

**THE EVALUATION OF HIGH TANNIN COTTON LINES FOR THEIR USE IN
BREEDING FOR RESISTANCE TO *XANTHOMONAS AXONOPODIS* PV.
MALVACEARUM, *PYTHIUM APHANIDERMATUM* AND *RHIZOCTONIA SOLANI***

A Dissertation

by

RAYMOND MATTHEW KENNETT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2010

Major Subject: Plant Breeding

**THE EVALUATION OF HIGH TANNIN COTTON LINES FOR THEIR USE IN
BREEDING FOR RESISTANCE TO *XANTHOMONAS AXONOPODIS* PV.
MALVACEARUM, *PYTHIUM APHANIDERMATUM* AND *RHIZOCTONIA SOLANI***

A Dissertation

by

RAYMOND MATTHEW KENNETT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Chairs of Committee, C. Wayne Smith

Committee Members, Steve Hague

Peggy Thaxton

James Starr

Department Head, David Baltensperger

December 2010

Major Subject: Plant Breeding

ABSTRACT

The Evaluation of High Tannin Cotton Lines and Their Use in Breeding for Resistance to *Xanthomonas axonopodis* pv. *malvacearum*, *Pythium aphanidermatum* and *Rhizoctonia solani*.

(December 2010)

Raymond Matthew Kennett, B.S., Cornell University;

M.S., Texas A & M University

Chair of Advisory Committee: Dr. C. Wayne Smith

Xanthomonas axonopodis pv. *malvacearum*(Smith), *Pythium aphanidermatum*(Edson) and *Rhizoctonia solani*(Kuhn) have all been shown to cause significant yield losses in cotton. Previous work has demonstrated that a set of high tannin cotton germplasm lines developed and released in 1989 by Texas A&M AgriLife Research may possess resistance to these three diseases. In this research, the usefulness of these high tannin lines in breeding for resistance to these pathogens as well as the role of tannin in conferring this resistance were examined. The high tannin lines were screened for their resistance to *Xanthomonas axonopodis* pv. *malvacearum* and five highly resistant lines were subjected to generation means and diallel analysis in order to determine the relative importance of different types of gene action in conferring resistance as well as which lines would be most useful in breeding for resistance. The effect of selection for *R. solani* resistance was measured and selected lines were subjected to diallel analysis. Lines showing elevated resistant to *P. aphanidermatum* were subjected to a single cycle of selection and the effect of selection was measured. Lines possessing high degrees of *P. aphanidermatum* resistance were subjected to generation means and diallel analysis. The

importance of tannin content in conferring resistance to *X. axonopodis* and *R. solani* was also measured.

Four high tannin lines were found to be not different from Tamcot Sphinx in their resistance to *Xam*. Generation means analysis for *Xam* resistance showed that in the five high tannin lines tested, additive gene effects were of the greatest importance. No correlation between tannin content and *Xam* resistance was detected. Under the conditions of this study tannin content was also shown to be unimportant in *R. solani* resistance. Two rounds of recurrent phenotypic selection were effective in increasing the resistance of selected HT lines to *R. solani*. One cycle of phenotypic selection for *P. aphanidermatum* resistance was found to produce significant improvements in seven of the most resistant HT lines. Generation means analysis for *P. aphanidermatum* resistance showed that in the HT lines tested, additive genetic effects were by far the most important.

AKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. Smith and all the members of my committee for all of their guidance, assistance and especially their patience in helping me conduct my research. I would also like to thank at the Cotton Improvement Lab. Without their help and friendship this research would not have been possible.

I would like to thank my parents, brothers, grandparents and my friends all of whom have helped to make me the person I am, and without whom I would never have made it this far. Lastly, I would like to thank my wife, Beth, who is always there for me no matter what and who every day helps me to be a better person.

TABLE OF CONTENTS

	Page
ABSTRACT.....	iii
ACKNOWLEDGEMENTS.....	v
TABLE OF CONTENTS.....	vi
LIST OF TABLES.....	ix
1. INTRODUCTION AND REFERENCES.....	1
1.1 Bacterial Blight: <i>Xanthomonas axonopodis</i> pv <i>malvacearum</i>	1
1.2 Seedling Disease: <i>Pythium aphanidermatum</i> and <i>Rhizoctonia solani</i>	3
1.3 Tannin.....	8
1.4 High Tannin (HT) Cotton Germplasm Lines.....	12
2. RESEARCH OBJECTIVES.....	14
3. MATERIALS AND METHODS.....	15
3.1. <i>Xanthomonas axonopodis</i> pv <i>malvacearum</i>	15
3.1.1 <i>Xam</i> Resistance Screening.....	15
3.1.2 <i>Xam</i> Generation Means Analysis.....	16
3.1.3 <i>Xam</i> Diallel Analysis.....	17
3.1.4 Tannin Measurement with Reference to <i>Xam</i> Resistance.....	17
3.1.4.1 Correlation of Tannin Content with Resistance and Disease Severity Across All HT Lines.....	18
3.1.4.2 Correlation of Tannin and Variation in Disease Severity within a Single HT Line.....	19
3.1.4.3 Correlation of Tannin Content with Time, Pathogen Inoculation and Disease Progression.....	19
3.2. <i>Rhizoctonia solani</i>	20
3.2.1 Tannin Measurement and <i>R. solani</i> Resistance.....	20
3.2.2 Progress from Selection for <i>R. solani</i> Resistance.....	21
3.2.3 Diallel Analysis for <i>R. solani</i> Resistance.....	22
3.3. <i>Pythium aphanidermatum</i>	22
3.3.1 Development of a More Efficient Method of <i>Pythium</i> Resistance Screening.....	22
3.3.2 Selection for <i>P. aphanidermatum</i> Resistance.....	23
3.3.3 <i>P. aphanidermatum</i> Generation Means Analysis.....	24
3.3.4 <i>P. aphanidermatum</i> Diallel Analysis.....	25

	Page
4. RESULTS AND DISCUSSION.....	26
4.1. <i>Xanthomonas axonopodis</i> pv <i>malvacearum</i>	26
4.1.1 <i>Xam</i> Resistance Screening.....	26
4.1.2 <i>Xam</i> Generation Means Analysis.....	26
4.1.3 <i>Xam</i> Diallel Analysis.....	28
4.1.4 Tannin Measurement with Reference to <i>Xam</i> Resistance.....	28
4.1.4.1 Correlation of Tannin Content with Resistance and Disease Severity Across All HT Lines.....	28
4.1.4.2 Correlation of Tannin and Variation in Disease Severity within a Single HT Line.....	29
4.1.4.3 Correlation of Tannin Content with Time, Pathogen Inoculation and Disease Progression.....	29
4.2. <i>Rhizoctonia solani</i>	30
4.2.1 Tannin Measurement and <i>R. solani</i> Resistance.....	30
4.2.2 Progress from Selection for <i>R. solani</i> Resistance.....	30
4.2.3 Diallel Analysis for <i>R. solani</i> Resistance.....	32
4.3. <i>Pythium aphanidermatum</i>	32
4.3.1 Development of a More Efficient Method of <i>Pythium</i> Resistance Screening.....	32
4.3.2 Selection for <i>P. aphanidermatum</i> Resistance.....	33
4.3.3 <i>P. aphanidermatum</i> Generation Means Analysis.....	34
4.3.4 <i>P. aphanidermatum</i> Diallel Analysis.....	35
5. CONCLUSIONS.....	37
REFERENCES.....	39
APPENDIX.....	48
VITA.....	68

LIST OF TABLES

TABLE	Page
<p>1. ANOVA for <i>Xam</i> resistance of HT lines as well as the resistant and susceptible controls. Seedlings were inoculated with two race mixtures of <i>Xam</i> and each was assigned a resistance score based on its reaction to both of the race mixtures. Experiments were performed in growth chambers at 30°C. Seedlings were established in March, April, May and June of 2007.....</p>	48
<p>2. Mean percent resistance of HT lines and controls to four races of <i>Xam</i> applied as two race mixtures. Means were separated using Fisher's LSD at $p = .05$. Experiments were performed in growth chambers at 30°C. Seedlings were established in March, April, May and June of 2007.....</p>	49
<p>3. Estimates of gene effects for <i>Xam</i> resistance. Resistance scores were between one and four and corresponded to the number of races of <i>Xam</i> each line was resistant to. Seedlings were established in the growth chamber between December 2009 and April 2010.....</p>	50
<p>4. Analysis of variance of diallel crosses for <i>Xam</i> resistance among five HT lines. 8 replications of 24 F2 seedlings were grown in a growth chamber at 30C in 2010. Plants were rated on a scale of 1-3, with 1 being the most susceptible and 3 being the most resistant.....</p>	51
<p>5. Estimates of general combining ability (GCA) effects of five HT lines for resistance to <i>Xam</i>. Estimates with different letters indicate that the effects are different at an LSD of .05. 8 replications of 24 F2 seedlings were established in the growth chamber between December 2009 and April 2010. Plants were given resistance scores of 1-3 with 1 being the most susceptible and 3 being the most resistant.....</p>	52
<p>6. ANOVA for the effect of genotype and replications on tannin content at 7 days post infection across all HT lines. Three replications of three seedlings for each of the HT lines were established in the growth chamber in May 2008.....</p>	53
<p>7. ANOVA for the effect of inoculation, time and disease progression on tannin content. Seedlings of TAM 86 DD 16 were established in March of 2010 in a growth chamber. Tannin content was measured in inoculated and uninoculated seedlings over a period of six days.....</p>	54

TABLE

Page

8. ANOVA for the effects of genotype on tannin content in *R. solani* infected hypocotyls. Tannin measurements were taken from hypocotyl tissue bordering developing *R. solani* lesions. Three replications of three plants each for each HT line were established in the growth chamber in September and October of 2007.....55
9. ANOVA for the effects of pedigree, selection and replication on lines selected for *R. solani* resistance. Two replications of 60 plants each were established in May through August of 2007 in a growth chamber and percentage survival was measured.....56
10. Mean separation (LSD = .05) for C₀, C₁ and C₂ lines selected for resistance to *R. solani*. While not included in the ANOVA the mean of Tamcot SP21 has been added for comparison purposes. Two replications of 60 seedlings each were established in May through August of 2007.....57
11. Analysis of variance of diallel crosses for *R. solani* resistance among seven HT lines. Plants were rate on a 1-3 scale, with 1= most susceptible and 3 = to the most resistant. Eight replications of twenty four seedlings each were grown in a growth chamber at 27°C in 2010.....59
12. Estimates of general combining ability (GCA) effects of seven HT lines for resistance to *R. solani*. Estimates with different letters indicate that the effects are different at an LSD of .05. Plants were rate on a 1-3 scale, with 1= most susceptible and 3 = to the most resistant. Eight replications of twenty four seedlings each were grown in a growth chamber at 27C in 2010.....60
13. Analysis of variance for the effects of genotype, replication and method used for *Pythium* resistance screening. The mean percentage survival of eight genotypes was measured after infection by *P. aphanidermatum* using the Howell method and the dry method using 200 ml of water per 10cm petri dish of inoculum. 3 replications of 50 seeds per genotype were grown in an incubator in September of 2007 in College Station.....61
14. Mean percentage survival of eight genotypes after infection by *P. aphanidermatum* using the Howell method and the dry method using 200 ml of water per 10cm petri dish of inoculum. 3 replications of 50 seeds per genotype were grown in an incubator in September of 2007 in College Station.....62

TABLE

Page

15. ANOVA for percent survival after 1 cycle of selection for resistance to *P. aphanidermatum*. Data are from the C₀ and C₁ generations. 3 replications of 72 seeds of each genotype were performed in an incubator at 30°C in November and December of 2009.....63
16. LSD at a level of .05 for mean percent survival of C₀ and C₁ lines selected for resistance to *P. aphanidermatum*. The resistant and susceptible controls, while omitted from the ANOVA, were grown along with the C₀ and C₁ lines and their data has been included here for comparison. 3 replications of 72 per genotype were performed in an incubator at 30° C in November and December of 2009.....64
17. Estimates of gene effects for *P. aphanidermatum* resistance. Eight replications were performed and seedlings were established in the growth chamber between December 2009 and April 2010. Population sizes per replication were 6 for both parents and the F1, 12 for both backcrosses and 24 for the F2.....65
18. Analysis of variance of diallel crosses for *P. aphanidermatum* resistance among seven HT lines. Percentage survival was measured for 8 replications of 24 F2 seeds were grown in an incubator at 30°C in 2010.....66
19. Estimates of general combining ability (GCA) effects of seven HT lines for resistance to *P. aphanidermatum*. Estimates with different letters indicate that the effects are different at an LSD of .05. 8 replications of 24 seeds per genotype were established in an incubator between December 2009 and April 2010.....67

1. INTRODUCTION AND REFERENCES

1.1 Bacterial Blight: *Xanthomonas axonopodis* pv *malvacearum*

The causal organism of bacterial blight in cotton is *Xanthomonas axonopodis* pv *malvacearum* (Smith). The original name of this bacteria was *Psuedomonas malvacearum* which was first changed to *Bacterium malvaceraum*, then to *Xanthomonas malvacearum* and then to *X. campestris* pv *malvacearum* before being changed to the current *X. axonopodis* pv. *malvacearum* (Xam). It is a gram negative bacteria and is classified as a non-fluorescent pseudomonad. The first description of the disease was by Atkinson in 1891 (Atkinson). *Xam* is known to affect all above ground parts of the plant, leading to a number of symptoms. These include angular leaf spot, vein blight, black arm, boll rot and plantlet burning. Symptoms start as water soaked lesions that later turn black, often with red or yellow margins. Severe infections can cause boll rot, defoliation and or discoloration of the lint.

The pathogen is favored by warm wet conditions. In the past, yield losses in the United States of up to 10% were not uncommon (Delannoy et al., 2005). Currently in the US, bacterial blight is controlled by the use of acid delinting, which eliminates early season inoculum occurring on the seeds themselves. Though it is not a problem in the US at the moment, bacterial blight can still cause yield losses of up to 35% in many parts of the world (Delannoy et al., 2005). Beyond using acid delinted seed, the main avenue of control has been resistant cultivars with heavy reliance upon the combination of the B₂ and B₃ resistance genes (Brinkerhoff et al., 1984).

Races of *Xam* were originally classified based on their ability to cause disease on a set of differential cotton genotypes. Nineteen races were recognized under this system with several additional highly virulent races being later identified in Africa. These highly virulent races are virulent on all of the differential host lines and are classified as races 20, 21 and 22 (Follin, 1983; and Delannoy et al., 2005). All documented resistance to *Xam* in cotton conforms to the gene-for gene model of resistance in which an avr protein originating in the pathogen is recognized by an R gene product in the plant that leads to resistance. In the case of *Xam*, it appears that resistance is conferred by the recognition of proteins produced by genes belonging to the avrBs3 family of genes. This has been confirmed by host cell death upon expression of avrBs3 gene products within the host cell. AvrBs3 genes were shown also to cause water and nutrient leakage from the cells of citrus, which is similar to the characteristic water soaked lesions seen with many *Xanthomonas* pathogens (Yang et al., 1996). This gene family encodes proteins that are unique to *Xanthomonas* (Leach and White, 1997). AvrBs3 proteins are injected into the host cell via type 3 secretion systems (Buttner and Bonas, 2003). There are multiple copies of avrBs3 genes in the *Xam* genome and the number of copies present tends to be correlated with the level of virulence in any specific race. Highly virulent races of the pathogen tend to have fewer copies as there are few targets for R genes to recognize (Chakrabarty et al., 1997).

Resistance to *Xam* in cotton is through the hypersensitive response. In a susceptible plant, an expanding water soaked lesion forms and tissue death does not occur for a week or more. Membranes and organelles degenerate and the intercellular space fills with bacteria. In resistant plants exhibiting a hypersensitive response, infected tissue collapse, becomes necrotic and is desiccated within two days. During this process, the plant produces a burst of reactive oxygen compounds and begins to accumulate phytoalexins, which are low molecular weight

antimicrobial compounds produced by the plant. The resistant response is localized and results in a small bacteriostatic zone around the site of infection (Essenberg et al., 1979A; and Essenberg et al., 1979B).

Resistance to *Xam* varies across the *Gossypium* genus. *Gossypium arboreum* and *G. herbaceum* exhibit near immunity while *G. barbadense* is susceptible. Upland cotton, *G. hirsutum*, tends to show the widest variation in resistance, from highly susceptible to highly resistant (Delannoy et al., 2005). Eighteen genes or gene complexes have been identified that provide resistance to *Xam*. Collectively, these genes are known as B genes and no single gene provides resistance to all races of the pathogen. In almost all cases, B genes have been shown to be controlled by simple dominant gene action (Luckett, 1989; Delannoy et al., 2005; Haidar et al., 2007; Innes and Brown, 1974). As was stated earlier, the major resistance in commercial cultivars resulted from pyramiding the B₂ and B₃ genes. In addition to this pyramiding, the combinations of B₂ and B₆, as well as B₂, B₃, B_{9L} and B_{10L} combinations have been used to some extent in Africa, though neither combination is resistant to the highly virulent African isolates of the pathogen (Innes, 1974; and Delannoy et al., 2005). Although a number of resistance genes have been identified, in cotton only the B₂, B₃, B₆ (actually a group of four distinct QTL) and B₁₂ have been mapped. An additional B gene was identified that was localized to chromosome 14 (Rungis et al., 2002).

1.2 Seedling Disease: *Pythium aphanidermatum* and *Rhizoctonia solani*

Cotton seedling disease complex (CSDC) is caused by a number of different soil-borne, fungal, pathogens, including *Rhizoctonia solani* (Kuhn), *Pythium* sp., *Fusarium* sp. and *Thielaviopsis basicola* (Berk. & Broome). These pathogens can cause a variety of symptoms such

as seed rot, pre-emergence damping-off, post-emergence damping-off, discolored roots, and dark lesions on the hypocotyl. Surviving plants often have weak, shallow root systems that leave plants susceptible to drought, secondary disease infection, and various other stresses. Infection can lead to thin, uneven stands of slow growing plants that yield poorly. Seedling disease caused an estimated yield loss of 3.3% in 2002 in the United States, which corresponds to over \$140,000,000 in lost farm income (Blasingame and Patel, 2003). Of the various pathogens that cause seedling disease, *Pythium* spp. and *R. solani* tend to be the most damaging (Garber et al., 1996).

Diseases caused by *Pythium* spp. are the single largest cause of pre-emergence damping off in cotton (Garber et al., 1996). There are approximately 120 species in the genus *Pythium* (Martin and Loper, 1999). It tends to infect through the roots tips, feeder roots, and most commonly, seedlings prior to or just after germination. *Pythium* spp. most often causes seed rot and pre-emergence damping off, though it can cause post-emergence damping-off, stunting, and chlorosis in later stages of plant development. Symptoms include watery, straw colored lesions and the rotting of root cortical tissue, leaving the central vascular stele of the tap root intact.

Pythium is an oomycete in the order Peronosporales. Oospores are the sexual spores of *Pythium* spp. They are the principle inoculum and also the life stage that survives unfavorable conditions (Agrios, 1997). Oospores possess thick walls and are able to survive long periods of soil desiccation (Martin and Loper, 1999). Dormancy in oospores is constitutive. Germination can be induced by the presence seed and root exudates (Howell 2002). Upon germination, the oospore will either infect the plant directly by means of a germ tube or indirectly by forming sporangia. The sporangia produce zoospores which are mobile in water and can infect plant

tissue. After infection occurs, oogonia can be fertilized by antheridia to produce dormant oospores (Martin and Loper, 1999).

Two of the more important *Pythium* spp. that cause seedling disease in cotton are *P. ultimum* (Trow) and *P. aphanidermatum* (Edson). *Pythium ultimum* tends to be a problem when conditions are cool. *Pythium aphanidermatum* is favored by warm temperatures and tends to be most prevalent in areas where soil temperatures in March and April are higher (DeVay et al., 1977). Both species are favored by moist soil conditions as moisture increases the distance traveled by root exudates and the mobility of zoospores. Increased soil moisture also leads to increased carbon dioxide concentrations, which increases the susceptibility of host tissue as well as reduces competition from other soil microorganisms, both of which give a competitive advantage to *Pythium* spp. Application of glyphosate as well as other herbicides has been shown to increase disease caused by *Pythium* spp. by increasing the amount of various exudates given off by the roots (Pankey et al., 2005 and Liu, 1997).

Planting high quality seed and not planting when conditions are favorable for disease development reduces losses due to *Pythium* spp. but the primary method of control is seed treatment with metalaxyl (Howell, 2007). Metalaxyl is a systemic, benzenoid fungicide specific to oomycetes (Thomson, 1997). There are also a number of different microorganisms that have been shown to be effective in the biocontrol of *Pythium* spp.

Though metalaxyl provides effective control, resistant cultivars, if available, could provide effective control without the added cost of fungicide treatments. There have been several studies that have tested a limited number of cultivars but many of these studies focused mainly or solely on *P. ultimum*. Garber et al. (1991) tested eight cultivars for *P. ultimum*

resistance but none produced adequate stands without fungicide treatment. Wang and Davis (1997) tested the resistance of 12 cultivars to *P. ultimum* and found two cultivars, Prema and Deltapine 6166, that had stand survival rates of 90% and 95%. Howell (2002) performed what might be the most comprehensive resistance screen, growing 22 cultivars in test tubes in soil naturally infested with both *P. ultimum* and *P. aphanidermatum*. Resistance was shown to vary greatly among the various cultivars with Tamcot Sphinx, Paymaster 1220 BG/RR, Paymaster 1244 RR and Deltapine 20B having survival rates of greater than 90%.

In addition to identifying new sources of *Pythium* resistance, it would be desirable to develop a quicker and easier screening procedure. The current accepted method for *Pythium* resistance screening is the method used by Howell (2002), which produces accurate results but is time consuming to the point of severely limiting the number of seedlings any one person can screen at one time. This method involves adding soil wetted with inoculum to a test tube, adding a seed and then adding more wetted soil. The major problem associated with this method is that it relies on using soil recently wetted with inoculum. Soil could be dried and stored for later use, but oospore germination is greatly affected by both aging and desiccation (Martin and Loper, 1999). Stored inoculum would have to be tested for oospore germination rates to make sure that a consistent concentration of viable inoculum. Wetted soil is difficult to measure due to the fact that wet soil can vary in compaction, whereas dry soil remains as individual particles. Aside from being difficult to measure, wet soil is hard to pour into test tubes. This is the single most time consuming step of the process since the wet soil must be forced through a funnel into the test tube. Since the seed is essentially inoculated as soon as it is added to the test tube, all steps must be completed in a short time span for accurate comparisons. A more ideal method would be adding dry soil to the test container, planting the seeds, and then adding inoculum at

convenience. This method would be preferable since less labor is required to screen a large number of plants or plant genotypes.

Rhizoctonia solani is the most common cause of post-emergence damping-off of cotton seedlings throughout the world (National Cotton Council of America, 2000). The pathogen causes a girdling lesion on the hypocotyl at or near soil level. Surviving plants are left weakened and often bare a canker, referred to as soreshin, from the lesion. *Rhizoctonia solani* is capable of infection at soil temperatures between 18-33°C and tends to be favored by warmer weather (Arndt, 1943). Infection is favored by moderately moist soil conditions, though unlike *Pythium* spp., high soil moisture can inhibit *R. solani* due to reduced oxygen availability (Johnson et al., 1969).

Rhizoctonia solani is the asexual stage of a basidiomycete fungus, *Thanatephorus cucumeris* (Frank). It does not produce any asexual spores, or conidia, and only occasionally produces sexual spores. In nature, *R. solani* reproduces asexually and exists primarily as vegetative mycelium and or sclerotia (Ceresini et al., 1999). Isolates of *R. solani* are classified based on a system known as anastomosis groups. The hyphae of members of the same anastomosis group are capable of fusion, unlike members of different groups. *R. solani* isolates capable of causing disease in cotton belong to anastomosis group 4 (Anderson, 1982).

Losses due to *R. solani* can be minimized by using high quality seed and planting when conditions are not favorable for the pathogen (Howell, 2007). *R. solani* can be effectively controlled by seed treatment with a variety of fungicides. While various fungicides will provide adequate control, products containing PCNB and/or iprodione tend to be the most effective (North Carolina Agricultural Chemicals Manual, 2010).

Resistant cultivars could provide effective control without the need for added chemical treatments. Unfortunately, while several studies have identified a number of lines with moderate resistance, no cultivars have been found that possess a high enough level of resistance to allow for the elimination of fungicide treatments. Hefner (1968) identified a single breeding line that had good emergence and survival when planted in soils infested with *R. solani*. Several studies have examined the Multiple Adversity Resistance (MAR) lines for *R. solani* resistance and while significant differences among lines have been found, no line has been identified as highly resistant (Bush et al., 1978 and Wallace et al., 1983). Wang and Davis (1997) tested 12 different cultivars for their resistance to *R. solani* and while they were able to identify significant differences, no cultivar had a final survival rate of over 57%.

1.3 Tannin

Tannins are astringent, bitter-tasting plant polyphenols that bind and precipitate proteins. The term tannin originated from the leather industry and was used to describe substances that were capable of turning animal hide into leather; however, the term is applied widely to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Tannins have molecular weights ranging from 500 to over 3,000.

Tannins fall into two categories: hydrolysable and condensed. Hydrolysable tannins, upon heating and the addition of hydrochloric or sulfuric acid, yield gallic or ellagic acids. As the name suggests, hydrolysable tannins are able to be hydrolyzed in the presence of weak acids or weak bases to produce carbohydrates and phenolic acids. Condensed tannins also are referred to as proanthocyanidins but upon heating and the addition of hydrochloric acid yield

phlobaphenes. Condensed tannins are made up of flavonoid polymers linked by carbon to carbon bonds, and while many are water soluble, they cannot be broken down by hydrolysis. (Bate-Smith and Swain, 1962). Condensed tannins tend to be far more abundant within plant tissues (Cowan, 1999).

Tannins are synthesized within the endoplasmic reticulum, accumulating in numerous small vacuoles, which then coalesce to form a much larger central vacuole. Tannins generally are located in vacuoles within cells of the endodermis and hypodermis and within scattered parenchyma cells of other tissues. Tannin concentrations greater than 20% of dry weight can occur in cotton in the buds, young leaves, young bolls, and young bracts (Bell, 1988).

The main function of tannin is to provide protection from herbivores, insects, and pathogen attack (Dixon et al., 2005). Swain (1979) considered tannins to be the most important class of secondary plant metabolites involved in plant defense against insects and disease. It is thought that tannin's antimicrobial mode of action is through nonspecific binding by way of hydrogen bonds, hydrophobic effects, and covalent bonding to various macromolecules. Because of such bonding, tannins are able to inactivate enzymes and transport proteins, disrupt microbial adhesion, and various other factors critical to disease development (Cowan, 1999). Another anti-microbial property of tannins is their ability to bind metals through complexes involving their o-diphenol groups (Dixon et al., 2005). Iron bioavailability can have a strong impact on microbial growth (House, 1999) and it has been suggested that tannins are capable of binding iron to a degree that limits bacterial growth (Scalbert, 1991). This is further supported by the fact that *Pseudomonas fluorescens*, which produces iron binding siderophores to aid in iron uptake, is one of the few microorganisms that has been shown to grow with tannin as its sole carbon source (Basaraba and Starkey, 1966).

Scalbert (1991), noted that tannin had been shown to be inhibitory to no less than 33 different microorganisms, including various bacteria, yeast, and filamentous fungi. Tannin synthesis in cotton increases greatly upon infection by *Verticilium dahlia*. Tannin production occurs more rapidly and to a greater concentration in resistant cotton genotypes, and tannin has been shown to have a strong antibiotic and somewhat weaker antispore effect against *V. dahlia*. Cotton cultivars released through the MAR program at Texas A&M University AgriLife Research, which were selected for resistance to several diseases, contain higher concentrations of tannins than most non-MAR lines, with increased concentrations in successive MAR germplasm releases (Bell, 1988). This increase in tannins occurred despite no direct selection for tannins, which could indicate that tannins are an important part of cotton disease resistance.

Tannin has been shown to be strongly correlated with resistance to *Pythium*, *Xam* and *R. solani*. Kantar et al. (1996) showed that seed coats in *Vicia faba* cultivars containing high levels of condensed tannin presented a significant barrier to *Pythium* when compared with low tannin cultivars. Pierce and Essenberg (1987) showed that mesophyll cells isolated from infected cotyledons had elevated concentrations of tannin as well as various other phenolics and phytoalexins. Donnelly (1983) observed that low tannin *Lespedeza cuneata* cultivars have greater susceptibility to *R. solani* when compared to high tannin cultivars and that disease severity was correlated with condensed tannin concentration.

Tannin is thought to suppress *R. solani* through the inactivation of polygalacturonase as well as other enzymes important in pathogenesis. Hunter (1974) showed that catechin, a flavan-3-ol that is a tannin precursor, oxidized by peroxidase extracted from healthy plants, inhibited polygalacturonase activity, a key enzyme involved in infection by *R. solani*. Un-oxidized catechin does not suppress polygalacturonase (Byrd et al., 1960). Condensed tannins in cotton

are polymers of catechin and gallo catechin with lesser concentrations of epicatechin and epigallocatechin as well as other flavan-3-ols. The ratio of catechin to gallo catechin varies from 4:1 to 1:1. Catechin and condensed tannin in general, when oxidized are converted to melanins, which are a characteristicly dark and somewhat reddish brown. The characteristic dark brown lesions associated with *Rhizoctonia*, is thought to be due to this oxidation of catechin and tannins into melanins (Bell et al., 1992).

Tannin concentrations increase as the plant ages from six to 12 days old and greatly increase 24 h after inoculation with *Rhizoctonia*, with greater post-infection increases in older plants (Hunter, 1974). When a concentration of catechin equivalent to what would be found in a 14-day old seedling is added to the growth media, growth of *Rhizoctonia* is strongly inhibited, while the concentration found in a five-day old plant has little effect (Hunter, 1978), suggesting that the increase in resistance seen as the plant ages could be due to tannin production.

Further evidence of the role of tannins in disease resistance, specifically resistance to seed-rot and pre-emergence damping off caused by *Pythium* sp., is seen in the manner in which pathogens infect the seed prior to germination. Infection in almost all cases occurs through the chalaza, which unlike the rest of the seed coat, is low in tannins and is permeable. The pathogen then infects the inner layer of the seed coat, which is also low in tannins. The nucellus, which is high in tannins, acts as a significant barrier to further infection, so much so that infection of the embryo can only take place in the end proximal to the chalaza, which contains a much lower tannin concentration (Hallowin, 1982).

Despite the various lines of evidence showing tannin's importance in disease resistance, modern cotton breeders generally have ignored condensed tannin as a source of resistance to

disease. This is most likely due to the fact that tannin has been shown to have a negative effect on the quality of cottonseed meal (Bell et al., 2010) but the levels of condensed tannins in cottonseed meal can be reduced by heat treatment which also has the beneficial effect of decreasing gossypol content (Yu et al., 1993). It appears that given the importance of tannin in cotton defense responses, this family of chemistry should not be ignored as a possible source of resistance.

1.4 High Tannin (HT) Cotton Germplasm Lines

The HT lines used in this research were from the 37 HT lines released by Smith et al. (1990a and 1990b) and Schuster et al. (1990) that exhibited elevated levels of condensed tannins in mature leaves as indicated by the HCl butanol assay. These lines were developed with the idea that an increase in tannin level might be a feeding deterrent to the bollworm complex, although later research showed that while condensed tannin slowed growth of bollworm it did not deter feeding (Navon et al., 1993). In addition to having been selected for high tannin levels based on the above plant analysis, the HT lines also were selected for resistance to two-spotted spider mite, *Tetranychus urticae* (Koch).

The HT lines have been previously evaluated for their resistance to *R. solani* and *P. aphanidermatum* (Kennett, 2009). No HT line was more resistant to *R. solani* than the resistant control, Tamcot SP21, although selected progeny from four HT lines were more resistant than their respective unselected HT parental population. This suggests that while there were no HT lines that possessed strong resistance to *R. solani*, selectable variation in the level of resistance exists within certain HT lines. This variation could be due to out-crossing between lines (open pollinated seed was used for all experiments), a lack of selection for tannins in the early stages of

growth in the development of the parental lines, or simply residual heterogeneity since cotton strains are rarely homozygous due to selection and maintenance procedures. This suggests that the HT lines may be useful in breeding for *R. solani* resistance.

Fifteen HT lines expressed resistance to *P. aphanidermatum* equal to the resistant control, Tamcot Sphinx. Tamcot Sphinx has been shown to have 100% resistance to *Pythium* in the field (Howell, 2002) and thus the HT lines may be a potent source of resistance to *P. aphanidermatum*.

Despite the fact that the only common factors shared by all the HT lines is elevated condensed tannin levels and some pedigree relationships, seed tannin content of uninfected seeds was not correlated with *P. aphanidermatum* resistance. This does not mean that there is no relationship between tannin and level of resistance. It is possible that tannin is produced at a later developmental stage. It is also likely that tannin is produced in response to infection and is not present in large amounts prior to actual infection by the pathogen but post-infection tannin measurements are made difficult due to tissue breakdown by the pathogen.

2. RESEARCH OBJECTIVES

The objectives of this research were to further examine the potential use of a set of high tannin cotton germplasm lines for their resistance to *Xanthomonas axonopodis* pv *malvacearum*, *Rhizoctnia solani*, and *Pythium aphanidermatum*.

Specific objectives for *Xam* were: 1. screen HT lines for blight resistance; 2. measure tannin content with respect to resistance in all HT lines 7 days post-infection and as infection progresses to see if tannin changes in response to pathogen infection and within certain lines to see if disease severity might correlate with tannin level; and 3. evaluate the genetics of resistance and identify the most desirable HT breeding lines through diallel and generation means analysis.

Specific objectives for *R. solani* were: 1. perform a second cycle of recurrent selection for resistance and measure the gain from selection; 2. measure tannin content of infected hypocotyls, and 3. evaluate the genetics of resistance and identify the most desirable HT breeding lines through diallel analysis.

Specific objectives for *Pythium* were: 1. develop and perfect a quicker and more convenient screening procedure; 2. perform selection for *P. aphanidermatum* and measure gains from selection; and 3. evaluate the genetics of resistance and identify the most desirable HT breeding lines through diallel analysis of *P. aphanidermatum* resistance.

3. MATERIALS AND METHODS

3.1 *Xanthomonas axonopodis* pv *malvacearum*

3.1.1 *Xam* Resistance Screening

Xam cultures were verified as *Xanthomonas* by growth on XS media, at 35°C, and a gram stain. Pathogen race was verified using a set of differential cotton genotypes.

The HT lines were evaluated for their resistance to *Xam* races 1, 2, 11 and 12. Seeds of each HT line were grown in peat pellets in a growth chamber at 30°C with 12 hours of light per day under high humidity growth conditions. Plants were grown for 7 days at which point the cotyledons were fully expanded. Seedlings were then inoculated with two different race mixtures of *Xam* using the toothpick method (Bird, 1986). Races 1 and 2 were combined as were 11 and 12. One inoculation was performed per cotyledon. Plants were grown for an additional 7 days and misted twice daily with water. After 7 days, disease reaction was assessed. Five replications of ten plants each were grown with Tamcot Sphinx and Pima S6 as the resistant and susceptible check, respectively. Seedlings were established in March, April, May and June of 2007. Each seedling was scored as 100% resistant if it was resistant to both race mixtures, 50% resistant if it was susceptible to one of the two race mixtures or 0% resistant if it was susceptible to both. The average resistance score per replication was then calculated. Data were subjected to analysis of variance using the General Linear Model procedure in SAS® and means were separated using Fisher's Protected LSD.

3.1.2 *Xam* Generation Means Analysis

Plants of five HT lines identified as most resistant to *Xam*, TAM 86 E 14, TAM 87 N 4, TAM 86 E 8, TAM 86 DD 18, and TAM 86 DD 16, and Tamcot Sphinx were crossed to a fully susceptible HT line, TAM 86 CC 17. The F₂ generation was developed by selfing the F₁ and the F₁ was backcrossed to each parent to produce the BC₁F₁a (crossed to the resistant parent) and BC₁F₁b (crossed to the susceptible parent) generations.

Seedlings of each generation, parental, F₁, F₂, BC₁F₁a and BC₁F₁b, were planted and inoculated with four separate *Xam* races, 1, 2, 11 and 18. Race 18 was used as opposed to race 12 because our original race 12 cultures was lost, and at some point mutated to race 18. By the time this was realized, there was no race 12 remaining in our culture. The switch to race 18 occurred shortly after the original screening procedure finished and was verified by a set of differential host cotton lines. While not planned, this did not impact the dissertation research because, in most cases, cotton genotypes susceptible to race 18 are susceptible to all other races. Reaction to each race was assessed 7 days post infection. Eight replications were performed with 6 seedlings of the parents and F₁, 12 seedlings of the backcrosses and 24 seedlings of the F₂. Seedlings were established at various dates between December 2009 and April of 2010. Seedlings were given rated using a 0-4 scale based on the number of races of *Xam* to which they were resistant to, with 4 being completely resistant and 0 being completely susceptible.

The individual scaling test (Mathers and Jinks, 1971) was used to test the assumption that generation means depend only on additive and dominance gene effects. This test uses the terms A, B, and C determined from the means of various generations and if all three terms are not

found to be significantly different from 0, a simple 3 parameter model consisting of additive (a) and dominance (d) gene action along with the mean effects (m), is said to be adequate.

Based on the results of the scaling test, the components of the 3 parameter model or a 6 parameter model were found using the methods used by Gamble (1962). The 6 parameter model consist of m , a , and d as defined for the three parameter model, plus the amount of variation among the means attributed to additive x additive epistasis (aa), the amount of variation among the means resulting from additive x dominance epistasis (ad), and the amount of variation among the means resulting from dominance x dominance epistasis (dd). The significance of these components was tested using a t test (Mathers and Jinks, 1971).

3.1.3 Xam Diallel Analysis

Plants of five HT lines identified as most resistant to *Xam*, TAM 86 E 14, TAM 87 N 4, TAM 86 E 8, TAM 86 DD 18, and TAM 86 DD 16, and one HT line identified as susceptible, TAM 86 CC 17, were crossed in all possible combinations to produce F_1 seed. F_2 seed was then generated.

Diallel analysis was restricted to using the F_2 generation. Seedlings were grown and inoculated with four separate races, 1, 2, 11, and 18. Disease reaction was assessed 7 days post infection. Eight replications of 24 seeds of each F_2 were grown. Data were analyzed using DIALLEL-SAS (Zhang and Kang, 1997).

3.1.4 Tannin Measurement with Reference to Xam Resistance

Seedlings were inoculated in a similar manner as was described for the screening procedure except that plants were inoculated with a mixture of all four races in an effort to

maximize disease response. Tannin was measured in infected cotyledons since tannin content has been shown to increase in response to disease infection. Leaf discs, 5 mm in diameter, were taken from uninfected portions of infected cotyledons. Each leaf disc was weighed and placed in a screw cap vial containing 5 ml of extracting solution consisting of 5% HCl and 95% butanol by volume. The closed vials were then placed in a 98° C water bath for 1 hour, and then refrigerated (3 +/-1° C) overnight. Absorbance of the supernatant at 550 nm was measured the following day using a Spectronic 20™ Colorimeter. Absorbance readings were converted to tannin content in g kg⁻¹ fresh weight using the following equation:

$$\text{Tannin} = \{[(\text{optical density at 550 nm})(5 \text{ ml reagent per vial})(1/\text{sample wt.})]/240\} \times 10 \text{ (Lege et al., 1992).}$$

Data were subjected to analysis of variance using the General Linear Model procedure in SAS® to determine significant differences among entries and means were separated using Fisher's Protected LSD. Tannin concentration was correlated with *Xam* resistance scores using PROC CORR in SAS®.

3.1.4.1 Correlation of Tannin Content with Resistance and Disease Severity Across All HT Lines

Several different experiments were performed in order to better examine any potential relationship between tannin and disease resistance. In the first experiment, tannin was measured across all lines and the controls and then correlated with the level of resistance present in each line. Three replications consisting of three leaf discs for each HT line and the controls were tested. Disease severity was assessed in an attempt to better correlate tannin with the level of disease. Disease severity was assessed on a scale of 1-5, with 1 being no symptoms and the

wound healed normally, 2 = no lesion but poor healing, 3 = minor lesions, 4 = large lesions, and 5 indicated large lesions that expanded well away from the initial inoculation site. Each replication consisted of three plants per entry.

3.1.4.2 Correlation of Tannin and Variation in Disease Severity within a Single HT Line

A second set of experiments were performed in order to examine the relationship between tannin and disease severity within lines. It is possible that tannin might not be the determining factor in resistance but may be essential in slowing disease progression. Symptoms within specific HT lines tend to be variable and differences in disease severity within lines could be due to differences in environment, tissue age, or, most likely, heterogeneity. There is a certain amount of heterogeneity present within the HT lines since they were originally derived from F3 lines and because these lines have been maintained using open pollinated increase blocks. While environment and developmental stage are assumed to be constant, they are both factors that strongly influence tannin production and could therefore influence disease severity.

For this set of experiments measuring tannin content within lines, TAM 86 E 9 was used because of its observed variation in the level of disease severity within reps. Three replications each containing 40 plants of TAM 86 E 9 were established in the growth chamber in May of 2008. Disease ratings were similar to those stated previously with the exception that a category 6 was added. Plants rated 6 exhibited lesions on uninoculated portions of leaves.

3.1.4.3 Correlation of Tannin Content with Time, Pathogen Inoculation and Disease Progression

The third set of experiments examined tannin production over the course of infection. Tannin content was measured in a single line, TAM 86 DD 16, which was chosen because it had

previously shown superior but not complete resistance. This seemed to be more in line with what might be expected from resistance due to an induced chemical response, such as tannin production in response to infection. One hundred and fifty plants were established with 100 being inoculated and 50 remaining uninoculated. The reason for there being more inoculated plants is that extras were needed to ensure that individuals with representative disease progression for the entire population of plants could be selected for each day. Tannin was measured in eight plants per day on days 0, 2, 4 and 6 post inoculation for both the inoculated and uninoculated plants.

For all three tannin measurement experiments, seedlings were established in January through March of 2010 in a growth chamber at 30°C.

3.2. *Rhizoctonia solani*

3.2.1 Tannin Measurement and *R. solani* Resistance

Seeds were grown in peat pellets in a growth chamber at 27° C for three days, followed by inoculation with 4 ml of *R. solani* (strain J1) inoculum (obtained from C. Howell, USDA-ARS at College Station, TX in 2004). Inoculum was prepared by growing a 0.5 cm diameter plug of *R. Solani* on potato dextrose agar (PDA) in a 6 cm petri dish for three days at 25° C. Media consisted of 15 g of PDA and 15 mg of rifampicin liter⁻¹ of water. After three days, 100 ml of reverse osmosis (RO) water was added to each plate, followed by maceration in a blender for 30 sec. Four ml of inoculum solution was pipetted onto each emerged seedling. Visual symptoms appeared by the third day and measurements were taken at four days post inoculation. Since *R. solani* destroys the tissue that would need to be examined, measurements need to be taken after symptoms have developed but before the hypocotyls are damaged beyond use for accurate

tannin measurements. A 1 cm segment of each hypocotyl was cut from the region bordering the developing lesion. Hypocotyl segments were weighed and tannin content determined as described above. Three replications of three plants each were evaluated in September and October of 2007. Data were subjected to analysis of variance using the General Linear Model procedure in SAS® to determine significant differences among entries and means were separated using Fisher's Protected LSD. Tannin concentration was correlated with *R. solani* resistance scores using PROC CORR in SAS®.

3.2.2 Progress from Selection for *R. solani* Resistance

Two rounds of recurrent selection were performed on four HT lines (C_0), TAM 86 J 1, TAM 86 III 11, TAM 86 III 11 and TAM 86 III 24, resulting in 14 C_1 and 35 C_2 lines. For each cycle of selection, plants were grown and inoculated as described above for tannin measurement except that 2.5 plates of inoculum were used per 100 ml of water. The first cycle of selection was made from fifty inoculated C_0 plants and the second cycle was made from one hundred inoculated C_1 plants. Surviving plants were grown to maturity and selfed to give rise to the subsequent generation. To measure the gain from selection, plants of each generation along with the resistant control, Tamcot SP21, were grown and inoculated as previously described with a single 6 cm plate of *R. solani* used per 100 ml of water. Disease reaction was assessed 7 days post inoculation. Two replications of 60 plants each were established of each genotype and each cycle. Plants were established in May and August of 2007. Progress from selection was analyzed using the General Linear Model procedure in SAS and means were separated using Fisher's Protected LSD.

3.2.3 Diallel Analysis for *R. solani* Resistance

Seven C₂ lines identified as numerically the most resistant in the gain from selection experiment, plus the susceptible parent, TAM 96WD-18, were crossed in all possible combinations. Plants used as parents for the diallel analysis were obtained from the surviving plants of the second replication of the gain from selection experiment. F₁ seeds obtained from these crosses were grown under greenhouse culture to produce F₂ seed. F₂ seeds were grown and inoculated as previously described with a single 6 cm culture of *R. solani* being used per 100 ml of water. Disease reaction was assessed seven days post-inoculation. Plants were rated 1-3 with 3 = a healthy upright plant, 2 = a plant that has lodged due to a lesion but is still turgid and expected to survive, and 1 = a dead or clearly dying plant. Experimental units were 24 plants in each of 8 replications. Data was analyzed using DIALLEL-SAS (Zhang and Kang, 1997).

3.3. *Pythium aphanidermatum*

3.3.1 Development of a More Efficient Method of *Pythium* Resistance Screening

P. aphanidermatum cultures were obtained from C. Howell, USDA-ARS at College Station, TX in 2004.

The ideal time to add inoculum would either be immediately after the seed is added to dry soil as discussed above. This would allow a significant portion of the experiment to be set up ahead of time. This method will be referred to as the dry method. In the Howell method, one mycelia mat of a 10-day old culture of *P. aphanidermatum* is mixed with 100 ml of RO water and macerated in a Waring™ 12 volt two speed blender on high for 30 sec. *P. aphanidermatum* cultures were grown on 25 ml of liquid V8/cholesterol media consisting of 177 ml of V8 juice, 823 ml of water, 3 g of CaCO₃, 2 ml of a 0.3 g ml⁻¹ cholesterol in ethanol solution and 15 mg of

rifampicin. This mixture is then applied to 1 kg of a soil mixture containing 60% field soil and 40% sand. Five grams of the inoculated soil mixture was added to a test tube, one seed is added, 5 grams of inoculated soil is added and finally 1 ml of water is added. The dry method involves preparing identical inoculum and then adding the soil to the test tube, then the seed, inoculum, soil and then water. The amount of each ingredient per test tube remains the same but the assembly is different. The small difference represents a significant savings in time.

To test this new dry method, seed of Tamcot Sphinx (resistant), SureGrow747 (SG 747)(highly susceptible), two HT lines showing elevated resistance, TAM III 26 and TAM N 6; two HT line showing moderate resistance, TAM III 11 and TAM DD 12; and two lines showing high susceptibility, TAM E 4 and TAM III 31, were planted according to both the Howell method and the dry method. Several inoculum concentrations for the dry method were used in order to obtain a rate of survival similar to that of the Howell method, these were 1 mycelia mat per 100 ml, 200 ml and 400ml of water. One ml of the prepared inoculum was added for each treatment in the dry method. Three replications of 25 seeds each were completed in September of 2007 and the rate of survival was measured. Data were subjected to analysis of variance using the General Linear Model procedure in SAS® to determine differences among treatments and means were separated using Fisher's Protected LSD. The inoculum concentration giving results most closely resembling the Howell method will be used as the standard or 1X inoculum concentration.

3.3.2 Selection for *P. aphanidermatum* Resistance

Seven HT lines possessing a high degree of resistance to *P. aphanidermatum*, TAM 86 E 8, TAM 86 III 11, TAM 86 III 26, TAM 87 N 4, TAM 87 N 5, TAM 87 N 6 and TAM 87 N 7,

were inoculated with twice the standard concentration of inoculum (2X) and grown as described in the dry method above. Surviving plants were grown to maturity and seed (C_1) were obtained.

Generation C_0 and C_1 seeds were grown along with seed from Tamcot Sphinx and SG 747 and inoculated with 2X inoculum and grown according to the dry method described earlier. After 7 days the number of surviving plants was counted. Three replications of 72 plants each were performed. Progress from one cycle of selection was analyzed using General Linear Model procedure in SAS® and means were separated using Fisher's Protected LSD.

3.3.3 *P. aphanidermatum* Generation Means Analysis

C_0 seed from the HT lines TAM 86 E 8, TAM 86 III 11, TAM 86 III 26, TAM 87 N 4, TAM 87 N 5, TAM 87 N 6 and TAM 87 N 7, were crossed to a susceptible control, SG 747, to obtain F_1 seed, which were grown under selfing conditions in a greenhouse and produced F_2 seed and were utilized as parents to produce backcrosses to each parent.

Seedlings of the parental, F_1 , F_2 , BC_1F_1a and BC_1F_1b generations were planted and inoculated with the 1X concentration of inoculum and grown according to the dry method. Reaction to *P. aphanidermatum* was assessed seven days after planting. Eight replications were performed with six seedlings of the parents and F_1 , 12 seedlings of the backcrosses and 24 seedlings of the F_2 per experimental treatment arranged in a randomized complete block design in a growth chamber.

The individual scaling test (Mathers and Jinks, 1971) was used to test the assumption that generation means depend only on additive and dominance gene effects. Based on the results of the scaling test, the components of the 3 parameter model or a 6 parameter model were found

using the methods used by Gamble (1962) and the significance of these components was tested using a t test (Mathers and Jinks, 1971).

3.3.4 *P. aphanidermatum* Diallel Analysis

C₀ seed from the seven HT lines used in the selection experiment, i.e., TAM 86 E 8, TAM 86 III 11, TAM 86 III 26, TAM 87 N 4, TAM 87 N 5, TAM 87 N 6 and TAM 87 N 7, and SG 747 were crossed in all possible combinations to produce F₁ seed. F₂ seed was then generated.

F₂ seedlings were grown and inoculated with 1X inoculum and grown according to the dry method. 8 replications of 24 seeds of each F₂ were grown. Data was analyzed using DIALLEL-SAS (Zhang and Kang, 1997).

4. RESULTS AND DISCUSSION

4.1. *Xanthomonas axonopodis* pv *malvacearum*

4.1.1 *Xam* Resistance Screening

Tamcot Sphinx was 100% resistant while Pima S6 was 0% resistant as was expected. The HT lines differed significantly in their level of resistance to *Xam* races used in this study. (Table 1). The HT lines varied in their resistance to *Xam* ranging from 100% of the plants tested being resistant for TAM 86 E 14 and TAM 87 N 4, to just 13% resistant for TAM 86 CC 17 (Table 2). The combination of races of the pathogen used as well as the interaction of the race and the specific HT line being test were both significant at $p < 0.0001$ and $p < 0.0008$, respectively. This suggests that resistance is race specific, which would fit with the accepted gene for gene model of resistance (Delannoy et al., 2005).

Four HT lines, TAM 86 E 14, TAM 87 N 4, TAM 86 E 8, and TAM 87 N 3, were found to be not different from Tamcot Sphinx in their resistance to *Xam* (Table 2). Since there is no overlap in the pedigrees among the HT lines and all known sources of resistance to *Xam*, it is quite possible that these lines represent new sources of resistance to the disease. These lines could prove quite useful in future efforts to breed for resistance to *Xam*.

4.1.2 *Xam* Generation Means Analysis

The scaling test indicated that in three of the lines tested, TAM 86 E 8, TAM 87 N 4, and TAM 86 DD 16, a three parameter model was adequate to explain the gene action of *Xam* resistance indicating that epistatic interactions are not involved in the inheritance of resistance for these lines when crossed with TAM 86 CC 17 (Table 3). The remaining three, TAM 86 E

14, TAM 86 DD 18, and Tamcot Sphinx, required a six parameter model indicating that epistatic interactions are important in the inheritance of resistance for these traits. Among these five highly resistant HT experimental lines, only additive gene action was significant. Dominant gene action was significant for Tamcot Sphinx when crossed with the highly susceptible HT line as was additive x dominant and dominant x dominant effects. These significant interactions suggest dominance effects at heterozygous loci with alleles contributed by both parents, suggesting that the HT line and Tamcot Sphinx carries different resistance genes. If one assumes that the *Xam* resistance in the HT lines is a new source and its resistance genotype is therefore different than that of Tamcot Sphinx, then this result might be expected since the susceptible parent used in this study. TAM 86 E 8, TAM 86 DD 16 and TAM 86 DD 18, having additive gene action significant at a .05 level of probability and TAM 87 N 4 and TAM 86 E 14 having additive gene action significant at a .01 probability level. The significant negative additive x dominance in TAM 86 E 14 and TAM 86 DD 18 and significant negative dominance x dominance gene effect with Tamcot Sphinx also suggest different alleles for resistance

Further evidence that the HT lines represent new sources of resistance to *Xam* can be deduced from these data since Tamcot Sphinx was the only genotype to show significant dominant gene action as well as dominant x dominant epistasis. This fits with the gene for gene model of resistance, which is to be expected since Tamcot Sphinx carries the B₂, B₃, and B₆, resistance genes (Bird, 1982). The negative dominant x dominant interaction is to be expected as there is redundancy in the resistance provided by this gene combination. The fact that additive type gene action is of primary importance in the HT lines as opposed to the primarily dominant gene action seen in Tamcot Sphinx supports the hypothesis that the resistance conferred by the

HT lines is different than what has been previously described since all previously described resistance is due to dominant gene action.

4.1.3 *Xam* Diallel Analysis

Analysis of variance found entry to be significant ($p < 0.0001$) and replications to be non-significant (Table 4). The estimates for general combining ability (GCA) for the five HT lines tested are given in Table 5. TAM 86 E 14 and TAM 87 N 4 gave the highest GCA values, which were both significant at probability levels of < 0.0001 . TAM 86 DD 18 and TAM 86 DD 16 gave non-significant GCA's and TAM 86 E 8 gave a slightly negative though significant GCA. An LSD at a p-level of .05 was able to differentiate the five GCA values into four groups. These results indicate that TAM 86 E 14 and TAM 86 87 N 4 would be the most useful lines in breeding for resistance to *Xam*. These results are in agreement with those of the generation means analysis as these two lines produced the most significant genetic effects.

4.1.4 Tannin Measurement in Reference to *Xam* Resistance

4.1.4.1 Correlation of Tannin Content with Resistance and Disease Severity Across All HT Lines

Tannin concentration varied ($p < 0.001$) across genotypes but not across replications in an exploratory evaluation of tannin content in 2008 (Table 6). A Pearson's correlation analysis indicated that disease severity and tannin content were negatively related ($p = 0.012$) with an r value of -0.23 , that is, as disease severity increases, average tannin content decreases.

Resistance, as a percentage of plants within each HT line showing no symptoms, on the other hand did not show a significant relationship with tannin content. The small but significant relationship between tannin content and disease severity with a lack of significant relationship

between resistance and tannin content suggests that tannin may only play a secondary role in disease resistance.

4.1.4.2 Correlation of Tannin and Variation in Disease Severity within a Single HT Line

No relationship ($p < 0.05$) was found between tannin content and within line variation in disease severity for the resistance of TAM 86 E 9. The Pearson's correlation was a non-significant ($p = .72$) value of .034. This is not particularly surprising given the previous finding that tannin most likely only plays a minor role in *Xam* resistance.

4.1.4.3 Correlation of Tannin Content with Time, Pathogen Inoculation and Disease Progression

In this experiment to show the effects of time and disease progression, time was the only factor found to be significant (Table 7), with a p-value of .018. This too is not surprising since tannin content has been shown to increase as the plant grows and matures (Bell, 1988). Both disease progression and infection, i.e., whether or not a plant was inoculated with the pathogen, were found to be non-significant. The lack of relationship between pathogen infection and tannin content further suggests that tannin content is not the determining factor in conferring resistance to *Xam* in the HT lines, although, despite the number of tests that have been done in this study relating to tannin content and *Xam* infection and resistance, it still cannot be ruled out. Pierce and Essenberg (1987) found the highest concentration of tannin in infected leaf cells and in those cells immediately surrounding the infection. Leaf tissue used in this study was from uninfected portions of infected leaves. The decision to use uninfected tissue was made because of the difficulty in quantifying the number of infected cells in any one leaf sample. *Xam* lesions, as with any pathogen infection, tend to be irregular, and obtaining a consistent number of

infected cells would be nearly impossible using available methods. Even in the Pierce and Essenberg study (1987), tannin levels were not actually quantified but compared subjectively on a per cell basis using the fluorescence due to tannin. They only were able to give relative quantities of tannin in various cells of infected leaves and not give detailed comparisons across lines. Thus, with the number of tests in this study to link tannin content and resistance, it is unlikely that tannin content plays a significant role in conferring resistance to *Xam*.

4.2. *Rhizoctonia solani*

4.2.1 Tannin Measurement and *R. solani* Resistance

Genotypes, HT lines plus controls, varied ($p < 0.01$) according to the analysis of variance in their response to infection by *R. solani*. (Table 8). However, no significant association as indicated by Pearson correlation was found between *R. solani* resistance and tannin content. The value of the correlation was .025 and was non-significant with a p value of .80. These data follow the pattern reported above for *Xam* and suggests that tannin content as a poor predictor of resistance. While it has been previously stated that the characteristic dark brown color of *R. solani* lesions is likely due to oxidized tannin (Bell et al., 1992), the presence of tannin may serve only to prevent secondary infection by opportunistic pathogens that would not normally be able to infect in the absence of such a wound.

4.2.2 Progress from Selection for *R. solani* Resistance

Pedigree (the original parent population of each line designated C_0), selection, and replications varied ($p < 0.05$) in response to infection with *R. solani* (Table 9). The mean resistance scores along with LSD significance grouping ($p < 0.05$) are presented in Table 10. Lines starting with RC1 are from C_1 and lines beginning with RC2 are C_2 lines. C_1 lines were

derived from independent plant selections from within several HT lines (referred to here as C_0). C_2 lines were derived from independent plant selections from within C_1 lines. The progenitor for each line is shown in the pedigree column. Forty-two lines, either C_0 , C_1 , C_2 , were not different ($p < 0.05$) than the resistant check, Tamcot Sp21 (Table 10). Of these twenty-four HT lines, or selections thereof, are in the top LSD significance grouping, which also contains Tamcot SP 21. Of these 21 lines, 18 were from C_2 and 3 were from C_1 . The fact that simple phenotypic selection for resistance could provide such gains is probably due to the wild type parent in the pedigree and the fact that the HT lines were derived from single F_3 plants during a standard pedigree program. This means that there should have been 12.5% residual heterozygosity in each of the F_3 plants selected, which could lead to heterogeneity within each HT line. A lesser, but still viable, source of variation could have come from outcrossing during the strain evaluation and strain maintenance phases. Since their original release, the seed of these lines used for these experiments has gone through several generations of open pollinated seed increase in the field. While out-crossing rates in cotton are much lower than in a cross pollinated crop, cross pollination does occur, which would lead to an increase in variation. Many of these lines are segregating for a number of easily observable phenotypic traits such as glandlessness, nectariless, plant architecture and pubescence. Several lines are even segregating for a green color in the lint. With this level of variation, it is not surprising that gains in resistance were accomplished. Also since tannin levels and resistance to *R. solani* appear to not be correlated, there would not have even been any inadvertent selection for *R. solani* resistance in the original development of these lines.

Even though none of these lines shown immunity to *R. solani*, the levels of resistance present, could prove very useful in breeding for resistance to the pathogen. As was stated earlier,

all cotton germplasm that has yet been examined shows relatively high susceptibility to *R. solani*. Using only two cycles simple recurrent selection for resistance lines showing resistances of up to 92% resistance were produced, suggesting great potential for the use of the HT lines in future efforts for breeding for *R. solani* resistance.

4.2.3 Diallel Analysis for *R. solani* Resistance

Analysis of variance showed both entry and replication to be significant (Table 11). Estimates for the GCA of seven C₂ HT *R. solani* resistant lines are presented in Table 12. Three Recurrent Cycle 2 selections used to estimate the GCA for *R. solani* resistance produced GCA estimates ($p < 0.05$) that indicated that they could be used as parents in a breeding program designed to develop improved resistance. Selected lines RC 2-14, -16, -21 had GCA estimates ranging from 0.199 to 0.344. RC2-14 and RC2-16 were found to be 87 and 80 % resistant respectively (Table 12) and with their GCA estimates, these two lines would seem to be of the most use for breeding for *R. solani* resistance., at least among the lines tested. RC2-17 and 28 combined with these parents for increased levels of *R. solani* as indicated by negative and significant GCA for resistance.

4.3 *Pythium aphanidermatum*

4.3.1 Development of a More Efficient Method of Pythium Resistance Screening

In preliminary tests, the 100 ml and 400 ml of water per plate of inoculum concentrations appeared to differ considerably from the results generated by the Howell method while the 200 ml concentration gave nearly identical results. Because of this the 100 ml and 400 ml concentrations were eliminated and the number of seeds analyzed for the two remaining

treatments, the Howell method and the 200 ml concentration, was doubled to 3 reps of 50 seed each.

Genotypes differed ($p < 0.001$) in their response to *P. aphanidermatum* infection while no differences were observed among replications (Table 13). More importantly, the methods used were not different ($p = 0.7259$) when applied to these eight genotypes. The two methods generated nearly identical overall means of 46.75% survival for the Howell method vs 46.46% survival for the dry method (Table 14). The LSD at .05 for the two means is 1.43, which is much greater than the .25% difference between the two.

While the two procedures seem relatively similar, the dry method represents a considerable time savings. Using the dry method takes about half the amount of time as the Howell method plus a greater number of entries can be managed by a single person because one can prepare a meaningful portion of the experiment ahead of time.

4.3.2 Selection for *P. aphanidermatum* Resistance

The analysis of variance showed that both entry and selection were significant at probability levels of 0.01 and < 0.0001 , respectively (Table 15). As was seen with *R. solani*, there appears to be selectable variation present for resistance to this pathogen of cotton seedlings (Table 9). The data reported in Table 16 are more exciting relative to using a simple recurrent selection program for single plant selection for *P. aphanidermatum* resistance. All seven C_1 populations, created by selection for resistance, were more resistant than the population from which they were selected, the C_0 populations. The resistant and susceptible checks were not included in the selection analysis, but were grown and inoculated alongside the C_0 and C_1

populations for purposes of comparison. Tamcot Sphinx had a survival rate of 47% and SureGrow 747 had a survival rate of 3%.

These results show that the HT lines show great potential as sources for *P. aphanidermatum* resistance. Tamcot Sphinx has been demonstrated to be the cultivar that is most resistant to *Pythium spp.* in the field (Howell, 2002) and according to these results the selected HT lines show greater resistance to at least *P. aphanidermatum*. Since Tamcot Sphinx provides 100% resistance to *Pythium spp.* under field conditions, the HT lines may in fact be completely immune to *Pythium* attack under normal field conditions. While more research has to be done to measure the resistance of the HT lines to other species of *Pythium*, as well as their resistance under field conditions, these results are promising. With a single round of simple selection for resistant plants, the least resistant C₁ lines was still more resistant than the most resistant C₀ line and the resistant control. If this level of resistance under lab conditions translates well to field conditions, it could easily be enough to be able to eliminate the need for metalaxyl seed treatments. Also while *Pythium* resistance is poorly documented, there is no overlap between the HT lines and Tamcot Sphinx or any MAR program line for that matter, which indicates that this may be a unique source of resistance.

4.3.3 *P. aphanidermatum* Generation Means Analysis

With the exception of TAM 86 E 8, a three parameter model was adequate to explain the variation in the means among the six generations of the seven lines tested (Table 17), indicating the lack of importance of epistatic gene interactions in conferring resistance to *P. aphanidermatum* among these lines. TAM 86 E 8 required a six parameter model, with both additive gene action and additive x additive gene action being significant. The additive x

additive gene action is negative indicating that there may alleles from both parents contributing to resistance (Mathers and Jinks, 1971). While this seems unlikely, since SG747 is completely susceptible, there may be a gene or genes in SG747 that are only expressed when in a TAM 86 E 8 background. Additive gene effects were significant in all seven lines while dominance effects were not significant.

The importance of additive gene action and residual heterozygosity in the HT lines would explain the dramatic improvements seen after only a single generation of selection. In the case of additive gene action, individuals homozygous for resistance genes would be easy to select as opposed to dominant gene action where homozygotes and heterozygotes would have the same phenotype. This also indicates that *Pythium* resistance coming from the HT lines would be useful to breeders as it would be quicker to select for individuals where the resistance genes had already become fixed.

4.3.4 *P. aphanidermatum* Diallel Analysis

Analysis of variance showed both entry and replication to be significant (Table 18). Estimates for the GCA of seven HT *P. aphanidermatum* resistant lines are presented in Table 19. Three of the seven HT line used to estimate the GCA for *P. aphanidermatum* resistance produced positive GCA estimates but only two of those lines were significantly different from zero ($p < 0.05$). This indicates that these lines, TAM 86 E 8 and TAM 87 N 6 could be useful as parents in a breeding program designed to develop improved resistance. GCA estimates for the seven lines ranged from .096 for TAM 86 E 8 and TAM 87 N 6 to -.091 for TAM 87 N 5. TAM 86 E 8 and TAM 87 N 6 were found to be 70 and 78% resistant respectively (Kennett, 2009) and

with their GCA estimates, these two lines would seem to be of the most use for breeding for *P. aphanidermatum* resistance., at least among the lines tested.

5. CONCLUSIONS

As a result of this research the following conclusions have been formed:

1. Four HT lines, TAM 86 E 14 TAM 87 N 4 TAM 86 E 8 TAM 87 N 3, were found to be not different from Tamcot Sphinx in their resistance to *Xam*. Since there is no overlap in the pedigrees between the HT lines and all known sources of resistance to *Xam*, it is quite possible that these lines represent new sources of resistance to the disease.
2. Generation means analysis for *Xam* resistance showed that in the five HT lines tested additive gene effects were of the greatest importance. This is useful to breeders since having a high percentage of additive genetic increases selection efficiency.
3. Diallel analysis showed that of the lines tested, TAM 86 E 14 and TAM 86 87 N 4 had the highest GCA values and would therefore be the most effective lines to use in breeding for resistance to *Xam*.
4. While tannin content was shown to be negatively correlated to disease severity of *Xam*, no correlation between tannin content and *Xam* resistance was detected.
5. Under the conditions of this study tannin content was also shown to be unimportant in *R. solani* resistance.
6. Two rounds of recurrent phenotypic selection were effective in increasing the resistance of selected HT lines to *R. solani*.
7. Diallel analysis for *R. solani* resistance showed that of the 7 C₂ lines tested, RC2-14 and RC2-16 were found to have the highest GCA estimates and therefore would likely prove to be the most effective in breeding for *R. solani* resistance.

8. A modification of the procedure standard procedure for *Pythium* resistance screening using dry soil instead of wetted, preinoculated soil, was found to produce nearly identical results in substantially less time.
9. One cycle of phenotypic selection for *P. aphanidermatum* resistance was found to produce significant improvements in seven of the most resistant HT lines. C₁ lines were significantly more resistant than their parent C₀ lines.
10. Generation means analysis for *P. aphanidermatum* resistance showed that in the HT lines tested, additive genetic effects were by far the most important. With exception of TAM 86 E 8, which also had a significant additive by dominance effect, additive gene action was the only source of genetic variation shown to be significant. This further proves the usefulness of HT line in breeding for *P. aphanidermatum* resistance as selection efficiency is improved with higher percentages of the genetic variation being due to additive gene action.
11. Diallel analysis of seven HT lines showed significant differences for GCA with TAM 86 E 8 and TAM 87 N 6 both having GCA values of .096 ($p < .01$).

REFERENCES

- Agrios, G. N. 1997. Plant Pathology. Academic Press: London.
- Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. Ann. Rev. Phytopathology. 20:329-47
- Arndt, C. H. 1943. Temperature as a factor in the infection of cotton seedlings by ten pathogens. Plant Dis. Rptr. Supp. 246:63-84.
- Atkinson, G. F. 1891. The black rust of cotton. Ala. Agric. Exp. Stn. Bull. 27:1-16
- Basaraba, J., and R. Starkey. 1966. Effect of plant tannins on decomposition of organic substances. Soil Sci. 101:17-23.
- Bate-Smith, E. C., and T. Swain. 1962. Flavonoid compounds. Vol III. p. 75-809. In Florkin M. Mason H.S. (eds) Comparative biochemistry. Academic Press: New York.
- Bell, A. A. 1988. Developmental changes in the tannin content of cotton leaves: implications in breeding for pest resistance. p. 31. In Proc of Beltwide Cotton Prod. Res. Conf. New Orleans, LA. 3-8 Jan. 1988. Natl. Cotton Council of America, Memphis, TN.
- Bell, A. A., C. R. Howell, and R. D. Stipanovic. 2010. Cotton host-microbe interactions. Physiology of Cotton. 2010: 187- 205.
- Bell, A. A., K. M. El-Zik, and P. M. Thaxton. 1992. Chemistry, biological significance and genetic control of proanthocyanidins in cotton (*Gossypium* spp.). p. 571-595. In Hemingway R.M., Lacks P.E., (eds.) Plant Polyphenols. Plenum Press: New York.

- Bird, L. S. 1986. Half a century dynamics and control of cotton disease: bacterial blight. p. 24-33. *In Proc. Beltwide Cotton Prod. Res. Conf. Las Vegas, NV. 5-9 Jan, 1986.* Natl. Cotton Council of America, Memphis, TN.
- Bird, L. S. 1982. The MAR (multi-adversity resistance) system for genetic improvement of cotton. *Plant Dis.* 66: 172-176.
- Blasingame, D., and M. V. Patel. 2003. Cotton disease loss estimate committee report. p. 130-131. *In Proc Beltwide Cotton Prod. Res. Conf. New Orleans, LA. 6-10 Jan. 2003.* Natl. Cotton Council of America, Memphis, TN.
- Brinkerhoff, L. A., L. M. Verhalen, W. M. Johnson, M. Essenberg, and P. E. Richardson. 1984. Development of immunity to bacterial blight of cotton and its implications for other diseases. *Plant Dis.* 68:168-73.
- Bush, D. L., L. S. Bird, and F. M. Bourland. 1978. Variation in susceptibility to *Rhizoctonia solani* among MAR cotton cultivars. p. 21. *In Proc Beltwide Cotton Prod. Res. Conf. Dallas, TX. 6-11. Jan 1978.* Natl. Cotton Coun. Am., Memphis, TN.
- Buttner, D., and U. Bonas. 2003. Common infection strategies of plant and animal pathogenic bacteria. *Curr. Opin. Plant Biol.* 6:312-19.
- Byrd, R. J. W., A. H. Fielding, and A. H. Williams. 1960. The role of oxidized polyphenols in the varietal resistance of apples to brown rot. p. 95-99 *In J.B. Pridham (ed.). Phenolics in Plants in Health and Disease.* Pergamon Press: London.
- Ceresini, P. C., H. D. Shew, and M. A. Cubeta. 1999. RFLP analysis of the PCR amplified ribosomal DNA regions ITS and IGS indicated that isolates of *Rhizoctonia solani* from

- potato and tobacco represent distinct groups within the anastomosis group 3. *Phytopathology*. 89:12.
- Chakrabarty, P. K., Y. P. Duan, and D. W. Gabriel. 1997. Cloning and characterization of a member of the *Xanthomonas avr/pth* gene family that evades all commercially utilized cotton R genes in the United States. *Phytopathology*. 87:1160–67
- Chana, M. H. 1987. Sabatical program performance. p: 138-164. *In* Smith, C. W. (ed). Annual Report: Cotton Genetic and Breeding Investigations. Texas Agricultural Experiment Station Department of Soil and Crop Sciences: College Station.
- Cowan, M. M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*. Oct. 1999: 564-582.
- Delannoy, E., B. R. Lyon, P. Marmey, A. Jalloul, J. F. Daniel and J. L. Montillet. 2005. Resistance of cotton towards *Xanthomonas campestris* pv. *malvacearum*. *Annu Rev Phytopathol*. 43:63–82.
- DeVay, J.R., D. R. Matheron, and H. Garber. 1977. Survival and assay of *Pythium* species in samples of field soil. National Cotton Council, Memphis, TN, USA. *Proceedings Cotton Diseases Council*. 37: 21-22.
- Dixon, R. A., D. Y. Xie, and S. B. Sharma. 2005. Proanthocyanidins: a final frontier in flavonoid research? *New Phytol*. 165:9–28.
- Donnelly, E. D. 1983. Breeding low-tannin *Sericea*. I. Selection for resistance to *Rhizoctonia* ssp. *Crop Science*. 23:14-16.

Essenberg, M., E. T. Cason, B. Hamilton, L. A. Brinkerhoff, R. K. Gholson, and P. E.

Richardson. 1979(A). Single cell colonies of *Xanthomonas malvacearum* in susceptible and immune cotton leaves and the local resistant response to colonies in immune leaves. *Physiological Plant Pathology*. 15:53-68.

Essenberg, M., B. Hamilton, E. T. Cason, L. A. Brinkerhoff, R. K. Gholson, and P. E.

Richardson. 1979(B). Localized bacteriostasis indicated by water dispersal of colonies of *Xanthomonas malvacearum* within immune cotton leaves. *Physiological Plant Pathology*. 15:69-78.

Follin, J. C. 1983. Races of *Xanthomonas campestris* pv. *malvacearum* (Smith) Dye in Western and Central Africa. *Coton Fibres Trop*. 38:277-80.

Gamble, E. E. 1962. Gene effects in corn I. Separation and relative importance of gene effects for yield. *Can J Plant Sci*. 42:339-348.

Garber, R. H., J. E. DeVay, R. J. Wakeman, and R. Vargas. 1991. The role of cultivar tolerance in cotton seedling disease control. p. 163-165 *In Proc. Beltwide Cotton Prod. Res. Conf. National Cotton Council of America Memphis, TN.*

Garber, R.H., J.E. Devay, P.B. Goodell, and P.A. Roberts. 1996. Cotton diseases and nematodes. p. 156-165. *In S. H. Johnson et al. (ed) Cotton Production Manual Publ 3352 Div. of Agric. and Natural Resources, University of California, Davis.*

Haidar, S., I. A. Kahn, S. Mansoor, and Y. Zafar. 2007. Inheritance studies of bacterial blight disease resistance genes in cotton (*Gossypium hirsutum L.*). *Pak. J. Bot.* 39(2): 603-608, 2007.

- Halloin, J. M. 1982. Ripening of cottonseed. *The New Phytopathologist*. 90:651-657.
- Hefner J. 1968. Screening cotton for resistance to damping-off by *Rhizoctonia solani*. p164-165.
In Proc Beltwide Cotton Prod. Res. Conf., Hot Springs, AR. 9-10 Jan 1968. Natl. Cotton Council of America, Memphis, TN.
- House, W. A. 1999. Trace element bioavailability as exemplified by iron and zinc. *Field Crops Research*. 60: 115–141.
- Howell, C. R. 2002. Cotton seedling preemergence damping-off incited by *Rhizopus oryzae* and *Pythium* spp. and its biological control with *Trichoderma* spp. *Phytopathology*. 92:177–80.
- Howell, C. R. 2007. Effect of seed quality and combination fungicide–*Trichoderma* spp. seed treatments on pre- and postemergence damping-off in cotton. *Phytopathology*. 97:66-71.
- Hunter, R. E., L. A. Brinkerhoff, and L. S. Bird. 1978. Development of a set of upland cotton lines for differentiating races of *Xanthomonas malvacearum*. *Phytopathology*. 58:830–32.
- Hunter, R. E. 1974. Inactivation of pectic enzymes by polyphenols in cotton seedlings of different ages infected with *Rhizoctonia solani*. *Physiological Plant Pathology*. 4: 151–159.
- Innes, N., and S. J. Brown. 1974. Genetical and environmental variation for resistance to bacterial blight of upland cotton. *Heredity*. 32(1): 53-72.
- Innes, N. 1974. Resistance to bacterial blight of cotton varieties homozygous for combinations of B resistance genes. *Ann. Appl. Biol.* 78:89-98.

- Johnson, L. F., A. Y. Chambers, and J. W. Measells. 1969. Influence of soil moisture, temperature and planting date on the severity of cotton seedling blight. Tennessee Agricultural Experiment Station Bulletin. 461: 5-28.
- Kantar, F., P. D. Hebblewaite, and C. J. Pilbeam. 1996. Factors influencing disease resistance in high and low tannin *Vicia faba*. Journal of Agricultural Science. 127:83-88.
- Kennett, R. 2009. The evaluation of high tannin cotton lines for resistance to *Rhizoctonia solani* and *Pythium aphanidermatum*. Masters Thesis, Texas A & M University, College Station.
- Leach, J. E., and F. F. White. 1997. Avirulence genes. p. 61–98 In G Stacey, NT Keen (ed.) Plant-Microbe Interactions. Chapman & Hall: New York.
- Lege, K. E., C. W. Smith, and J. T. Cothren. 1992. Genotypic and cultural effects on condensed tannin concentration of cotton leaves. Crop Science. 32: 1024–1028.
- Lockett, D. 1989. Diallel analysis of yield components, fibre quality and bacterial blight resistance using spaced plants of cotton. Euphytica. 44 : 11-21, 1989.
- Liu, L. 1997. Altered root exudation and suppression of induced lignifications as mechanisms of predisposition by glyphosate of bean roots (*Phaseolus vulgaris* L.) to colonization by *Pythium spp.* Physiol. Mol. Plant Pathol. 51:110-127.
- Martin, F. N., and J. E. Loper. 1999. Soilborne plant diseases caused by *Pythium spp.*: Ecology, epidemiology, and prospects for biological control. Critical Reviews in Plant Sciences. 18: 21-111.
- Mather, K., and J. L. Jinks. 1971. Biometrical Genetics. Chapman & Hall: London.

- National Cotton Council of America. 2000. Cotton Seedling Disease Identification.
<http://www.cotton.org/tech/pest/seedling/identification.cfm>. Retrieved on Jan 5 2006.
- Navon, A., J. D. Hare, and B. A. Federici. 1993. Interactions among *Heliothis virescens* larvae, cotton condensed tannin and the CryIA(c) delta-endotoxin of *Bacillus thuringiensis*. *Journal of Chemical Ecology*. 19:2485–2499.
- North Carolina Agricultural Chemicals Manual. 2010. The College of Agriculture and Life Sciences, North Carolina State University: Raleigh.
- Pankey, J. H., J. L. Griffin, P. D. Colyer, R. W. Schneider, and D. K. Miller. 2005. Preemergence herbicide and glyphosate effects on seedling diseases in glyphosate-resistant cotton. *Weed Technology*. 19:312–318.
- Pierce, M., M. Essenberg. 1987. Localization of phytoalexins in fluorescent mesophyll cells isolated from bacterial blight-infected cotton cotyledons and separated from other cells by fluorescence-activated cell sorting. *Physiological and Molecular Plant Pathology*. 31:273-290.
- Rungis, D., D. Llewellyn, E. S. Dennis, B. R. Lyon. 2002. Investigation of chromosomal location of bacterial blight resistance gene in present in an Australian cotton cultivar. *Aust. J. Agric. Res.* 53:551-60.
- Scalbert, A. 1991. Antimicrobial properties of tannins. *Phytochemistry*. 30: 3875–3883.
- Schuster, M. F., C. W. Smith, and G. A. Niles. 1990. Registration of nine upland cotton germplasm lines having elevated levels of condensed tannins. *Crop Science*. 30:1375.

- Smith, C. W., M. F. Schuster, and G. A. Niles. 1990a. Registration of 11 upland cotton germplasm lines having elevated levels of condensed tannins. *Crop Science*. 30: 1374.
- Smith, C. W., M. F. Schuster and G. A. Niles. 1990b. Registration of 17 upland cotton germplasm lines having elevated levels of condensed tannins. *Crop Science*. 30: 1374–1375.
- Swain, T. 1979. Phenolics in the environment. p 624-637. *In* T.Swain, J. B. Harborne, C.F. Van Sumere (eds). *Recent Advances in Phytochemistry*. Vol 12. Plenum Press: New York.
- Thomson, W. T. 1997. *Agricultural Chemicals. Book IV—Fungicides*. W. T. Thomson, ed. Thomson Publications, Fresno, CA.
- Wallace, T. P., K. M. El-Zik, and L. S. Bird. 1983. Response of cotton cultivars to the seedling disease complex and stand establishment under two soil temperatures. p. 35. *In* Proc Beltwide Cotton Prod. Res. Conf. San Antonio, TX. 2-6 Jan 1983. Natl. Cotton Council of America, Memphis, TN.
- Wang, H., and R. M. Davis. 1997. Susceptibility of selected cotton cultivars to seedling disease pathogens and benefits of chemical seed treatments. *Plant Dis*. 81:1085–1088.
- Yang, Y., Q. Yuan, and D. W. Gabriel. 1996. Water soaking function(s) of *Xcm* H1005 are redundantly encoded by members of the *Xanthomonas* avr/pth gene family. *Mol. Plant Microbe Interact*. 9:105–13.
- Yu, F., T. N. Barry, P. J. Moughan, and G. F. Wilson. 1993. Condensed tannin and gossypol concentrations in cottonseed and in processed cottonseed meal. *Journal of the Science of Food and Agriculture*. 63: 7–15.

Zhang, Y., and M. S. Kang. 1997. DIALLEL-SAS: A SAS program for Griffing's diallel analyses. *Agron. J.* 89:176-182.

APPENDIX

Table 1: ANOVA for *Xam* resistance of HT lines as well as the resistant and susceptible controls. Seedlings were inoculated with two race mixtures of *Xam* and each was assigned a resistance score based on its reaction to both of the race mixtures. Experiments were performed in growth chambers at 30C. Seedlings were established in March, April, May and June of 2007.

Source	df	MS	F-value
Genotype	38	50.631	44.05***
Race	1	3.169	104.77***
Enrty*Race	38	2.287	1.99**
Rep	4	1.386	11.45***
Error	308	9.316	

** , *** Significant at .01 and < 0.001 respectively

Table 2: Mean percent resistance of HT lines and controls to four races of *Xam* applied as two race mixtures. Means were separated using Fisher's LSD at $p = .05$. Experiments were performed in growth chambers at 30C. Seedlings were established in March, April, May and June of 2007.

Genotype	Resistance	
TAM 86 E 14	100%	A
TAM 87 N 4	100%	A
Sphinx	100%	A
TAM 86 E 8	99%	A
TAM 87 N 3	95%	Ab
TAM 86 DD 16	93%	Bc
TAM 86 DD 18	93%	Bc
TAM 86 CC 11	83%	Cd
TAM 86 III 22	79%	De
TAM 86 J 1	79%	Def
TAM 86 CC 12	79%	Def
TAM 86 E 4	76%	Def
TAM 86 III 8	74%	Ef
TAM 86 DD 11	73%	Efg
TAM 86 E 7	72%	Efg
TAM 87 N 7	69%	Efg
TAM 86 CC 7	69%	Efgh
TAM 86 III 16	69%	Efgh
TAM 86 E 3	68%	Fgh
TAM 86 E 6	61%	Ghi
TAM 86 CC 13	61%	Ghi
TAM 86 III 11	56%	Hij
TAM 87 N 5	53%	Ijk
TAM 86 III 26	51%	Ijkl
TAM 86 III 24	50%	Ijkl
TAM 86 E 19	50%	Ijkl
TAM 86 CC 18	49%	Ijklm
TAM 86 III 7	48%	Ijklm
TAM 86 DD 12	47%	Ijklm
TAM 86 E 20	42%	Jklm
TAM 86 E 9	42%	Klmn
TAM 86 III 31	41%	Lmn
TAM 87 M 41	35%	Mno
TAM 87 N 6	34%	No
TAM 86 III 15	21%	O
TAM 86 DD 17	17%	P
TAM 87 M 48	16%	P
TAM 86 CC 17	13%	P
Pima S6	0%	Q

Table 3: Estimates of gene effects for *Xam* resistance. Resistance scores were between one and four and corresponded to the number of races of *Xam* each line was resistant to. Seedlings were established in the growth chamber between December 2009 and April 2010.

Genotype	<i>M</i>	<i>A</i>	<i>D</i>	<i>aa</i>	<i>ad</i>	<i>Dd</i>
TAM 86 E 8	2.87**	1.92*	2.60	-	-	-
TAM 87 N 4	3.33**	1.87**	0.14	-	-	-
TAM 86 DD 16	2.54**	0.69*	0.93	-	-	-
TAM 86 E 14	2.86*	1.28**	0.10	-1.74	-0.72*	3.72
TAM 86 DD 18	2.41*	0.88*	-0.35	-1.69	-1.12**	4.42
Tamcot Sphinx	3.63**	0.41	2.42*	0.57	-1.59**	-3.99*

*, ** Significant at the 0.05 and 0.01 probability level on the basis of t test with $n - 1 = 5$ degrees of freedom, respectively.

- indicates that the 3 parameter model was adequate to explain the 6 generation means

Table 4: Analysis of variance of diallel crosses for *Xam* resistance among five HT lines. 8 replications of 24 F₂ seedlings were grown in a growth chamber at 30C in 2010. Plants were rated on a scale of 1-3, with 1 being the most susceptible and 3 being the most resistant.

Source	df	MS	F	
Genotype	14	2.64	20.78	***
Replication	7	0.25	1.93	
Error	98	0.13		

*** Significant at < 0.001.

Table 5: Estimates of general combining ability (GCA) effects of five HT lines for resistance to Xam. Estimates with different letters indicate that the effects are different at an LSD of .05. 8 replications of 24 F₂ seedlings were established in the growth chamber between December 2009 and April 2010. Plants were given resistance scores of 1-3 with 1 being the most susceptible and 3 being the most resistant.

Genotype	Estimate	p-value
TAM 86 E 14	0.539a	<0.0001
TAM 87 N 4	0.536a	<0.0001
TAM 86 DD 18	0.165b	0.159
TAM 86 DD 16	0.009c	0.939
TAM 86 E 8	-0.252d	0.041

Table 6: ANOVA for the effect of genotype and replications on tannin content at 7 days post infection across all HT lines. Three replications of three seedlings for each of the HT lines were established in the growth chamber in May 2008.

Source	df	MS	F-value
Genotype	38	75.04	5.5***
Replication	2	4.33	0.32
Error	76	13.64	

*** Significant at < 0.001 .

Table 7: ANOVA for the effect of inoculation, time and disease progression on tannin content. Seedlings of TAM 86 DD 16 were established in March of 2010 in a growth chamber. Tannin content was measured in inoculated and uninoculated seedlings over a period of six days.

Source	df	MS	F-value
Infection	1	0.06	1.32
Time	3	0.18	3.60*
Disease Progression	1	0.051	1.03
Error	58	0.05	

* Significant at 0.05.

Table 8: ANOVA for the effects of genotype on tannin content in *R. solani* infected hypocotyls. Tannin measurements were taken from hypocotyl tissue bordering developing *R. solani* lesions. Three replications of three plants each for each HT line were established in the growth chamber in September and October of 2007.

Source	df	MS	F-value
Genotype	33	2.58	2.11**
Replication	2	1.16	0.95
Error	66	1.22	

** Significant at 0.01

Table9: ANOVA for the effects of pedigree, selection and replication on lines selected for *R. solani* resistance. Two replications of 60 plants each were established in May through August of 2007 in a growth chamber and percentage survival was measured.

Source	df	MS	F-value
Pedigree	3	0.088	3.42*
Selection	2	0.48	18.71***
Replication	1	8.4	327.42***
Error	99	0.026	

*, *** Significant at .05 and <0.0001 respectively

Table 10: Mean separation (LSD = .05) for C₀, C₁ and C₂ lines selected for resistance to *R. solani*. While not included in the ANOVA the mean of Tamcot SP21 has been added for comparison purposes. Two replications of 60 seedlings each were established in May through August of 2007.

Entry	Pedigree	Resistance	
RC2-17	RC1-10	0.92	a
RC2-21	RC1-10	0.88	ab
RC2-14	RC1-3	0.87	abc
RC2-23	RC1-11	0.83	abcd
RC2-16	RC1-10	0.80	abcde
RC2-28	RC1-5	0.77	abcde
Tamcot SP21		0.77	
RC2-18	RC1-10	0.75	abcdef
RC2-15	RC1-3	0.73	abcdef
RC2-34	RC1-4	0.72	abcdefg
RC2-19	RC1-10	0.70	abcdefgh
RC2-25	RC1-11	0.70	abcdefgh
RC2-30	RC1-1	0.70	abcdefgh
RC2-12	RC1-2	0.68	abcdefghi
RC2-26	RC1-11	0.65	abcdefghij
RC2-29	RC1-5	0.65	abcdefghij
RC2-2	RC1-12	0.63	abcdefghij
RC2-36	RC1-3	0.63	abcdefghij
RC1-4	TAM 86 III 16	0.63	abcdefghij
RC1-6	TAM 86 J 1	0.63	abcdefghij
RC2-3	RC1-13	0.62	abcdefghij
RC2-20	RC1-10	0.62	abcdefghij
RC2-38	RC1-8	0.62	abcdefghij
RC1-3	TAM 86 III 16	0.62	abcdefghij
RC1-5	TAM 86 J 1	0.62	abcdefghij
RC2-10	RC1-9	0.60	acdefghij
RC2-35	RC1-6	0.60	bcdefghij
RC2-13	RC1-3	0.58	bcdefghijk
RC2-27	RC1-5	0.58	bcdefghijk
RC2-32	RC1-2	0.58	bcdefghijk
RC1-1	TAM 86 III 16	0.58	bcdefghijk
RC2-7	RC1-14	0.58	bcdefghijk
RC2-31	RC1-2	0.57	cdefghijk
RC2-9	RC1-8	0.55	defghijkl
RC2-33	RC1-3	0.55	defghijkl
RC1-11	TAM 86 III 11	0.55	defghijkl
RC1-12	TAM 86 III 24	0.55	defghijkl
RC2-4	RC1-13	0.53	defghijkl
RC2-11	RC1-1	0.52	efghijkl
RC2-37	RC1-9	0.52	efghijkl
RC1-13	TAM 86 III 24	0.52	efghijkl
RC1-10	TAM 86 III 11	0.50	efghijkl
RC2-24	RC1-11	0.45	fghijkl
RC1-2	TAM 86 III 16	0.43	ghijkl

Continued on next page

Table 10 (continued)

Entry	Pedigree	Resistance	
TAM 86 J 1		0.42	hijkl
RC1-8	TAM 86 III 11	0.40	hijkl
RC2-6	RC1-14	0.40	hijkl
RC1-9	TAM 86 III 11	0.38	ijkl
RC2-5	RC1-14	0.37	jkl
RC1-14	TAM 86 III 24	0.35	jkl
TAM 86 III 16		0.28	kl
TAM 86 III 24		0.27	l
TAM 86 III 11		0.25	l
RC1-7	TAM 86 J 1	0.25	l

Table 11: Analysis of variance of diallel crosses for R. solani resistance among seven HT lines. Plants were rate on a 1-3 scale, with 1= most susceptible and 3 = to the most resistant. Eight replications of twenty four seedlings each were grown in a growth chamber at 27C in 2010.

Source	df	MS	F	
Entry	27	1.82	48.19	***
Replications	7	0.12	0.0042	**
Error	188	0.04		

** , *** Significant at .01 and < 0.001 respectively

Table 12: Estimates of general combining ability (GCA) effects of seven HT lines for resistance to *R. solani*. Estimates with different letters indicate that the effects are different at an LSD of .05. Plants were rate on a 1-3 scale, with 1= most susceptible and 3 = to the most resistant. Eight replications of twenty four seedlings each were grown in a growth chamber at 27C in 2010.

Genotype	Estimate		p-value
RC2-14	0.344	a	<.0001
RC2-16	0.319	a	<.0001
RC2-21	0.199	b	0.0036
RC2-18	0.103	c	0.083
RC2-23	0.102	c	0.132
RC2-28	-0.196	d	0.0011
RC2-17	-0.217	d	0.0015

Table 13: Analysis of variance for the effects of genotype, replication and method used for *Pythium* resistance screening. The mean percentage survival of eight genotypes was measured after infection by *P. aphanidermatum* using the Howell method and the dry method using 200 ml of water per 10cm petri dish of inoculum. 3 replications of 50 seeds per genotype were grown in an incubator in September of 2007 in College Station..

Source	df	MS	F-value
Genotype	7	5401	898.52***
Replication	2	16.75	0.12
Method	1	0.75	2.79
Error	37		

*** Significant at <0.0001

Table 14: Mean percentage survival of eight genotypes after infection by *P. aphanidermatum* using the Howell method and the dry method using 200 ml of water per 10cm petri dish of inoculum. 3 replications of 50 seeds per genotype were grown in an incubator in September of 2007 in College Station.

Genotype	Howell Method	Dry Method
Tamcot Sphinx	65.33	64
SureGrow 747	0	0
TAM III 26	80	78
TAM N 6	73.33	75
TAM III 11	54.66	51.33
TAM DD 12	60.66	63.33
TAM E 4	32	32
TAM III 31	8	8
Overall Mean	46.75	46.46

Table 15: ANOVA for percent survival after 1 cycle of selection for resistance to *P. aphanidermatum*. Data are from the C₀ and C₁ generations. 3 replications of 72 seeds of each genotype were performed in an incubator at 30°C in November and December of 2009.

Source	df	MS	F-value
Rep	2	61.15	.57
Selection	1	13094.53	121.19***
Genotype	6	344.65	3.20**
Error	32	108.04667	

** , *** Significant at .01 and < 0.001 respectively

Table 16: LSD at a level of .05 for mean percent survival of C₀ and C₁ lines selected for resistance to *P. aphanidermatum*. The resistant and susceptible controls, while omitted from the ANOVA, were grown along with the C₀ and C₁ lines and their data has been included here for comparison. 3 replications of 72 per genotype were performed in an incubator at 30 C in November and December of 2009.

Genotype	Percent Survival
TAM 87 N 7 C ₁	97.2% A
TAM 86 III 11 C ₁	89.8% ab
TAM 87 N 4 C ₁	81.5% bc
TAM 86 III 26 C ₁	81.0% bc
TAM 86 E 8 C ₁	78.7% Bcd
TAM 87 N 6 C ₁	78.7% Bcd
TAM 87 N 5 C ₁	68.5% D
TAM 87 N 7 C ₀	56.5% E
TAM 87 N 4 C ₀	51.9% Ef
TAM 87 N 6 C ₀	51.4% Ef
TAM 86 III 11 C ₀	50.0% Ef
Tamcot Sphinx	47.2%
TAM 86 III 26 C ₀	42.1% Fg
TAM 87 N 5 C ₀	41.7% Fg
TAM 86 E 8 C ₀	34.7% G
SG747	2.8%

Table 17: Estimates of gene effects for *P. aphanidermatum* resistance. Eight replications were preformed and seedlings were established in the growth chamber between December 2009 and April 2010. Population sizes per replication were 6 for both parents and the F₁, 12 for both backcrosses and 24 for the F₂.

Genotype	m	A	d	aa	ad	dd
TAM 86 E 8	0.94**	0.57**	-0.53	-1.07*	0.19	0.98
TAM 86 III 11	0.70**	0.63**	-0.94	-	-	-
TAM 86 III 26	0.62**	0.29*	0.32	-	-	-
TAM 87 N 4	0.82**	0.43*	-0.95	-	-	-
TAM 87 N 5	0.83**	0.42*	-0.72	-	-	-
TAM 87 N 6	0.83**	0.55*	-0.01	-	-	-
TAM 87 N 7	0.72**	0.41*	-0.08	-	-	-

*, ** Significant at the 0.05 and 0.01 probability level on the basis of t test with $n - 1 = 5$ degrees of freedom, respectively.

- indicates that the 3 parameter model fitted the 6 generation means

Table 18: Analysis of variance of diallel crosses for *P. aphanidermatum* resistance among seven HT lines. Percentage survival was measured for 8 replications of 24 F₂ seeds were grown in an incubator at 30C in 2010.

Source	df	MS	F	
Entry	27	0.065	7.09	***
Replications	7	0.068	7.46	***
Error	189	0.0092		

*** Significant at < 0.001

Table 19: Estimates of general combining ability (GCA) effects of seven HT lines for resistance to *P. aphanidermatum*. Estimates with different letters indicate that the effects are different at an LSD of .05. 8 replications of 24 seeds per genotype were established in an incubator between December 2009 and April 2010.

Genotype	GCA Estimate	p-value	
TAM 86 E 8	0.096	0.0075	a
TAM 87 N 6	0.096	0.0024	a
TAM 87 N 7	0.049	0.12	b
TAM 86 III 26	-0.002	0.94	c
TAM 86 III 11	-0.029	0.42	c
TAM 87 N 4	-0.065	0.07	d
TAM 87 N 5	-0.091	0.011	d

VITA

Name: Raymond Matthew Kennett

Address: TAMU 2474: Cotton Improvement Lab

College Station, Texas 77843-2474

Email Address: rkennett@ag.tamu.edu

Education: B.S., Plant Sciences, Cornell University, 2004

M.S., Plant Breeding, Texas A & M University, 2009

PhD, Plant Breeding, Texas A & M University, 2010