

[1]Treatment details can be found in Table 1

[2]Data were analyzed with JMP 14 Pro (SAS I74nstitute Inc., Cary NC), values with the same letter within a column are not significantly different, where percent stand was analyzed across locations and by location using Mixed Model and Fit Model – Standard

Least Squares procedure, respectively – Tukey HSD means separation with alpha =0.1

Table 4: 2019 Percent Emergence Data

Trt[1]	AL	AR	GA	LA1	LA2	MS1	MS2	NC	TN	TX1	TX2	VA	Mean
1	31 b[2]	79 ab	64	52 c	46	55	83	42	71 c	40 bc	26 b	73	54 d
2	34 ab	81 ab	72	72 ab	49	63	82	52	74 bc	29 c	41 a	75	59 cd
3	32 ab	74 b	76	71 b	56	66	84	59	78 abc	42 bc	26 b	70	60 bc
4	35 ab	81 ab	84	80 ab	61	74	83	55	78 abc	55 ab	38 ab	77	66 ab
5	39 ab	81 ab	75	81 ab	53	76	81	63	85 a	45 abc	39 ab	75	65 abc
6	40 ab	74 b	82	77 ab	56	79	88	53	82 ab	55 ab	33 ab	69	64 abc
7	44 a	80 ab	71	78 ab	53	71	85	65	83 ab	50 ab	33 ab	75	64 abc
8	39 ab	80 ab	75	80 ab	60	70	82	59	83 a	57 ab	33 ab	72	65 abc
9	43 ab	95 ab	75	83 ab	58	72	83	57	82 ab	57 ab	32 ab	72	68 abc
10	37 ab	84 ab	75	82 ab	58	73	83	53	86 a	57 ab	31 ab	71	66 abc
11	36 ab	81 ab	94	81 ab	49	79	81	57	83 ab	49 ab	37 ab	76	67 abc
12	35 ab	85 ab	80	82 ab	60	76	82	51	83 ab	63 a	35 ab	71	68 abc
13	40 ab	90 ab	73	75 ab	51	77	85	56	80 abc	49 abc	35 ab	75	67 abc
14	42 ab	89 ab	82	82 ab	57	71	89	67	81 ab	64 a	39 ab	69	71 a
15	42 ab	85 ab	88	79 ab	60	56	87	58	80 abc	51 ab	35 ab	67	67 abc
Avg	38	83	78	77	55	70	84	56	80	51	34	72	65

[1]Treatment details can be found in Table 2

[2]Data were analyzed with JMP 14 Pro (SAS Institute Inc., Cary NC), values with the same letter within a column are not significantly different, where percent stand was analyzed across locations using Mixed Model – Tukey HSD means separation with alpha = 0.05 and by location using the Fit Model – Standard Least Squares procedure – Tukey HSD means separation with alpha =0.1

Table 5: 2018 Planting and Soil Temperature Overview

Site ID	Planted	Sampled	Counted	Row counted	Seed planted	Soil temp.[1]
AR	5/1	5/31	5/31	20	160	75 (65)
AU	4/13	5/14	5/14	25	100	73 (71)
LA1	4/13	5/14	5/14	25	125	68 (56)
MS1	4/20	5/23	5/1	70	280	67 (62)
MS2	4/20	5/23	5/1	70	280	67 (62)
OK	5/30	7/1	7/1	20	80	83 (70)
LA2	4/16	5/16	5/16	25	175	65 (51)
TX1	5/16	5/30	6/18	60	240	70 (33)
TX2	5/15	5/29	6/14	60	240	39 (39)
TX3	5/5	5/19	6/15	60	240	75 (63)
UT	5/2	6/1	5/14	60	300	68 (62)
VA	5/14	6/13	6/13	60	225	79 (71)

[1]Mean (Minimum) soil temp. (°C); 3-day average following planting

Table 6: 2019 Planting and Soil Temperature Overview

Site ID	Planted	Sampled	Counted	Row counted	Seed planted	Soil temp.[1]
AL	4/17	5/22	5/22	25	100	22 (18)[2]
AR	5/29	7/1	7/1	50	250	27(21)
GA	4/4	5/15	5/15	25	75	27(21)[2]
LA1	4/16	5/14	5/14	20	100	19(14)[2]
LA2	4/17	5/20	5/20	25	100	19(16)[2]
MS1	4/24	5/28	5/22	70	280	24(23)[2]
MS2	6/10	7/12	7/11	70	280	27(24)
NC	5/9	6/6	6/6	40	160	23(14)
TN	4/23	5/23	5/22	60	240	20(19)[2]
TX1	5/15	6/13	6/13	25	100	26(22)[2]
TX2	5/6	6/5	6/3	36	144	20(16)[2]
VA	5/8	6/12	6/12	60	240	22(17)

[1]Mean (Minimum) soil temp. (°C); 3-day average following planting.
[2]Weather data collected from National Weather Service weather station.

Table 7: 2019 Isolation Frequencies (%)

Location	*Pythium* spp.[1]	*T. basicola*[1]	*Fusarium* spp.[1]	*R. solani*[2]	*Pythium* spp. CFU/g[3]	*T. basicola* CFU/g[3]
AR	96	72	16	3.6	61	12.8
AU	54	100	61	**	347	--
LA1	56	96	82	**	228	--
MS1	98	0	44	**	131	--
MS2	93	3	28	**	278	--
OU	78	33	43	2.2	231	7.3
LA2	88	100	90	**	422	--
TX1	86	100	53	3.6	67	0.3
TX2	86	18	51	2.9	72	3.8
TX3	88	100	69	15.8	111	4.7
TN	84	92	30	10.1	308	--
VA	56	56	69	15.8	103	13.9
Average	80	64	53	7.7	197	7.1

[1]-Isolation Frequencies based on 20-30 seedling per location

[2]- Isolation frequency based on soil baiting: propagules/100 cm^3

[3]- Isolation frequency based on soil dilution plating

*-Information not available

Table 8: 2019 Isolation Frequencies

Isolation frequency (%)[1]						
Location	*Pythium* spp.[2]		*T. basicola*	*Fusarium* spp.	*R. solani*[2]	
AL*	37	0	100	63	32.4	202
AR*	67	25	35	67	27.4	5
GA	27	35	60	100	31	80
LA1*	13	10	55	100	25.2	15
LA2	30	15	50	93	32.4	25
MS1	27	15	0	90	12.2	0
MS2	0	0	0	100	25.2	0
NC	7	0	95	100	32.4	0
TN*	17	5	90	90	14.4	0
TX1*	13	0	20	100	33.1	0
TX2*	40	0	65	97	24.5	0
VA	30	0	0	100	31.7	1
Avg	26	8.8	48	92	26.8	16.1

[1] All isolations from seedlings, except first column of *R. solani* are from soil and is reported as propagules/100 cm^3

[2] Gray column indicates ELISA isolation frequency

*Indicates locations with significant standresponse

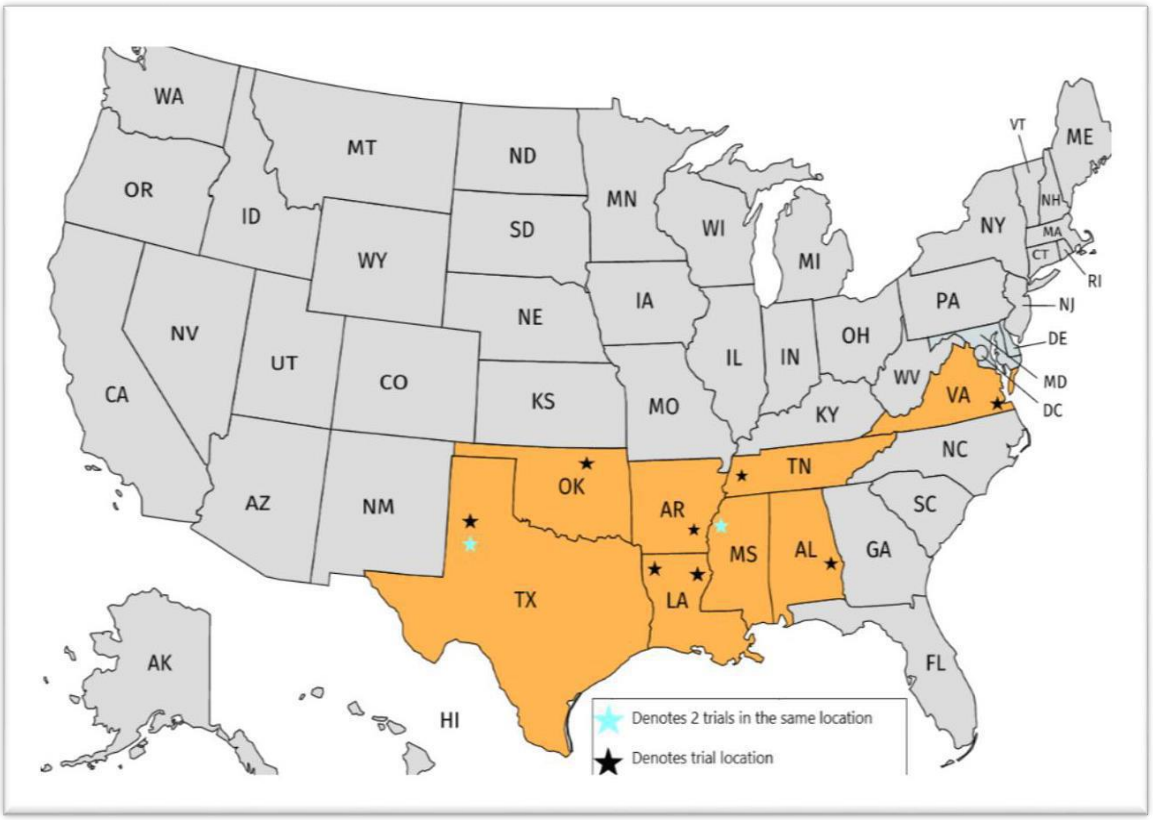


Figure 1. 2018 NCST Field Trial Locations

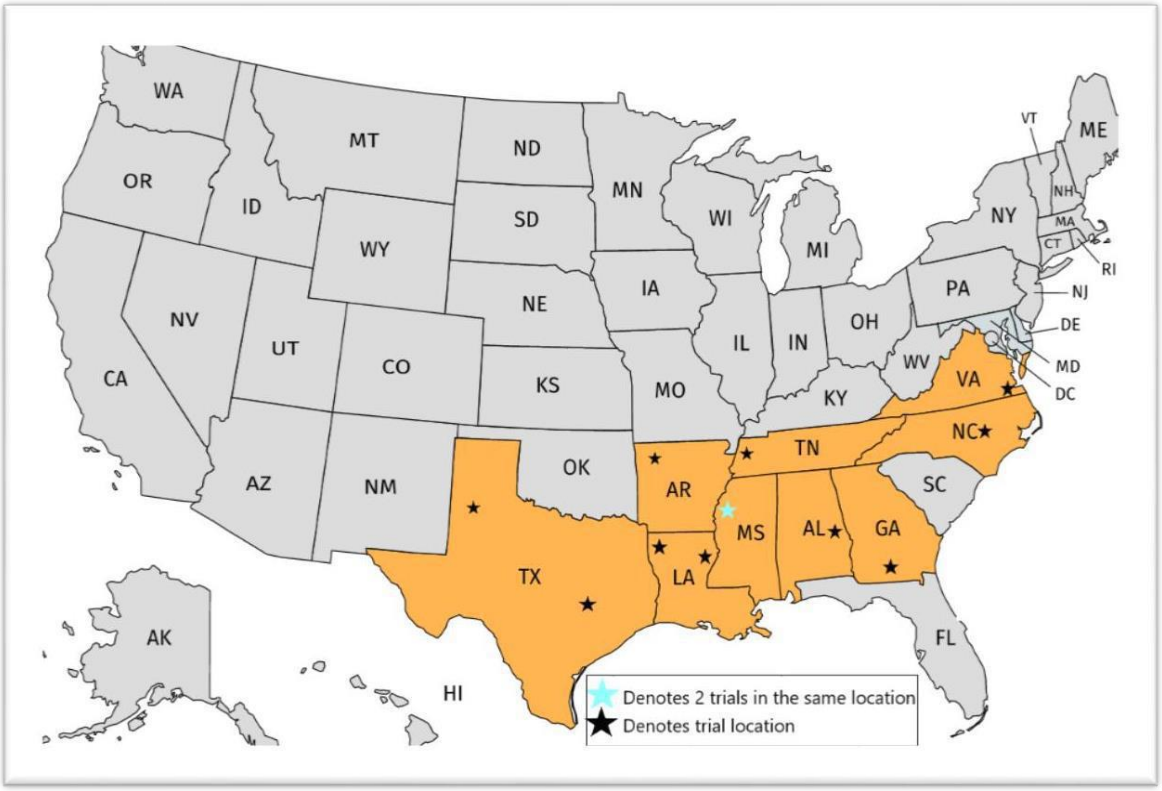
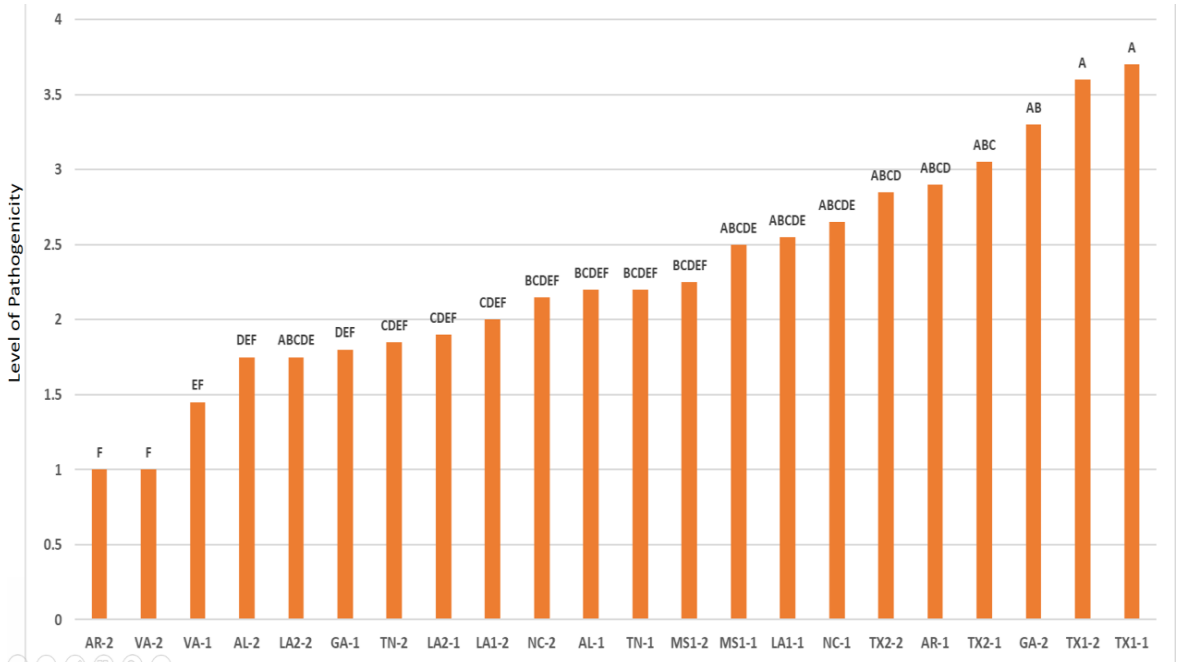


Figure 2. 2019 NCST Field Trial Locations



Pathogenicity was sorted across locations with Mixed Model – Tukey HSD means separation with alpha = 0.05

Figure 3. Pathogenicity Results for Isolates from Locations in 2019

VITA

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