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Selection for resistance to pythium ultimum trow within four cultivars of cotton (Gossypium hirsutum L.)

Gary K. Palmer

To the Graduate Council:

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Leander F. Johnson, Major Professor

We have read this thesis and recommend its acceptance:

V. H. Reigh, M. R. McLaughlin

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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and recommend its acceptance:

M. R. McLaughlin

Vernon H. Reich

Accepted for the Council:

L. Evans Roth

Vice Chancellor
Graduate Studies and Research

SELECTION FOR RESISTANCE TO *PYTHIUM ULTIMUM* TROW. WITHIN FOUR CULTIVARS OF COTTON (*GOSSYPIUM HIRSUTUM* L.)

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Gary K. Palmer

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DEDICATION

This thesis is dedicated to Raymond Sutton, who made it possible for me to continue my education, to Dr. Charles Hadden who encouraged me to apply to the Department of Entomology and Plant Pathology, to my family whose encouragement and support is deeply appreciated, and to my fiancée, Rhonda Hux, whose faith in me and encouragement helped me immensely in my efforts.

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ABSTRACT

Four commercial cotton (Gossypium hirsutum L.) cultivars were chosen for an experiment involving selection for resistance to Pythium ultimum Trow. Two cultivars, Auburn M and Delcot 277J, previously had been found to be slightly more susceptible than Coker 310 and Dixie King 3. The potential for inheritance of resistance was based on the large variation that occurred among plants within the cultivars.

The relative susceptibility or resistance found previously was confirmed in a field test with the four cultivars. Pathogens were isolated with frequencies similar to that recorded in the literature.

Parent seeds of the four cultivars were planted in sterilized sand and grown at 27 C for eight days before being inoculated with P. ultimum. Inoculated seedlings were incubated at 18 C and rated for disease severity after seven days. Surviving seedlings of different ratings were transplanted to sterilized soil and grown to maturity. Plants were self-pollinated and progeny seeds obtained were planted in sterilized sand. Seedlings obtained were inoculated as before and rated for disease severity after seven days.

Heritability of each cultivar was calculated by three methods. Values obtained by the regression method were not different from zero at the 5% level of probability. Heritability values for Delcot 277J and Coker 310 were significantly different from zero at the 10% level. The partition of variance method was thought to contain too much error due to environmental effect and was included for comparison only. The realized heritability method was used to determine the response to one

generation of selection. From values obtained by this method, Auburn M could have the most potential of the four cultivars in a future breeding program. Values for realized heritability can be employed to predict future response to selection. Coker 310 met the criteria necessary for accurate predictions.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Seedling pathogens are responsible for significant annual reduction of cotton yields. From 1975 to 1979, losses averaged 2.68% nationally and ranged from 1.48% in 1977 to 3.64% in 1976 (5, 6, 18, 19, 20). In 1976 and 1977, estimated reductions in yields due to seedling disease were 373,778 and 356,562 bales respectively (5, 19).

In Tennessee, estimates of yield reduction caused by seedling disease are often considerably higher than the national average. The average loss during the five year period from 1975 - 1979 was 12.6% (5, 6, 18, 19, 20). The greatest loss occurred in 1976 with an estimated reduction in yield of 20% (18). In 1978 and 1979, losses were again high with an estimated 15% both years (5, 6). These losses are considered serious since they are reflected in monetary loss to cotton growers.

Fusarium spp., Rhizoctonia solani, and Pythium spp. are the most frequently isolated pathogens from diseased seedlings, although the isolation of Thielaviopsis basicola is becoming more frequent (26, 30). These as well as other species of fungi can cause a variety of disease symptoms referred to as the cotton seedling disease complex. Seed rot, pre-emergence damping-off, post-emergence damping-off, and stem canker (soreshin) make up the components of the seedling disease complex. The seedling disease complex is associated with low temperatures and high soil moisture levels which often occur during and/or following the normal cotton planting dates in Tennessee.

Control measures currently consist of fungicide seed treatment and in-furrow application of fungicides. Fungicide applications, however,

are often ineffective in controlling seedling disease. Although resistance to seedling disease is not present in commercial cotton cultivars, Johnson (24) demonstrated significant but small differences in resistance to infection by Pythium ultimum, a major pathogen in Tennessee. He indicated that lines of cotton with more resistance to Pythium spp. might be produced by selection and propagation of the more resistant plants. The objectives of this study were to determine if genetic advances in resistance to P. ultimum among four cultivars of Gossypium hirsutum L. were attainable with one generation of selection and if differences in genetic advance exist among individual plants in the four cultivars.

CHAPTER I

LITERATURE REVIEW

Seedling Disease Complex

Atkinson (3), in 1892, was the first researcher to publish a description of symptoms on cotton seedlings caused by Rhizoctonia solani. Since that time other workers have added several seed and seedling disease microorganisms to the list of pathogens. This list includes R. solani and Fusarium spp. as major causes of seed rot as well as weaker parasites of seeds such as Rhizopus spp. and Aspergillus spp. Colletotrichum gossypii and Xanthomonas malvacearum have also been implicated in seed rot. Rhizoctonia solani and Pythium spp. are most often the major causes of both pre and post-emergence damping-off. Thielaviopsis basicola, C. gossypii, Aschochyta gossypii, and other less important pathogens also may cause post-emergence damping-off. Rhizoctonia solani can also cause stem canker (soreshin) (2, 25, 27, 31, 36). Because of the many pathogens involved which can cause related but distinct effects on seedlings, the term "seedling disease complex" has been applied to this major cotton growing problem.

Seedling Disease in Tennessee

In Tennessee, over a seven year period (1962 - 1968), Fusarium spp. R. solani, and Pythium spp. were isolated most often from diseased seedlings (27). Either R. solani or Pythium spp. was isolated most frequently depending on the year. Both were considered to be major causes of seedling disease. A second study in Tennessee confirmed these results and identified the Pythium spp. as P. irregulare, P. sylvaticum, and P.

ultimum (26). However, in the second study, Thielaviopsis basicola was obtained in addition to the three genera previously mentioned. Since these studies, T. basicola has been found to cause seedling disease with increasing frequency (31).

Influence of Environmental Factors on Incidence of Seedling Disease

The occurrence of environmental factors associated with the early planting of cotton often favors the occurrence of seedling disease. Cool, wet weather stresses young, tender cotton seedlings and reduces natural resistance (27).

No significant correlation was found between isolation frequency of R. solani and soil temperature in Tennessee (27). However, in studies in Louisiana negative correlations were found (38). Isolation frequency of Pythium spp. is often highly negatively correlated with soil temperature. This is evident in the high frequency of isolation of Pythium spp. and low frequency of R. solani from cotton planted during April in Tennessee. The reverse occurs during May plantings (27).

The incidence of seedling disease is also correlated with soil moisture (27, 29). A decrease in stand under high soil moisture conditions often results. Frequency of Pythium spp. isolated from diseased seedlings was highly correlated with soil moisture, but a similar response was not found for R. solani. Poor stands can occur under very dry soil conditions as well as very wet conditions. Fusarium spp. are implicated as possible important seedling blight organisms under hot, dry conditions (27).

Chemical Control of Seedling Disease

One of the most common methods of controlling seedling disease has been the use of fungicides. The effectiveness is often low for seed rot and pre-emergence damping-off and little, if any, control is usually achieved for post-emergence damping-off (17, 30).

In recent years only slight progress has been made toward effective fungicide seed treatments (30). As cotton seedlings emerge and grow away from the area of first protection, very little protection is given against post-emergence damping-off.

In field evaluations of experimental fungicides Davis (21) noted the potential for development of effective fungicides. In greenhouse tests of fungicides against Pythium ultimum, Rhizoctonia solani, and Thielaviopsis basicola by DeVay, et al. (22), several fungicides had high fungicidal activity against seedling disease. A combination that appears to be especially promising for control of P. ultimum, R. solani, and T. basicola is a Ridomil, CGA 64251 mixture. Other combinations of fungicides appear to have potential as seed treatments.

Application Methods

When methods of fungicide application were compared, very little difference in effectiveness was noted. In some tests, however, application of granular formulations of several fungicides significantly increased stands when compared to in-furrow sprays (15, 17). Chambers (14) also noted slight improvement in seedling disease control in a five-year study of methods of applying soil fungicides in cotton.

Soil Solarization

One of the newest methods of controlling seedling disease is soil solarization, a process in which clear plastic tarps are used to induce solar heating of soil to temperatures lethal to soil-borne organisms. The effectiveness of this non-chemical method has been documented in several experiments. Enhanced growth response in cotton has been attributed to soil solarization (22, 34, 40). In an experiment by Pullman, et al. (35), seedling disease control in cotton was significantly improved for one year following growth of safflower on tarped soil.

Breeding for Resistance to Seedling Disease

Resistance as a means of disease control is more economical and more effective than chemical control measures. Niles (32) considered the advantages of crop plant resistance to consist of the following:

1. It stabilizes production levels and quality
2. Genetic resistance is relatively permanent.
3. It obviates the need for recurring pest control measures.
4. Genetic resistance against various pests may be additive.
5. Use of pest resistant cultivars reduces pesticidal contamination of the environment.

In 1895, a South Carolina grower began the first planned program of breeding for disease resistance in cotton. Since that time breeding cotton for resistance to seedling disease has progressed very slowly. However, researchers have detected possible resistance to the major seedling pathogens (9, 24, 30). Bird (13) found that selecting for seedling disease escape gave resistance to other disease as well as earliness and higher yield potential. He defined escape "to include all mechanisms, whether they result in reduced penetration, protection,

klenducity, or alterations of the environment creating an unfavorable host-pathogen interaction" (8).

Bird (7) proposed the hypothesis that multi-resistant genes exist in cotton which causes either resistance or escape from six major diseases. These genes influence other desirable agronomic characteristics as well. The term "adversity-multi-disease resistance," later shortened to "multi-adversity resistance," was given to the hypothesis. This hypothesis gives rise to the assumption that each step in genetic improvement will lead to cultivars possessing more resistance to major diseases. Bird (11) suggested that identification of several key traits involved in the system could permit direct selection for disease, insect, and stress resistance collectively.

Exudates and competitive microorganisms may be instrumental in multi-adversity resistance. The components of exudates and competitive organisms may favor yield and earliness while causing reduction in seedling disease severity and losses due to insects. Under consideration are plans to alter the microorganisms that comprise the natural exoflora and endoflora of root and other tissue to microorganisms which are antagonistic to pathogens and insects (12).

Sappenfield, et al. (37), conducted cotton breeding experiments in Missouri with three objectives: "(a) breeding for resistance to a single disease, (b) for multiple disease resistance, or (c) multi-adversity resistance." The major objectives in Missouri cotton improvement research as stated by Sappenfield, et al., reflect similar goals set by Bird (9, 10) for the Texas multi-adversity resistance program. This Trend toward development of multiple resistant cultivars may well become the goal of cotton breeding programs in general.

CHAPTER II

MATERIALS AND METHODS

Field Test

Production of Parent Seeds

Four cultivars of cotton were chosen based on a high degree of difference in Johnson's disease ratings relative to a reference cultivar, Stoneville 603 (24). Cultivars Coker 310 and Dixie King 3 appeared to be more resistant and cultivars Delcot 277 and Auburn M more susceptible than Stoneville 603. Delcot 277J was substituted for Delcot 277 due to the nonavailability of seeds of the latter.

Fungicide treated seeds of the four cultivars were obtained from P. E. Hoskinson, Associate Professor, Department of Plant and Soil Science, West Tennessee Experiment Station, Jackson, Tennessee. Seeds were planted five per pot in eight inch pots containing a sterile soil mix. After emergence, plants were thinned to one per pot. A total of thirty-one pots were planted per cultivar. Plants were self-pollinated by placing parchment corn tassel bags over squares prior to opening. Bags were secured with a staple. Cotton was collected and seeds were removed by hand, bulked by cultivar, and acid delinted according to the method of Andrews (1).

Germination Test

A test for viability of the seeds was made with a germination test suggested by Dr. M. A. Newman, Associate Professor, Department of Entomology and Plant Pathology, West Tennessee Experiment Station, Jackson,

Tennessee, and described by McNew (28). Random samples of fifty seeds of each cultivar were used in two replications. Seeds in each fifty-seed sample were placed approximately 3 cm apart and positioned between two sheets of germination paper saturated with distilled water. The two sheets were rolled loosely in a sheet of wax paper. Each end of the roll was secured loosely with a rubber band. Each roll was inserted into a plastic bag which was sealed with a rubber band to prevent evaporation. Two rolls of each cultivar were placed in each of two incubators, one maintained at 15 C and one at 25 C. After seven days, data on germination were recorded. The test was repeated.

Disease Severity

A preliminary field test was conducted at the West Tennessee Experiment Station with the aid of A. Y. Chambers, Associate Professor, The Department of Entomology and Plant Pathology, The University of Tennessee. Utilizing a Latin square design random samples of 167 selfed seeds of each of the four cultivars were planted in plots consisting of a single row 4.6 meters in length spaced 1 meter from adjacent plots. After four weeks, data were collected on emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off.

Isolation of Pathogens

Twenty-five live seedlings were collected from each plot, wrapped in a moistened paper towel, covered with wax paper to prevent desiccation, and placed in an ice chest for transporting to the laboratory in Knoxville. One Centimeter sections were cut from diseased hypocotyls. Hypocotyl sections from each plot were washed separately for three hours

in flowing tap water. After three hours a small amount of the non-ionic detergent, "Tween 20," was added and hypocotyls were washed in flowing tap water for thirty minutes. Individual hypocotyls were transferred through three sterile distilled water baths and placed on sterile filter paper to remove excess moisture. Each hypocotyl was placed in a petri plate containing water agar and 10 µg/ml of Aureomycin. Plates were examined three to six days later, and identifications of isolates made.

Selection for Resistance

Rating of Parent Plants

Cotton seeds were planted in sterile sand in four inch sterile plastic pots. Five seeds were planted per pot and incubated in a growth chamber at 27 C for eight days. Pots were arranged in random groups of four with each cultivar represented once per group. A total of twelve groups or forty-eight pots were treated in this manner.

Previous research indicated that resistance to Rhizoctonia solani and Pythium ultimum increased with the age of the seedling (23, 24). To reduce error that could occur due to this effect, seedlings emerging later than the sixth day were removed and discarded.

On the eighth day, hypocotyls of seedlings were inoculated with agar disks, 6 mm in diameter, cut from a five-day-old culture of Pythium ultimum Trow. The isolate of P. ultimum was obtained from Dr. L. F. Johnson, Professor, The University of Tennessee, Knoxville, Tennessee. To reduce injury to the hypocotyls, a hole was made adjacent to the hypocotyl with a stream of water from a plastic wash bottle. The disk was placed against the hypocotyl, and sand was pressed gently against the plant to cover and hold the disk in place. Inoculated plants were

incubated at 18 C in a growth chamber for seven days.

After seven days, each pot was inverted gently to remove plants and sand. The plants were suspended in water to allow gravity to gently separate the sand from the roots. Disease ratings were made as follows:

0 - No visible symptoms

1 - Small pin-point lesion

2 - Lesion less than 0.5 cm in length

3 - Lesion 0.5 cm or more in length

4 - Plant wilted

5 - Plant dead

A photograph illustrating examples of each category is contained in figure 1. Five repeated tests were made as described above. Seedlings with ratings of 0, 1, 2, and 3 were transplanted individually to sterilized soil in eight inch pots, grown to maturity in the greenhouse, and self-pollinated for futher genetic analysis. A maximum of twelve seedlings were transplanted for each cultivar and rating. Seedlings of each cultivar and rating were obtained from at least two repeated tests. The maximum number of seedlings for transplanting was obtained with all four cultivars with ratings of 1, 2, and 3. The numbers obtained for the 0 rating were, Auburn M, 3; Coker 310, 3; Delcot 277J, 2; and Dixie King 3, 5. Thus, the total number of seedlings transplanted to eight inch pots for selfing was 157. Identity of individual plants was maintained with respect to repeated test, position in growth chamber of pot from which obtained, cultivar, position in pot, and rating.

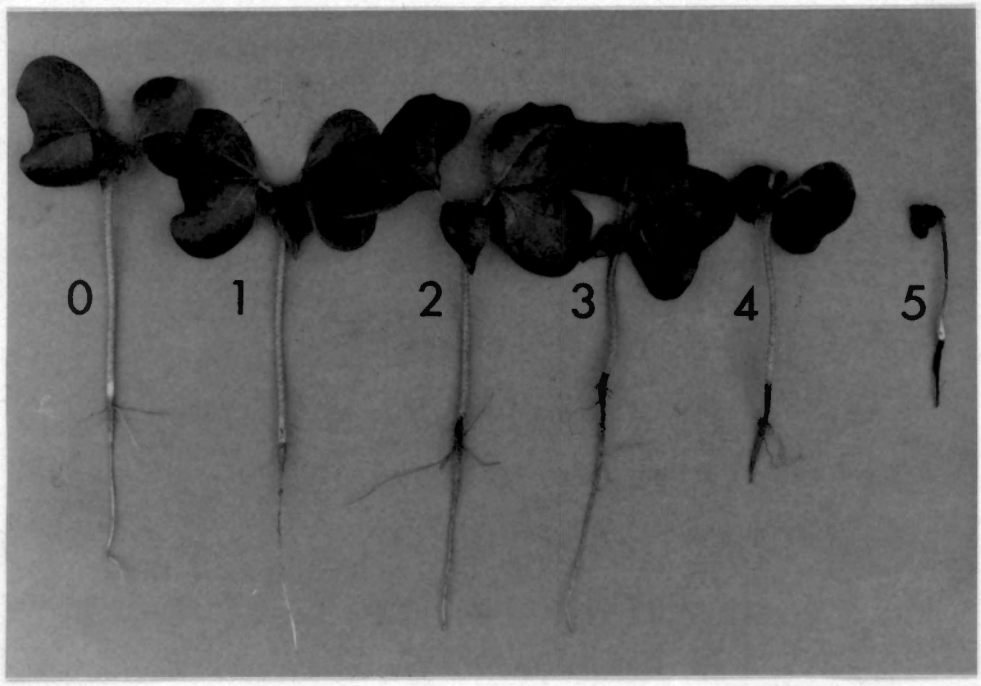

Figure 1. Seedling blight of cotton caused by Pythium ultimum. Infection classes of 0 - 5 are based on disease severity, where 0 = no visible symptoms (far left) and 5 = a dead plant (far right).

Rating of Progeny

Seeds obtained from self-pollinated plants were bulked by parent plant and delinted. Four inch pots were filled with sterilized sand and divided into quadrants by crosses made from plastic pot markers which were inserted so that they were partially buried in the sand. Dividers were used to prevent seed shift during watering and to aid in plant identification. Two progeny seeds were planted in each quadrant. One parent plant was represented per quadrant selected at random. Parent seeds were planted in quadrants selected at random. Plants were incubated at 27 C in a growth chamber, thinned to one seedling per quadrant after six days, and incubated as before. Inoculated seedlings were incubated at 18 C for seven days. Seedlings were removed from the sand, rinsed, and rated as before. This procedure was repeated twelve times and a total of twelve progeny were evaluated from each parent.

Estimation of Heritability

Three methods were used to estimate heritability (h^2) of resistance within each cultivar. Method one, regression, was computed in the Statistical Analysis System (SAS) with the general linear models procedure (GLM). Method two, partition of variance using components of variance, was computed by SAS with the variance component estimation procedure (VARCOMP). The response to one generation of selection was calculated by method three, the realized heritability method, using data from progeny of parents rated 1.

CHAPTER III

RESULTS

Field Test

Seed Germination

There were no significant differences among cultivars in the parent seed germination at 25 C ($P \leq 0.01$) (Table 1). At 15 C Coker 310 had a significantly lower percent germination than the other three cultivars. In all cases percent germination at 15 C was lower than that at 25 C, but the difference was statistically significant only with Coker 310.

Disease Severity

Significant differences occurred among cultivars in all categories evaluated (Table 2). Seedling emergence was less for Delcot 277J than the other three cultivars. No significance existed in post-emergence damping-off for Coker 310, Auburn M, or Delcot 277J. The most post-emergence damping-off occurred in Dixie King 3. Percentages ranged from 9% to 21% for post-emergence damping-off.

Pre-emergence damping-off was used as a collective term to include seed rot. Pre-emergence damping-off and a combination of pre and post-emergence damping-off followed similar patterns with the highest incidence of each occurring in Delcot 277J. Coker 310 had significantly less pre-emergence damping-off and total damping-off than in the other cultivars. Auburn M and Dixie King 3 were not significantly different in emergence and damping-off.

TABLE 1

GERMINATION OF PARENT SEEDS OF FOUR COTTON CULTIVARS[a]

Cultivar	Temperature	1st Replication No. Germination	%	2nd Replication No. Germination	%	Average	%[b]
Auburn M	15 C	45	90	44	88	44.5	89yz
	25 C	48	96	45	90	46.5	93yz
Delcot 277J	15 C	46	92	41	82	43.5	87yz
	25 C	49	98	48	96	48.5	97z
Coker 310	15 C	37	74	23	46	30.0	60x
	25 C	43	86	47	94	45.0	90yz
Dixie King 3	15 C	43	86	35	70	39.0	78y
	25 C	48	96	43	86	45.5	91yz

[a]Seed lots consisted of 50 seeds each and were evaluated seven days after initiation of experiment.

[b]Means followed by the same letter are not significantly different ($P \leq 0.01$) according to Duncan's New Multiple Range test.

TABLE 2

DISEASE SEVERITY OF SEEDLINGS OF FOUR COTTON CULTIVARS IN A FIELD TEST AT JACKSON, TN,[a] EMERGENCE DATA ARE ADJUSTED TO THE GERMINATION RATE AT 25 C

Cultivar	(%)[c] Emergence	Damping-off (%)[b] Pre-emergent	Post-emergent	Total
Auburn M	53xy	47y	16xy	63y
Delcot 277J	34y	66x	9y	75x
Coker 310	74x	26z	18xy	44z
Dixie King 3	55xy	45y	21x	66y

[a]Plots consisted of four replicated rows of 167 seeds for each cultivar. Data were taken four weeks after planting.

[b]Percent of plants that did not emerge (Pre-emergent) and emerged plants that died after emergence (Post-emergent).

[c]Means in each column followed by the same letter are not significantly different at a 5% level of significance.

Pathogens Isolated

Fusarium spp. were isolated most frequently from necrotic hypocotyl sections of plants in the field test (Table 3). Since Johnson, et al. (26) considered *Fusarium* spp. as saprophytic secondary invaders or weak parasites of injured stem tissue, other more pathogenic fungi were considered the primary parasites when found in conjunction with *Fusarium* spp. The frequencies of occurrence of *Rhizoctonia solani*, *Thielaviopsis basicola*, and *Pythium* spp. were 13.5%, 5%, and 4% respectively. There were no significant differences in frequency of occurrence among cultivars.

Selection for Resistance

Parent Seedling Ratings

Mean disease ratings of parent seedlings inoculated with *P. ultimum* were analyzed by Duncan's New Multiple Range test (Table 4). Delcot 277J was significantly more susceptible than the other three cultivars. No significant difference was found among ratings for Auburn M, Coker 310, and Dixie King 3.

Progeny Seedling Ratings

Calculations of mean ratings of progeny seedlings revealed significant differences among cultivars (Table 5). Auburn M was significantly more susceptible to *Pythium ultimum* than Dixie King 3 or Coker 310. Dixie King 3 was significantly more resistant than the other three cultivars. No significant differences were found between Delcot 277J and Auburn M or between Delcot 277J and Coker 310, although measurable differences were obtained. Disease severity of progenies was not

TABLE 3

FREQUENCIES OF FUNGI ISOLATED FROM 400 DISEASED HYPOCOTYLS OF COTTON FROM A FIELD TEST AT JACKSON, TN

Pathogen	No.	%
Fusarium spp.	307	76.75
Rhizoctonia solani	54	13.50
Thielaviopsis basicola	20	5.00
Pythium spp.	16	4.00
Phoma spp.	2	0.50
Alternaria spp.	1	0.25

TABLE 4

MEAN SEEDLING DISEASE SEVERITY OF PARENT PLANTS IN FOUR CULTIVARS INOCULATED WITH *PYTHIUM ULTIMUM*[a]

Cultivar	Replicate test[b]					Mean[c]
	1	2	3	4	5	
Auburn M	2.05	2.09	1.84	2.67	2.92	2.38y
Delcot 277J	2.10	2.45	2.12	2.69	3.64	2.62x
Coker 310	1.95	2.44	2.00	2.39	2.78	2.37y
Dixie King 3	2.05	1.91	2.64	2.22	2.92	2.26y

[a]Each figure is an average rating based on a acale of 0 - 5, where 0 = no visible symptoms and 5 = a dead plant.

[b]Numbers of seedlings inoculated were 20, 34, 25, 36, and 36 respectively for the repeated tests per cultivar.

[c]Means followed by the same letter are not significantly different at a 5% level of significance according to Duncan's New Multiple Range test.

TABLE 5

DISEASE SEVERITY OF PROGENY OF SELF-POLLINATED PARENTS OF FOUR COTTON CULTIVARS INOCULATED WITH *P. ULTIMUM*[a]

Cultivars	Progeny		Parent Control[d]
	Mean[b]	Standard error[c]	
Auburn M	3.20x	.3376y	3.16x
Delcot 277J	3.13xy	.3203y	3.14x
Coker 310	3.00y	.2947z	2.94xy
Dixie King 3	2.79z	.3729x	2.70y

[a]Means followed by the same letter in each column are not significantly different at a 5% level of significance according to Duncan's New Multiple Range test.

[b]Average rating of over 450 seedlings of each cultivar.

[c]Average standard error of 12 progeny from each of 36 parent plants.

[d]Average rating of 96 parent seedling controls.

significantly different from samples of the parents tested at the same time.

Comparisons of progeny ratings from parents differing in susceptibility were made with Duncan's New Multiple Range test (Table 6). A general trend of increasing susceptibility from rating 1 to rating 3 was found in all cultivars. However, the trends were only measurable with the exception of Auburn M where a significant difference existed between rating 3 and ratings 1 and 2. The rating 0 did not appear to conform to any trends.

Comparisons were made between Auburn M and Delcot 277J for each rating 0, 1, 2, and 3. There were no significant differences for ratings 1 or 2 and marginal significance for rating 3. Rating 0 was highly significant between the two cultivars. These same comparisons were made between Coker 310 and Dixie King 3. Significant differences existed between 1 ratings and between 2 ratings for the two cultivars. No differences existed between 0 ratings or between 3 ratings. Comparisons were also made between Auburn M and Delcot 277J collectively and Coker 310 and Dixie King 3 collectively. Differences between 1 ratings, 2 ratings, and 3 ratings were significant for the two groups of cultivars. There was no significant difference for 0 ratings.

Estimates of the regression coefficient obtained by regressing offspring on parental values were doubled to estimate heritability ($b = 1/2(V_A/V_P)$, where b = regression coefficient and V_A/V_P = heritability) (Table 7). Estimates for all four cultivars were measurable, but the heritability for Auburn M and Dixie King 3 was not significantly different from zero. Delcot 277J and Coker 310 were greater than zero at the

TABLE 6

DISEASE SEVERITY OF PROGENY OF PARENTS DIFFERING IN SUSCEPTIBILITY TO P. ULTIMUM[a]

Parent Rating	Cultivar[b] Auburn M	Delcot 277J	Coker 310	Dixie King 3	Standard Error[c]
0	3.56x	2.50y	2.83x	2.93x	
1	3.04y	3.12x	2.68y	2.96x	.3454x
2	3.07y	3.23x	2.72xy	3.03x	.3290xy
3	3.40x	3.16x	2.96xy	3.03x	.3198y

[a]Means followed by the same letter are not significantly different at a 5% level of significance according to Duncan's New Multiple Range test.

[b]Average rating of over 138 seedlings for each cultivar in each parent rating, except that fewer seedlings were tested in the 0 rating.

[c]Average standard error of 12 progeny from each of 48 parent plants.

TABLE 7

ESTIMATES OF HERITABILITY OF FOUR COTTON CULTIVARS BY THREE METHODS

Cultivar	Method		
	Regression	Partition of variance	Response in one generation
Auburn M	.115	.139	.058
Delcot 277J	.194[a]	.165	.019
Coker 310	.177[a]	.147	.012
Dixie King 3	.071	.166	-.010

[a]Significantly different from zero at .10.

10% level of significance.

Estimates of variance components obtained by determining the expected variance from analysis of variance were also used to estimate heritability. Estimates were calculated by dividing the estimate for variation due to progeny by the estimate due to error plus the estimate due to progeny (Var P/Var error + Var P, where Var P = estimate of variance due to progeny and Var error = estimate of variance due to error). Heritability was measurable for all four cultivars, but did not appear to follow the same trends obtained for heritability by regression. Tests were not conducted to determine difference from zero.

Realized heritability or response to one generation of selection was calculated using the formula $h^2 = R/i\sigma$, where R = the advance in one generation of selection, σ = the phenotypic standard deviation of the parental population, and i is the intensity of selection, a coefficient determined by the proportion of the population selected to be parents (39). In this method the progeny of the 1 ratings of the four cultivars was compared to the parent population of the same cultivar.

The realized gain from the parent generation to the next generation was higher for Auburn M than for the other three cultivars, yet still very low (Table 7). The gain for Auburn M was slightly lower than 1.5%. Low mean disease severity ratings coupled with greater diversity occurred more frequent in individual parent plants of Coker 310 than in Auburn M. These factors were found most frequent in Dixie King 3 (Tables 8, 9, 10, and 11, Appendix).

CHAPTER IV

DISCUSSION

From germination rates of parent seeds it was found that the seed quality of Coker 310 and Dixie King 3 may have been slightly lower than Auburn M and Delcot 277J, but the differences were not significant at a germination temperature of 25 C. In all growth chamber experiments the temperature was maintained at 27 C during germination. Therefore, germination rate differences were not considered to be a significant factor in growth chamber tests.

Differences in percent germination in the field test were considered and were used to adjust percentages of emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off. Information from the field test was attributed to the combined effects of the pathogens found. No single effect could be attributed to a specific pathogen.

Delcot 277J was significantly more susceptible in all categories of the field test than Dixie King 3. Similar results with these cultivars have been reported by others (24, 42, 43). A smaller percentage of post-emergence damping-off occurred in the Delcot 277J cultivar than in either Dixie King 3 or Auburn M. In all, 68% of seeds either failed to germinate or eventually succumed to seedling disease. Of the 68%, 62% was due to seedling disease.

The frequency and occurrence of cotton seedling pathogens observed in this study was consistant with that reported by other scientist (2, 25, 27, 31). Although isolated most frequently, Fusarium spp. are not

considered major pathogens. The high frequency of isolation was probably due to their tendency to overgrow primary invaders preventing isolation and identification of primary invaders (27).

In tests of parent seedlings inoculated with Pythium ultimum, Delcot 277J and Auburn M were more susceptible than Dixie King 3 and Coker 310 as previously found (24). In the present study, Auburn M was only measurably and not significantly more susceptible than Coker 310 and Dixie King 3.

Trends similar to that found for parent seedlings occurred when progeny seedlings were inoculated. Separations were measurable, but not significant. Coker 310 appeared to be the most resistant and Auburn M most susceptible.

When means of progeny ratings from specific parent ratings were evaluated, more resistance was found as ratings decreased, but the differences were not significant at a 5% level. This small difference may be an indication of potential, but it also reflects a low degree of heritability. A disassociation between the 0 rating and the other ratings was discovered when the data were evaluated. The 0 rating seemed to follow no trends and will be referred to as the "zero effect." Because of the lack of any noticeable conformity and the extreme range of means between cultivars with a 0 rating, it was concluded that the "zero effect" could be due to some type of disease escape of parent plants rather than a genetic effect. The "zero effect" was revealed when comparisons between cultivars and combinations of cultivars were made. The lower number of parents with 0 ratings in this experiment was also a possible contributing factor to the "zero effect."

Validity of estimates of heritability depends not only on the material to be studied, but also on the structure of the experiment. Differences from parent to the progeny are due in part to genetics and in part to environmental effects. This study was designed to minimize the effect of environment. Each progeny was represented once in each repeated growth chamber test and parent seeds were randomly dispersed as controls in the same growth chamber.

The use of regression to determine heritability was based on the assumption that progeny of parents with extreme ratings (0 and 3) tend to regress toward the mean. Close parent-progeny similarities would reflect large genetic effect and a small environmental effect. Although it can be shown that regression coefficients estimate narrow sense heritability, the method is not free from error (39).

The partition of variance method uses components of variation to determine heritability, and does not sufficiently separate additive genetic variance from that due to environmental effects. The method may give estimates of heritability which are more of a combination between narrow sense heritability and broad sense heritability than that of the regression method. Regression is considered to be a more secure approach to heritability than partition of variance. Data for partition of variance were included for comparison only.

The values for the two previously mentioned methods of determining heritability reflect the degree to which the variability of the rating of the parent seedling is transmitted to the progeny. Since the estimates are for a specific population in a specific experiment, they can not be applied directly to other experiments, but can be used as indicators of magnitude for future breeding programs (39).

The last method used to estimate heritability was response in one generation of selection which is referred to as realized heritability. Realized heritability was estimated with progeny whose parents had a rating of 1. The 1 rating was chosen instead of the 0 rating due to the error in the "zero effect" described previously. Calculations were made by multiplying the standard deviation of the parent population by the intensity of selection. This value was divided into the advance in one generation of selection to obtain an estimate of heritability.

From the realized heritability estimates obtained, Auburn M may have the most potential for future breeding programs involving Pythium ultimum. All figures, however, were low and genetic advance may be slow and difficult to achieve. Delcot 277J, Coker 310, and Dixie King 3 may have a heritability so close to zero that use of these cultivars in a breeding program would be futile.

The realized heritability estimates can be used to predict responses in future experiments with the formula $ih^2\sqrt{V}$ where i = 1.13 + .73 log 1/k, k is the fraction selected from the total population, h^2 is heritability, and V is variance. The success of the prediction depends on the selection of parents with signs of resistance from diverse populations, good control of the environment, and intense selection. The prediction works well if a good estimate of heritability has been made. If the prediction does not hold true, then the estimate for heritability is usually suspected as the cause (39).

The frequency of low mean disease severity ratings coupled with high diversity found in progeny of parent plants of Coker 310 could prove to be more useful in a selection program. These factors were most frequent

in Dixie King 3, but the realized heritability for this cultivar was thought to be essentially zero. Therefore, Dixie King 3 would not be a good choice for a breeding program.

CHAPTER V

SUMMARY

Experiments involving selection for resistance to Pythium ultimum Trow. were conducted with four cotton cultivars which were previously found to differ in susceptibility to P. ultimum.

Differences in resistance among the cultivars, Auburn M, Coker 310, Delcot 277J, and Dixie King 3 obtained in previous tests were confirmed in a field test. Frequencies of isolation and occurrence of individual pathogens were similar to that recorded in the literature.

Parent seeds were grown under controlled conditions and inoculated with P. ultimum. Individual plants were selected according to cultivar and disease severity rating. Plants were grown to maturity and self-pollinated.

Progeny of self-pollinated plants were grown under controlled conditions, inoculated with P. ultimum, and rated for disease severity.

Heritability of resistance was estimated by regression, partition of variance, and by response to one generation of selection. Significant differences from zero at the 10% level were determined for regression estimates. From analysis of response to one generation, realized heritability, it was concluded that Auburn M could be the most likely candidate of the four cultivars for a breeding program. However, Coker 310 met more criteria for a selection program.

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APPENDIX

TABLE 8

DISEASE SEVERITY OF PROGENY OF PARENT PLANTS OF CULTIVAR AUBURN M[a]

Rating	Progeny	Mean	Standard error
0	3	3.25	.329
	2	3.50	.452
	1	3.92	.379
1	8	2.42	.229
	10	2.50	.289
	2	2.67	.300
	4	2.75	.446
	3	2.91	.285
	11	2.92	.482
	1	3.00	.302
	12	3.17	.297
	9	3.25	.279
	7	3.50	.373
	6	3.58	.286
	5	3.92	.336
2	11	2.75	.329
	6	2.75	.250
	12	2.83	.405
	10	2.83	.241
	1	3.00	.326
	3	3.00	.301
	2	3.08	.379
	8	3.17	.297
	7	3.25	.372
	5	3.25	.372
	4	3.33	.355
	9	3.58	.379
3	7	2.75	.372
	3	3.08	.452
	1	3.17	.322
	6	3.17	.297
	5	3.17	.241
	8	3.27	.304
	2	3.42	.379
	10	3.64	.453
	9	3.73	.333
	11	3.73	.304
	12	3.75	.429
	4	4.00	.357

[a]Mean and standard error of twelve progeny plants.

TABLE 9

DISEASE SEVERITY OF PROGENY PARENT PLANTS OF CULTIVAR DELCOT 277J[a]

Rating	Progeny	Mean	Standard error
0	2	2.25	.279
	1	2.75	.329
1	5	2.40	.267
	4	2.55	.312
	9	2.91	.476
	2	2.92	.288
	12	3.00	.408
	1	3.08	.417
	7	3.17	.423
	8	3.17	.241
	3	3.33	.333
	6	3.36	.244
	11	3.50	.314
	10	3.83	.345
2	12	2.58	.313
	7	3.00	.302
	6	3.00	.234
	2	3.08	.229
	11	3.09	.315
	1	3.09	.285
	10	3.11	.455
	4	3.17	.405
	3	3.33	.333
	8	3.42	.452
	9	3.82	.296
	5	4.00	.326
3	8	2.58	.313
	7	2.58	.193
	1	2.83	.297
	10	2.83	.112
	3	3.00	.381
	6	3.18	.377
	9	3.18	.325
	12	3.25	.329
	4	3.33	.333
	5	3.50	.261
	11	3.67	.284
	2	3.92	.313

[a]Mean and standard error of twelve progeny plants.

TABLE 10

DISEASE SEVERITY OF PROGENY OF PARENT PLANTS OF CULTIVAR COKER 310

Rating	Progeny	Mean	Standard error
0	1	2.17	.297
	2	2.82	.325
	3	3.50	.379
1	10	2.36	.388
	5	2.36	.244
	8	2.42	.313
	9	2.45	.413
	1	2.67	.355
	7	2.73	.359
	4	2.75	.279
	2	2.83	.423
	3	2.83	.405
	6	2.83	.241
	12	2.83	.112
	11	3.09	.415
2	5	2.33	.188
	3	2.50	.314
	1	2.55	.434
	4	2.58	.149
	6	2.64	.203
	8	2.67	.284
	12	2.67	.284
	2	2.75	.179
	11	2.83	.366
	9	3.00	.302
	10	3.00	.302
	7	3.08	.358
3	7	2.55	.340
	3	2.58	.260
	1	2.72	.179
	4	2.83	.112
	8	2.92	.336
	6	2.92	.229
	9	2.92	.229
	11	3.00	.258
	2	3.08	.379
	10	3.22	.278
	5	3.42	.358
	12	3.45	.340

[a]Mean and standard error of twelve progeny plants.

TABLE 11

DISEASE SEVERITY OF PROGENY OF PARENT PLANTS OF CULTIVAR DIXIE KING 3[a]

Rating	Progeny	Mean	Standard error
0	3	2.10	.277
	1	2.75	.372
	4	3.00	.405
	2	3.18	.400
	5	3.50	.435
1	5	2.17	.297
	2	2.83	.441
	1	2.83	.386
	9	2.90	.314
	7	2.91	.456
	12	2.92	.484
	10	3.00	.426
	6	3.00	.326
	4	3.08	.336
	11	3.27	.469
	8	3.33	.414
	3	3.36	.310
2	3	2.09	.285
	4	2.58	.336
	12	2.83	.345
	1	2.83	.386
	8	3.00	.330
	9	3.09	.436
	5	3.09	.343
	11	3.25	.329
	6	3.25	.279
	7	3.36	.364
	2	3.50	.500
	10	3.60	.542
3	1	2.36	.279
	11	2.75	.329
	9	2.82	.377
	10	2.83	.271
	8	2.92	.417
	4	3.00	.330
	5	3.08	.417
	12	3.18	.400
	7	3.25	.305
	6	3.33	.355
	3	3.36	.411
	2	3.42	.398

[a]Mean and standard error of twelve progeny plants.

VITA

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