3-1978

Resistance of two cotton (Gossypium hirsutum L.) cultivars to Pythium ultimum Trow

Elizabeth Dian Sutherland

To the Graduate Council:

I am submitting herewith a thesis written by Elizabeth Dian Sutherland entitled "Resistance of two cotton (Gossypium hirsutum L.) cultivars to Pythium ultimum Trow." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

Leander F. Johnson, Major Professor

We have read this thesis and recommend its acceptance:

J. W. Hilty, F. F. Bell

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:

Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

RESISTANCE OF TWO COTTON (*GOSSYPIUM HIRSUTUM* L.) CULTIVARS TO *PYTHIUM ULTIMUM* TROW.

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Elizabeth Dian Sutherland

March 1978

DEDICATION

This thesis is dedicated to my parents, Jack and Jean Sutherland, whose encouragement and support is deeply appreciated.

ACKNOWLEDGEMENTS

The author is deeply indebted to Dr. L. F. Johnson of the Department of Agricultural Biology, The University of Tennessee, for suggesting the topic of this study and for his constructive criticism of the manuscript. Appreciation is also expressed to Dr. J. W. Hilty, Department of Agricultural Biology, and Dr. F. F. Bell, Department of Plant and Soil Science, The University of Tennessee, for their suggestions and criticism of the manuscript. Thanks are also due Dr. E. T. Graham, Department of Ornamental Horticulture and Landscape Design, and Dr. J. M. Stewart, United States Department of Agriculture Plant Physiologist, The University of Tennessee, for their aid and interest in this study.

ABSTRACT

Two commercial cotton (Gossypium hirsutum L.) cultivars were tested for their relative susceptibility to the fungal seedling blight pathogen, Pythium ultimum Trow. Cotton plants grew in sterilized sand for eight days, and then were inoculated with the pathogen. The seedlings were incubated at 18 C, and after a seven day period disease severity was rated. 'Dixie King 3' was consistently more resistant to P. ultimum than 'Delcot 277'.

Nutrient status of the fungus affected its pathogenicity. Disease severity on cotton seedlings was greater when the fungus grew on potato dextrose agar (PDA) than when it grew on dilute PDA or water agar.

Since the epidermal cell wall is the first physical barrier to invasion by a fungal pathogen, the two cultivars were compared for differences in cell wall thickness. There was no significant difference between the cultivars.

Pythium ultimum was cultured on various substrates to test linear growth responses. No differences in growth rates were found when the fungus was cultured on water agar mixed with ground hypocotyl tissue of each cultivar.

Gossypol, a terpenoid aldehyde, catechin, a polyphenol, and catechol, all previously implicated in plant resistance to other pathogens, were incorporated into culture media. Growth inhibition of P. ultimum occurred on such media containing catechol at 10 ppm and gossypol at 1,000 ppm. Catechin did not inhibit growth at

1,000 ppm, the highest concentration tested.

Cotton hypocotyl tissue was extracted and gossypol and catechin "equivalents" were measured with a spectrophotometer. Gossypol concentrations decreased in both cultivars after infection by P. ultimum. Concentrations of catechin "equivalents" increased threefold in the Pythium-tolerant cultivar 'Dixie King 3' following infection, but decreased in the susceptible cultivar 'Delcot 277'. This phytoalexin-like response in the resistant cultivar may be involved in cotton resistance to infection by Pythium ultimum.

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INTRODUCTION

A major problem in the cotton producing states is reduction of cotton yields by seedling pathogens. In 1975, an estimated 213,409 bales of cotton were lost due to seedling blight (12). Even greater cotton losses from seedling disease occurred in 1976 causing a decline of an estimated 333,778 cotton bales (13). In 1977, estimated reduction in yield from seedling disease totaled 212,140 bales (14).

The extent of reduced yield from seedling blight varies from state to state in the cotton producing areas. Seedling diseases caused a major loss in cotton in Tennessee in 1975, 1976, and 1977. There was an estimated 8% reduction in cotton yield in Tennessee as a result of seedling diseases in 1975 (12), 20% reduction in 1976 (13), and 5% reduction in 1977 (14). Seedling disease occurrence and severity was greater in 1976 than it had been in the previous 20 years. Chambers (11) attributed the high losses to early planting followed by cool, wet weather making the conditions optimum for injury by seedling pathogens. Cotton fields that were not replanted suffered from damaged roots which promoted invasion by other parasites and insects.

The disease symptoms that are associated with seedling parasites are seed rot, pre-emergence damping-off, post-emergence damping-off, and stem canker (soreshin) (35). Rhizoctonia solani, Pythium spp., and Fusarium spp. are commonly implicated in the seedling blight complex. Low temperatures and high moisture levels are environmental conditions generally associated with the occurrence of seedling diseases.

Recently it has been found that certain commercial cultivars might

differ in their resistance to Pythium ultimum Trow. (33). This finding led to a study of the mechanism of this resistance, and might yield knowledge that would benefit cotton growers by reducing yield losses. Therefore, the objectives of this study were to (a) measure the difference in resistance to P. ultimum of two Gossypium hirsutum L. cultivars grown commercially, (b) determine if these two cultivars differed in the thickness of their epidermal cell hypocotyl walls, and (c) determine if the cultivars differed in certain terpenoid aldehyde and polyphenol contents within their hypocotyl tissues.

CHAPTER 1

LITERATURE REVIEW

In 1892, Atkinson first recognized seedling blight as a major problem to cotton producers when he published a description of symptoms caused by Rhizoctonia solani. Since then, other scientists have identified a number of seedling disease pathogens. R. solani, Fusarium spp., Rhizopus spp., and Aspergillus spp. are often associated with seed rot. R. solani and Pythium spp. are usually isolated from seedlings with pre- or post-emergence damping-off. R. solani also causes canker formation on stems of seedlings causing a disease known as soreshin. These organisms mentioned compose what is known as the seedling blight complex (1, 35, 48).

Seedling Blight Complex in Tennessee

R. solani, Pythium spp., and Fusarium spp. were the most commonly isolated fungi from diseased cotton seedlings over a six year period (1963-1968) (35). R. solani and Pythium spp. were reported to be the major causal agents of seedling blight and depending on the year, one or the other was isolated more often. R. solani, Pythium spp., Fusarium spp., and in addition, Thielaviopsis basicola, were isolated from cotton seedlings in a second study performed in Tennessee (34). Again, R. solani and Pythium spp. were most commonly isolated. The Pythium spp. were identified as P. irregulare, P. sylvaticum, and P. ultimum.

Environmental Factors Affecting Severity of Seedling Blight

Environmental conditions commonly related to occurrence of seedling diseases are soil temperature and moisture. Early planting of cotton can be responsible for poor cotton stands by subjecting plants to adverse weather conditions. Poor stands were found to be highly susceptible to post-emergence damping-off (35).

In laboratory tests, the optimum temperature for germination and growth of cotton seedlings is from 27 to 35 C. Cool weather, especially when preceded by warm weather (49), damages cotton plants by lowering natural resistance and increases susceptibility to pathogen invasion. Although seedling blight is usually more severe at low temperatures, some isolates of R. solani have been reported to be pathogenic at high temperatures (35).

Soil moisture has been correlated with occurrence of seedling diseases (35, 43), and has been found to influence frequency of R. solani and Pythium spp. isolated from germinated but non-emerged cotton seedlings. When mean soil moisture readings were 11 to 12%, R. solani was isolated more often. An increase in soil moisture caused Pythium spp. to occur in higher numbers (35).

Factors Affecting Resistance of Cotton to Pathogens

Two factors considered in resistance of plants to fungal invasion and colonization are structural and chemical barriers of the host plant. Fungal penetration of a plant occurs directly through the surface, through natural openings, or through wounds (19). Pythium spp. most frequently enter their hosts through unwounded surfaces by means of

infection pegs or thin hyphae arising from the underside of slight swellings or appressoria. Occasionally penetration will also occur through natural openings (16). The importance of the epidermis as a physical barrier has long been debated, and there is conflicting evidence as to whether penetration by fungi is a mechanical or chemical process (19, 38).

Resistance of seedlings to diseases due to biochemical compounds may be affected by the following factors: (a) cotton growth may be slowed more than that of potential pathogens by low temperatures, (b) the stage of seedling susceptibility may be extended for a longer period of time, (c) biochemical changes in the cotton plant may be induced by cool weather making it a more suitable substrate for microorganisms (21), and (d) exudate quality and quantity may increase the inoculum potential of the pathogen (23). Lowered resistance of cotton in cool weather may partly be explained by biochemical changes in cotton plants.

When root temperatures of cotton seedlings were lowered, it was shown that sugar concentrations increased within seedlings (21, 22, 29). Guinn and Hunter (22) found that reducing and total sugars accumulated rapidly and reached a maximum after cotton seedlings were subjected to 15 C for two days. They also found that starch as well as sugar accumulated from low temperatures indicating that the increase did not occur from the breakdown of starches. Sugar content of seedlings can be manipulated (21). When air temperature was lowered from 27 to 16 C, sugar content of the roots and tops increased rapidly. When temperature was then increased to 27 C, sugar concentration returned

to the original quantities.

Amino acid content in chilled cotton seedlings was found to increase slightly at lower temperatures (21, 29), and the same relationship was found for soluble protein content (21). The concentration of total nitrogen in plant tissue did not appear to be correlated with root temperature (29), but an exogenous source of nitrogen was found to affect sugar content of cotton. At 25 C, addition of varying amounts of ammonium nitrate caused a decrease in sugar content of cotton. Only the highest level of ammonium nitrate caused decrease in sugar at 15 C (21).

R. solani grew better on extracts of chilled cotton tissue than on extracts of tissue subjected to higher temperatures (21, 29). It was concluded that the increase in sugar at low temperatures contributed to the increase in disease severity (29). Other studies have demonstrated the effects of outside sources of sugar on infection by R. solani. Weinhold and Bowman (54) added sugars to the growth medium of cotton to test virulence of R. solani on five-day-old cotton seedlings. Virulence of Rhizoctonia was reduced when they added sugars, except for arabinose.

Other nutrients that might influence pathogen growth have been found in cotton root exudates. Booth (8) compared two cotton cultivars for amounts of the vitamin B choline in exudates. The Verticillium-tolerant cultivar exuded more choline. When alanine was added to the nutrient solution of older plants, the tolerance characteristic was lost.

Nutrients exuded from plants may increase disease incidence by

increasing inoculum potential near potential infection sites. In experiments with zoospores of Pythium spp., exuded materials from roots of cotton were thought to be responsible for zoospore attraction and encystment (50).

Resistance of cotton to pathogenic microorganisms may be the result of chemicals produced by the host in response to the parasite. Those chemicals which inhibit the growth of microorganisms and are produced in response to a host-pathogen interaction are termed phytoalexins (47). Gossypol and related terpenoids are endogenous in Gossypium spp. (6, 51), and have been shown to increase in concentration when inoculated with Verticillium albo-atrum (3, 5, 6, 7, 51) or R. solani (30, 31).

The increased resistance of cotton to seedling pathogens with age has thought to be related to the increased ability to produce gossypol-related compounds. Very young hypocotyl tissues formed smaller amounts of terpenoid aldehydes than older tissues which were resistant to the seedling pathogens, R. solani and Pythium spp. (2). After stem tissues were inoculated with V. albo-atrum, quantities of gossypol-related compounds formed were directly related to age (2, 3).

When cotton seedlings of different ages were inoculated with R. solani, concentrations of gossypol-related compounds were higher in 12-day-old seedlings than 5- or 6-day-old seedlings (28, 30). Young stem tissues of resistant cultivars had greater potentials for production of gossypol-related compounds than young tissues of susceptible cultivars. This was also true for young root tissues (3). Concentration of phytoalexins formed in young root and stem tissues

are often only partially fungistatic (4). Production of terpenoid aldehydes appears to be a factor involved in the resistance of cotton to certain seedling diseases with age.

Gossypol and related compounds have been implicated in resistance of mature cotton plants, while catechins and flavolans have been thought to act in the juvenile defense system. A high level of juvenile defense compounds appears to inhibit or exclude terpene synthesis. Catechin and flavolan contents were greatest in young leaves, petioles, and stele tissues of both healthy and infected cotton plants and decreased in concentration with age (7). (+)-Catechin has been found to be the predominant polyphenol in hypocotyls of cotton seedlings (26, 28). Most of the (+)-catechin is polymerized into a condensed tannin as cotton plants age. Concentrations of tannins have been related to cultivar resistance to Verticillium wilt (5).

When cotton (Gossypium hirsutum) seedlings were infected with R. solani, the concentration of phenolic compounds, mostly catechin, increased in 6- or 12-day-old seedlings. The increase was greater in the 12-day-old seedlings, and was related to the increased resistance of cotton to R. solani as seedlings aged. The mechanism involved in resistance was due to the inactivation of an enzyme produced by R. solani, important in infection (26, 28).

An increase in peroxidase activity has also been related to decreased susceptibility of seedlings to R. solani. Oxidation of catechin by peroxidase increases in infected seedlings (52, 53), and this interaction has been related to the browning associated with soreshin lesions produced by R. solani (19, 51, 52, 53). Hunter (27)

tested the effect of catechin upon R. solani by adding a combination of 0.5% calcium chloride and 0.01% catechin to paper rolls of cotton seedlings four days before inoculation. This chemical combination consistently reduced disease incidence.

(+)-Catechin and other polyphenols may also affect resistance of young cotton plants to Verticillium dahliae. Howell et al. (25) found high concentrations of polyphenols in young leaves of a cotton cultivar that was resistant to V. dahliae. As the cotton plants aged, they became susceptible to V. dahliae. Older leaves were found to have lower concentrations of the polyphenols.

Control of Seedling Diseases

A review of practices associated with control of seedling blight has been made by Minton (43), and general cropping practices are mentioned that can promote disease and insect control. Breeding has played a part in the control of seedling pathogens. Efforts to incorporate new pools of variability into cotton have had some success in combating seedling disease problems, but greater efforts are needed to fully utilize this form of control.

The use of fungicides is the most commonly employed method in the prevention of seedling disease. Fungicides and rates are suggested annually for each of the cotton producing states (44, 45). Seed treatments have been effective in reducing seedling disease incidence when compared to non-treated seed (10, 18). The extent of this effectiveness is not necessarily pronounced and varies with method of application.

In a five year study by Chambers (9), only slight improvement in disease control was observed when granular soil fungicides were applied. An evaluation of three methods of fungicide application was made when seedling disease injury was very high (10). In-furrow spray did not appear to improve stands, but granular applications of different fungicides significantly increased stands.

In reviewing the methods of control, breeding for resistance offers the most effective and economical means of protection. Several millions of dollars are spent in the use of fungicides and replanting (9). However, there is little resistance found in commercial upland cottons to soil-borne plant pathogens. Fulton et al. (17) found some lines of 'Arkot' that exhibited resistance to P. ultimum, Colletotrichum gossypii, and T. basicola, and certain lines of Yugoslav cotton that had tolerance to P. ultimum and R. solani. In the cottons tested by Mathre and Otta (40), no resistant sources to the two most common damping-off pathogens, P. ultimum and R. solani, were discovered.

CHAPTER 2

MATERIALS AND METHODS

Measurement of Disease Severity

Seeds of two commercial cotton (*Gossypium hirsutum* L.) cultivars, 'Dixie King 3' and 'Delcot 277', were obtained from P. E. Hoskinson, West Tennessee Experiment Station. To minimize differences in seed quality, plants grew from these seeds to maturity in the greenhouse. Seeds used in this study were produced from selfed plants. They were finally acid-delinted before use. Cotton seeds were planted in sterilized sand in four inch sterilized plastic pots. Five cotton seeds were planted per pot, and incubated in a growth chamber at 27 C for eight days. Plants were then thinned to three seedlings per pot, and inoculated using the water-jet method suggested by Donald M. Gardner, Graduate Research Assistant, The University of Tennessee, Knoxville, Tennessee.

To minimize abrasion of the cotton hypocotyl, a hole was made at the base of each cotton seedling with a stream of water from a plastic wash bottle. Disks, 5 mm in diameter, were cut from three-day-old petri dish cultures of *Pythium ultimum* Trow. that grew on potato dextrose agar (PDA) (36). The isolate of *P. ultimum* was obtained from L. F. Johnson, Professor, The University of Tennessee, Knoxville, Tennessee. The inoculum disk was placed against the hypocotyl, and sand was gently pushed towards the plant to cover and hold the disk in place. Inoculated plants were then incubated at 18 C in a growth

chamber for seven days.

The seedlings were washed free of sand, and disease severity was rated. The rating system was as follows:

0-no symptoms

1-a small pinpoint dot

2-necrotic lesion less than 1 cm in length

3-necrotic lesion greater than 1 cm in length

4-plant wilted

5-plant dead

This experiment was repeated twice, and was analyzed statistically using a nested design.

An experiment was designed to determine if the nutrient status of the fungus would affect its pathogenicity. Cultures of P. ultimum were grown on five different types of media:

Medium 1-water agar (1.7% agar)

Medium 2-1:100 (PDA:water agar)

Medium 3-1:10 (PDA:water agar)

Medium 4-1:1 (PDA:water agar)

Medium 5-PDA

Disks, 5 mm in diameter, from each of the above substrates was used to inoculate eight-day-old seedlings in sterilized sand with the water-jet method. Disease severity was rated according to the above rating system after seven days of incubation at 18 C. A completely randomized design was utilized in analyzing the data.

Growth of Pythium ultimum on Hypocotyl Tissue and Other Substrates

An experiment was designed to determine if fungistatic substances were present in cotton hypocotyls. Eighty seeds of two cultivars, 'Dixie King 3' and 'Delcot 277', were planted in two flats of sterilized sand and placed in a growth chamber at 27 C. Cotton plants were harvested after eight days. A cork borer was used to cut 1 cm segments, measured from the center of the transition zone (root:stem). The segments were weighed, and 3.13 g of each cultivar was used as the sample. Segments were washed with sterilized distilled water three times, washed with Tween 20 and sterile water, and then rinsed twice with sterile water. The segments were then washed with 10% ethanol, and rinsed twice with sterile water.

The segments from each cultivar were ground aseptically with a mortar and pestle into a homogeneous mixture. The ground segments then were added to 45 ml each of water agar. Each bottle was shaken vigorously, and three petri dishes were poured for each cultivar. After the mixture had cooled, a 5 mm disk of P. ultimum was placed on the surface of the agar substrates 0.5 mm from the edge of the plates. Three measurements were made per dish. One measurement was made perpendicular to the edge of the disk. The other two measurements were made at 45^{o} angles to the left and right from the edge of the inoculum disk. Such linear growth measurements were made twice a day until the plates were covered with mycelia.

Certain phytoalexins were incorporated into water agar to determine if they were inhibitory to P. ultimum. Gossypol was obtained

from A. A. Bell, United States Department of Agriculture National Cotton Pathology Research Lab, College Station, Texas. A 1 ml solution of 10,000 ppm of gossypol in absolute ethanol was made and added to 99 ml of water agar. A dilution series was made with water agar and the following concentrations were prepared: 1,000, 100, 10, 1, and 0 ppm. Five plates of each concentration were poured, and each was inoculated with a disk of P. ultimum. Linear growth measurements were made once a day until the mycelia had grown across the plate. In order to separate the effects of gossypol and ethanol, absolute ethanol was incorporated into water agar at the following rates: 16, 8, 4, 2, 1, and 0%. Linear growth measurements were made daily.

To test the effect of (+)-catechin on P. ultimum, a 10 ml solution of 100,000 ppm of (+)-catechin was prepared. The solution was filtered through a sterile millipore filter, and added to 40 ml cooled water agar. The following concentrations of (+)-catechin were made in water agar: 1,000, 100, 10, 1, and 0 ppm. Five plates were poured from each concentration, and each was inoculated with a disk of P. ultimum. Linear measurements were made each day until the plates were covered with mycelia. This experiment was repeated. The growth response of P. ultimum to catechol was tested in a similar manner.

Epidermal Cell Wall Measurements

Seeds of the two cultivars were planted in sterilized sand. Seedlings grew in a growth chamber for eight days at 27 C. The cotton plants were then harvested, and 2 cm segments were cut so that 1 cm was on each side of the transition zone. The segments were fixed, dehydrated, and embedded in paraffin according to the techniques of Jensen

(32). Embedded tissue was sectioned to 7 µm thickness with a rotary microtome. The sections were stained with 1% safranin in water and stained a second time with 0.5% fast green in water (20) using a standard procedure (32). With this technique, cell walls were stained red. Eighteen slides were made with sections of each cultivar. Cell wall thickness was measured under high power (1,000X) of a compound microscope.

Isolation of Gossypol and Catechin "Equivalents" from Diseased Cotton Tissue

A total of 608 cotton seeds, 304 seeds each of cultivars 'Dixie King 3' and 'Delcot 277', were planted in sterilized sand in flats and incubated at 27 C. After seven days, plants were individually inoculated with 5 mm disks of P. ultimum using the water-jet method. Of those plants that germinated, approximately 475 were inoculated and about 80 were left to serve as the control for each test.

Plants were washed free of sand, and categorized with the following severity ratings.

0-no expressed symptoms

1-scattered, darkened spots indicating minor disease invasion

2-a dark, sunken lesion less than 1 cm in length

3-a dark, sunken lesion greater than 1 cm in length

4-wilted or dead plant

Figure 1 is a photograph illustrating examples of each category. One centimeter segments were cut measuring from the center of the transition zone for the control or 0 grouping, or from the center of the pinpoint lesions for the plants falling in the 1 category. For the

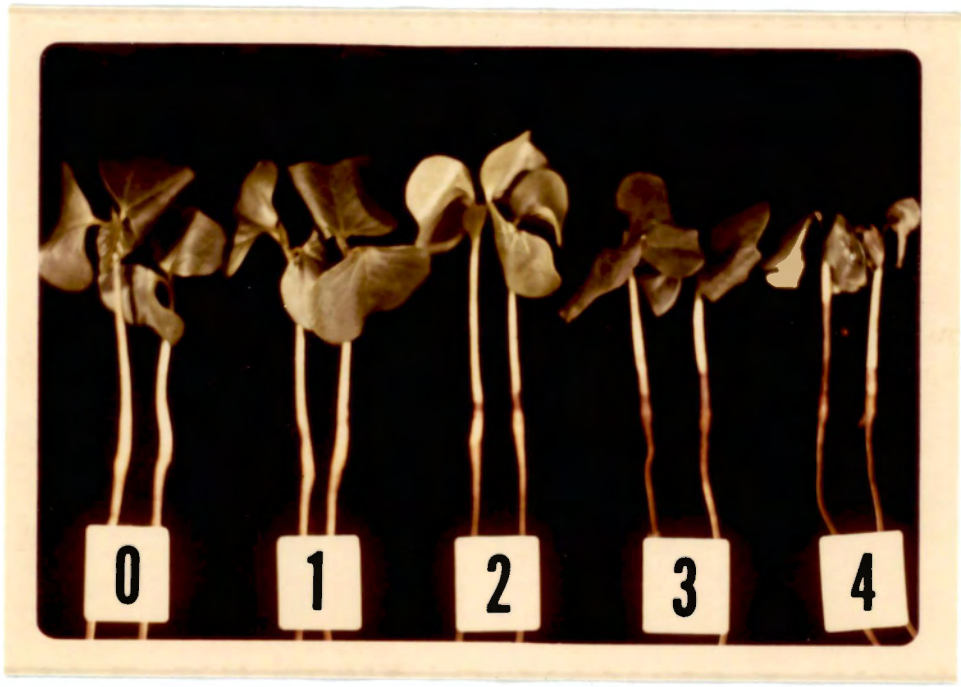

Figure 1. Seedling blight of cotton caused by Pythium ultimum. Infection classes of 0-4 are based on disease severity, where 0=no exhibited symptoms (far left) and 4=wilted or dead plants (far right).

other groups, segments of the area surrounding the lesion were cut.

The segments to be used for the gossypol analysis were left to air-dry. After drying, 0.1 g was weighed from each category and extracted according to a procedure suggested by Bell (7). The segments used in the catechin analysis were weighed, and 1 g of plant material was extracted for each category (7). The extracts for each grouping were quantified with a Perkin-Elmer 202 Spectrophotometer. Standard curves were determined by measuring known amounts of gossypol and (+)-catechin with the spectrophotometer.

CHAPTER 3

RESULTS

Disease Severity Evaluation

The cultivar tests demonstrated that 'Dixie King 3' was more resistant to invasion by *Pythium ultimum* than was 'Delcot 277'. Data from three replicated experiments are found in Table 1. Average infection classes were calculated for each cultivar. In each of the three experiments, 'Dixie King 3' was more resistant than 'Delcot 277' ($P=0.01$).

Nutrient status of the fungus was found to affect pathogenicity. Cultures of *P. ultimum* which grew on PDA caused more disease on both cultivars than did cultures which grew on water agar or dilute PDA (Table 2). 'Dixie King 3' was more resistant to *P. ultimum* that grew on water agar and the higher concentrations of PDA.

Growth of Pythium ultimum on Various Substrates

Pythium ultimum grew equally well on agar containing hypocotyl tissue of 'Dixie King 3' and agar containing hypocotyl tissue of 'Delcot 277'. All 9 cm plates were covered with mycelia after four days. There were no differences in growth rates of *P. ultimum* on media containing alcohol or (+)-catechin. These two chemicals apparently are not fungistatic in culture at the concentrations tested.

Gossypol was found to inhibit *P. ultimum* at a concentration of 1,000 ppm (Table 3). There was no inhibition of growth on media containing 0, 1, 10, and 100 ppm of gossypol. Hyphae grew to the edge of

TABLE 1

PATHOGENICITY OF *PYTHIUM ULTIMUM*
ON TWO COTTON CULTIVARS

Experiment	Cultivar	Number of plants/infection class 0[a]	1	2	3	4	5	Mean infection class
1	'Dixie King 3'	4	13	11	1	0	1	1.43
	'Delcot 277'	2	6	12	4	5	1	2.23
2	'Dixie King 3'	3	4	13	6	2	2	2.20
	'Delcot 277'	1	4	12	4	6	3	2.63
3	'Dixie King 3'	0	4	23	2	0	1	2.03
	'Delcot 277'	0	2	8	2	11	7	3.43

[a]Infection classes of 0-5 based on disease severity, where 0=no symptoms and 5=dead plant.

TABLE 2

EFFECT OF NUTRIENT STATUS OF *PYTHIUM ULTIMUM*
ON DISEASE SEVERITY OF TWO COTTON CULTIVARS

Growth medium	Mean infection class* 'Dixie King 3'	'Delcot 277'
Water agar (1.7% agar)	0.58[a] a	1.67 bc
1:100 (PDA:water agar)	1.25 ab	1.42 abc
1:10 (PDA:water agar)	2.25 cd	2.25 cd
1:1 (PDA:water agar)	2.83 d	3.83 e
PDA	3.00 de	3.83 e

*Infection classes of 0-5 based on disease severity, where 0=no symptoms and 5=dead plant.

[a]Means followed by the same letter are not significantly different (P=0.05) according to Duncan's Multiple Range test.

TABLE 3

LINEAR GROWTH OF PYTHIUM ULTIMUM ON AGAR MEDIA CONTAINING GOSSYPOL

Days of incubation	Concentration of gossypol (ppm)				
	0	1	10	100	1000
1	2.0[a]	2.0	2.0	2.0	0.0
2	4.7	4.5	4.7	4.4	0.0
3	6.7	6.7	6.9	6.5	0.0
4	7.2	7.2	7.2	7.2	0.3
5	7.2	7.2	7.2	7.2	0.7

[a]Each figure is the average in cm of measurements on three plates.

the plates (cm) within four days at these lower concentrations, but it was four weeks before hyphae grew this distance on 1,000 ppm of gossypol.

Catechol, a derivative of (+)-catechin, inhibited growth of P. ultimum at concentrations of 10 ppm or more (Table 4). Fungistasis was detected 24 hours after inoculation of plates with agar disks of P. ultimum. By the fourth day, petri dishes of media containing no catechol or 1 ppm of catechol were covered with mycelia. Complete growth inhibition of P. ultimum occurred at 1,000 ppm concentration of catechol.

Thickness of Epidermal Cell Walls

The average per plant thickness of epidermal cell walls of the hypocotyl ranged from 0.63 to 0.84 mμ. The average cell wall thickness of 'Dixie King 3' was 0.76 mμ and that of 'Delcot 277' was 0.72 mμ. This difference was not statistically significant.

Gossypol "Equivalents" in Hypocotyl Tissue

Gossypol and related compounds were found to decrease in concentration as disease severity increased (Figure 2). 'Dixie King 3' contained more gossypol than did 'Delcot 277' in each disease category, but the difference was minimal in healthy and slightly infected plants. The large cultivar differences in gossypol concentration in severely diseased plants did not occur when the experiment was repeated.

TABLE 4

LINEAR GROWTH OF *PYTHIUM ULTIMUM* ON AGAR MEDIA CONTAINING CATECHOL

Days of incubation	Concentration of catechol (ppm)				
	0	1	10	100	1000
1	2.6[a]	2.2	1.6	1.1	0.0
2	5.1	3.7	2.4	1.2	0.0
3	7.0	5.7	3.8	1.6	0.0
4	7.2	7.2	5.8	2.2	0.0

[a]Each figure is the average in cm of measurements on three plates.

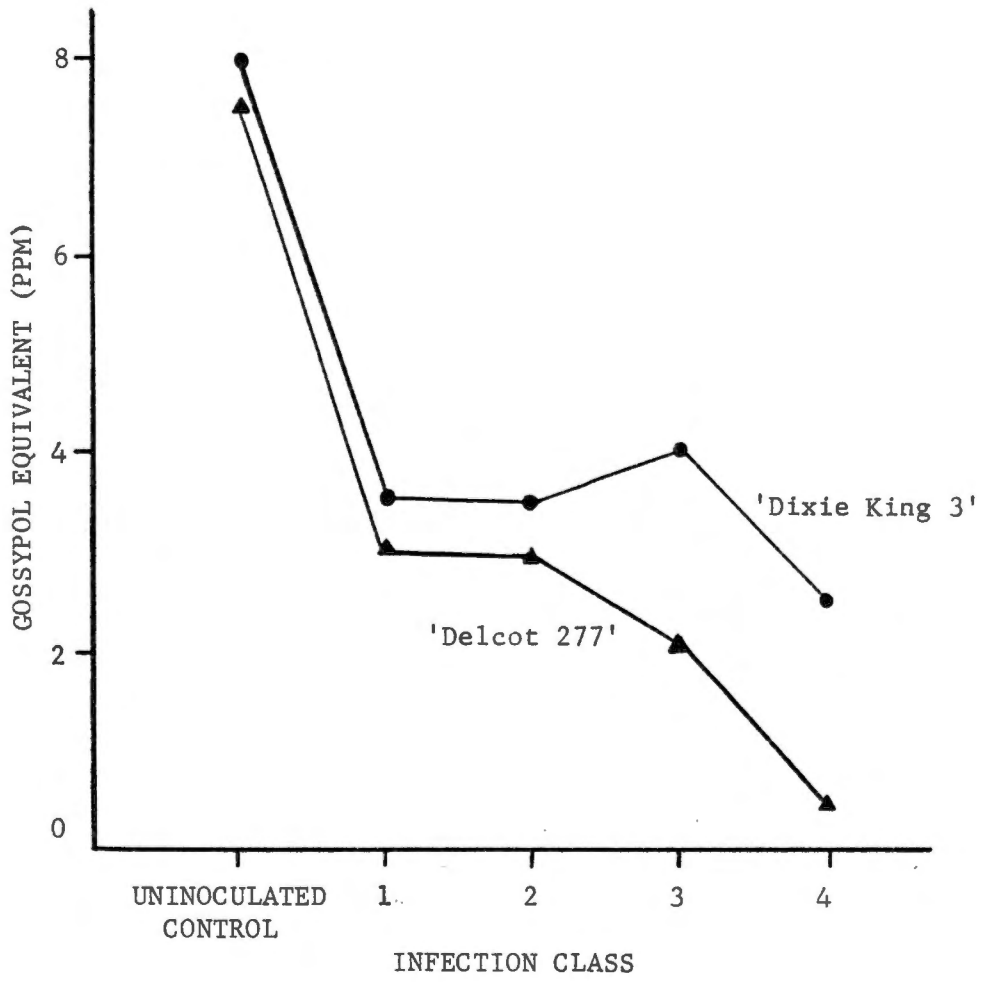

Figure 2. Concentrations of gossypol "equivalents" in hypocotyl tissue of two cotton cultivars. Infection classes of 1-4 are based on disease severity, where 1=slight symptoms and 4=wilted or dead plants.

Catechin "Equivalents" in Hypocotyl Tissue

Level of catechin increased almost threefold in slight to moderately diseased hypocotyl tissue of the resistant cultivar, 'Dixie King 3', but decreased in tissue of the susceptible cultivar, 'Delcot 277' (Figure 3). Quantities of catechin decreased in both cultivars in severely diseased plants. In severely diseased plants, there was no appreciable difference in catechin levels between the two cultivars.

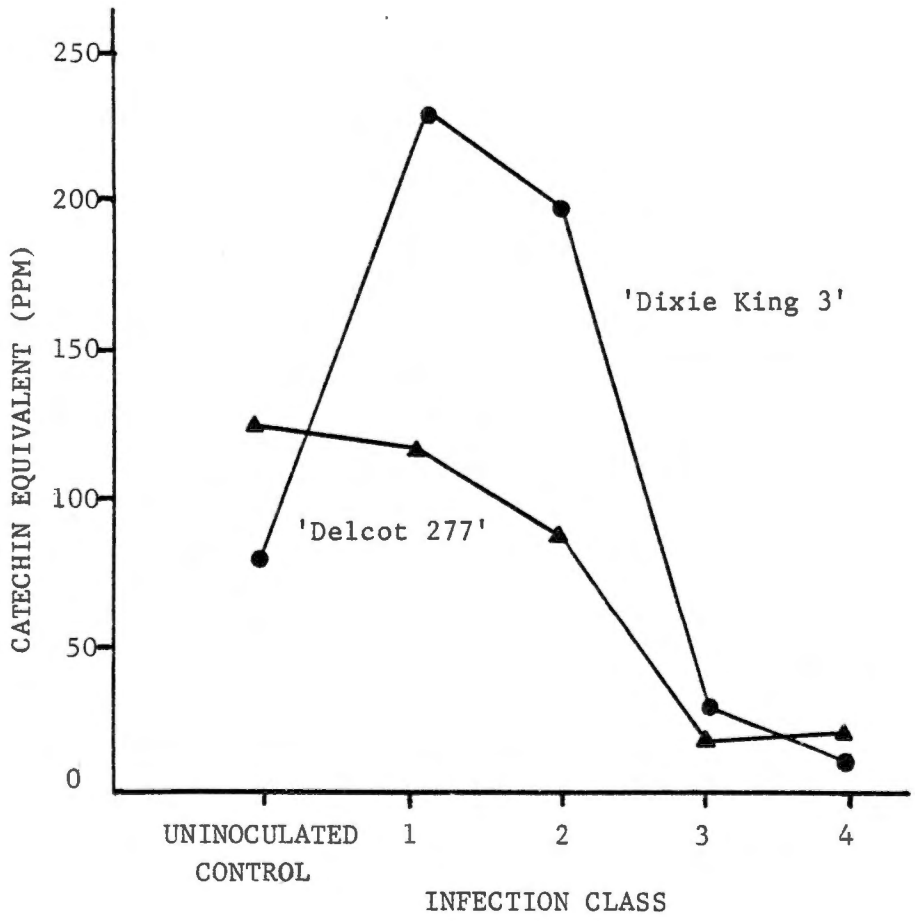

Figure 3. Concentrations of catechin "equivalents" in hypocotyl tissue of two cotton cultivars. Infection classes of 1-4 are based on disease severity, where 1=slight symptoms and 4=wilted or dead plants.

CHAPTER 4

DISCUSSION

In order to maintain a basis for cultivar comparison, cotton plants were grown under controlled conditions in growth chambers. This aided in maintaining the proper environment for pathogenicity studies. Although individual plants within the two cultivars tested varied considerably in susceptibility to Pythium ultimum, there was a measurable and statistically significant difference between the two cultivars. This is the first report of differences between commercial cultivars of cotton in resistance to P. ultimum.

Disease severity was influenced by the nutrient status of the fungus. These results substantiate pathogenicity studies performed by Mildenhall et al. (42) with Pythium spp. They had discovered that some isolates of Pythium caused more disease when cultured on corn extract than when cultured on potato-dextrose broth. Therefore, it is important to consider nutrient status of the fungus when evaluating disease severity.

The plant cuticle may occur intermittently below the soil surface (37), but there is no evidence as yet to show that Pythium spp. produce cutinolytic enzymes (16). Observation could not justify the presence of the cuticle in the area of the hypocotyl commonly infected by P. ultimum.

Thickness of hypocotyl cell walls were not different between the cultivars. The epidermal cell wall represents a highly complex defense

system (19), and chemical inhibition of the fungus during penetration of the epidermal cell wall should not be excluded from further consideration because of these results. In the case of Pythium, it would appear that production of phytoalexins would be the source of cotton cultivar resistance rather than naturally occurring inhibitors in the cell wall (16, 41, 46). Unsubstantial information regarding cell wall thickness might have been predicted since epidermal cell walls vary strikingly in thickness in different plants and in different parts of the same plant (39).

Segregating cotton plants into disease severity groups for chemical studies eliminates innate variation related to the age of the plants. When all plants are harvested at specific time intervals and grouped for chemical tests, it is not probable that all plants will exhibit the same degree of symptoms. In visually categorizing diseased seedlings, variation becomes confined to the indexed groups of plants. Once data is obtained from each of the designated categories, it can be utilized in estimating the degree of disease in plant populations. Although all plants in a given population are subjected to identical quantities of inoculum, genetic variability of individuals and disease escape phenomena influence the severity of infection.

Previous workers have emphasized the importance of rates of phytoalexin production when comparing resistance in cotton cultivars (2, 4). Early in disease infection, Verticillium-resistant cotton plants demonstrated faster rates of phytoalexin synthesis than susceptible cotton plants. After two weeks, phytoalexin content was inversely related to cultivar resistance (4). By harvesting and indexing plants when a wide

range of symptoms is available, continuous sampling over a time period is avoided.

It may be difficult to determine the validity of experiments testing fungal inhibition in culture. Howell and Bell (24) had found that gossypol was equally toxic to virulent and avirulent strains of Verticillium albo-atrum when tested in culture. Concentrations in excess of 100 ppm of gossypol were required to inhibit P. ultimum in culture. Only 7 to 8 ppm of gossypol and related compounds were found in samples of healthy hypocotyl tissue. Since gossypol "equivalents" decreased upon infection of the cultivars with P. ultimum, they do not appear to play a role in cultivar resistance to P. ultimum.

Phenolic compounds vary widely in their fungal toxicity (67). When tested in culture, catechol and (+)-catechin produced entirely different responses of P. ultimum. While catechol caused reduced growth of Pythium at concentrations of 10 ppm or more, (+)-catechin caused no inhibitory effects. However, (+)-catechin increased in concentration in slightly diseased hypocotyls. One reason for the discrepancy might be that (+)-catechin and other related compounds compose the catechin "equivalents" fraction measured by the spectrophotometer analysis. (+)-Catechin was used for the culture test and as a standard in the spectrophotometer tests. Therefore, the catechin levels detected in slightly diseased hypocotyls were probably not identical to the (+)-catechin used for growth studies.

The two cotton cultivars varied greatly in amounts of catechin contained in extracted plant tissue. In the case of 'Dixie King 3', catechin could be referred to as a plant phytoalexin. The obvious

distinction in catechin production between cultivars indicates that this may be a factor in cultivar resistance to P. ultimum. Further studies in this area are certainly warranted.

CHAPTER 5

SUMMARY

Two cotton (Gossypium hirsutum L.) cultivars were tested for resistance to Pythium ultimum Trow. 'Dixie King 3' was found to be more resistant to the fungal seedling blight pathogen than 'Delcot 277'. Nutrient status of the fungus affected pathogenicity. When P. ultimum grew on potato dextrose agar (PDA), disease severity was greater than when the fungus grew on dilute PDA or water agar.

Growth response of P. ultimum was measured on media containing water agar and healthy hypocotyl tissue of each cultivar in order to detect naturally occurring inhibitors. No inhibition was observed.

Chemicals implicated in disease resistance were tested for their fungistatic activity. Gossypol, catechin, and catechol were incorporated into culture media that was inoculated with agar disks of P. ultimum. Inhibition occurred at concentrations of 1,000 ppm of gossypol and 10 ppm of catechol. Catechin did not inhibit P. ultimum at concentrations of 1,000 ppm.

Uninoculated and inoculated hypocotyl tissue of 'Dixie King 3' and 'Delcot 277' cultivars were extracted and tested for gossypol and catechin "equivalent" concentrations. Gossypol concentrations decreased in both cultivars after infection occurred. Concentrations of catechin increased in the resistant cultivar following infection, but decreased in the susceptible cultivar. Catechin may act as phytoalexin in cotton plants of 'Dixie King 3', and may be responsible for differences in resistance observed between the cultivars.

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VITA

Elizabeth Dian Sutherland was born in Sewickley, Pennsylvania on September 27, 1953. She graduated from North Allegheny High School in June 1971. Miss Sutherland received a Bachelor of Science degree in Agriculture from The University of Tennessee in December 1975. In the summer of 1976 she accepted a research assistantship at The University of Tennessee, and began study towards a Master's degree.

The author is a member of Gamma Sigma Delta Agriculture Honor Society, Phi Kappa Phi National Honor Society, and The American Phytopathological Society. Miss Sutherland will be continuing her education at Michigan State University following graduation.