VERTICILLIUM WILT OF COTTON: 1) EFFECT OF MEDIA AND TEMPERATURE ON MORPHOLOGY AND PATHOGENICITY, 2) VASCULAR PLUGGING IN RESISTANT AND SUSCEPTIBLE COTTONS

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CHAPTER I

INTRODUCTION

The wilt of cotton incited by Verticillium albo-atrum Reinke and Berthold has become a very serious problem in many of the cotton growing states of the United States, and in many other countries.

V. albo-atrum, in addition to its wide distribution has an extensive host range, which includes at least 160 plant species belonging to 40 different families (31). Since the fungus is soil borne, and since cultural or chemical treatments have not proven economical for cotton production, resistant varieties seem to be the most promising method of controlling the disease.

Environmental factors influence the morphology and growth of the fungus on culture media. Disease severity is also highly influenced by the environment and often, in a population of susceptible plants, the disease does not develop uniformly.

Many theories are suggested as to the nature of the resistance mechanisms to vascular parasites (7): a) poor growth of a parasite because of inadequate nutrition or direct inhibition by the host, b) failure of an active parasite to produce specific toxic metabolites, c) an insensitivity of the host tissues to the toxic

metabolites of the fungus, or d) physical localization of the parasite.

The objectives of this investigation were to determine 1) the effect of different media and temperature regimes on morphology and pathogenicity of the fungus, 2) possible differences in the resistance mechanisms between resistant and susceptible cotton varieties.

CHAPTER II

REVIEW OF LITERATURE

History and Distribution

According to Presley (42), Nees von Esenbeck (37) established the genus, Verticillium, in 1816. In Germany, Reinke and Berthold (43) in 1879 isolated the fungus from diseased potato plants and established the species, Verticillium albo-atrum. In 1913 Klebahn (29) isolated a fungus from dahlia plants and named it Verticillium dahliae, saying that it differs from V. albo-atrum Reinke and Berthold in the production of abundant true microsclerotia in culture. But this has not been accepted by all investigators because of the argument that the original description of Reinke and Berthold also includes an illustration of true microsclerotia.

The pathogenicity of V. albo-atrum to cotton, Gossypium spp., was discovered in the United States in 1914 when Carpenter (13) observed two diseased cotton plants in a greenhouse at Arlington, Virginia, and isolated the fungus from them. Sherbakoff (50) in 1928 recorded the disease on cotton plantations in Tennessee. Since 1928 many investigators--Sherbakoff (50), Young (56), Herbert and Hubbard (23), Brown (11), Humphrey (24),

McLaughlin (35), Lehman and Gariss (30), Leyendecker (31), Presley (42) and others--have reported the occurrence of the disease in Mississippi, Tennessee, Arkansas, Texas, Arizona, California, New Mexico, Louisiana, and Oklahoma. According to Presley (42), the disease on cotton has been reported from Greece, Brazil, Peru, Africa, Central Asia and Bulgaria.

Effect of Environmental Factors

Most studies have shown that several factors influence the morphology or pathogenicity of V. albo-atrum, Rudolph (44) and Chaudhuri (14) have reported morphological changes by continuous cultivation on artificial media. Presley (42) states that V. albo-atrum is most pathogenic in heavier clay soils where soil reaction is neutral or slightly alkaline. He further mentions that Verticillium wilt is severe on soils high in organic matter. On the other hand, in soils with high amounts of potassium, the percentage of the wilt is decreased.

In 1949 Isaac (25), in a comparative study of pathogenic isolates of V. albo-atrum, stated that the optimum pH for the dark mycelial strains was pH 8.0 - 9.6 and for other strains 5.3 - 7.2. Malca et al. (33) reported that the growth of V. albo-atrum was best at a pH range of 5.9 - 6.3 and meager at less than pH of 4.

Temperature has been found to be an important factor

influencing morphology and pathogenicity of V. albo-atrum. Isaac (25) observed the optimum temperature in culture for all isolates to be 22.5 C. Kendrick and Middleton (28) reported that the maximum growth rate of the fungus occurred at 24 C, but good growth was made between 12 and 30 C. They further noticed increased disease severity on six varieties of pepper which have a maximum growth at an average air temperature of 24 C, and an average soil temperature of 30 C. They concluded that disease expression was more closely associated with growth of the pathogen than with growth of its host. Wilhelm (55) reported that a temperature difference of 3 - 6 C within the growing range (10 C - 31 C) of V. albo-atrum may produce marked differences in cultural appearance and in morphological characters, particularly of the resting stages. He found that colonies of the wild type grown at low temperatures (10 - 22 C) or during winter at Berkeley, California, are jet black and growth consists almost entirely of thick microsclerotial crusts. At higher temperatures (25 - 31 C), the colonies of the same fungus were reported to be creamy white and have only sparse development of microsclerotia.

Garber and Halisky (18) reported that the most favorable soil temperatures for disease expression in a wilt-susceptible cotton variety, Deltapine 15, to be 20 and 25 C. They mentioned that no wilt symptoms developed at a constant soil temperature of 35 C. They also made growth studies on potato dextrose agar (PDA) at constant

temperatures ranging from 12 to 39 C at 3 C intervals. In their study, the largest colony diameters were observed at 18, 21 and 24 C. But they did not get any measurable growth at 33, 36 and 39 C. The absence of symptom expression in plants with 35 C constant soil temperature was accounted to the lack of growth of the fungus at high temperatures.

Ludbrook (32) reported that maximum disease development in tomatoes occurred at soil temperature of 24 C with V. albo-atrum (microsclerotial type); 28 - 30 C was reported as the upper limit for disease expression. Schneider (48) found that in Guayule, Verticillium wilt was severe at soil temperatures of 18 - 22 C. The activity of the fungus was reported to be nil between 27 C and 31 C.

Leyendecker (31) stated that in the laboratory, V. albo-atrum formed microsclerotia at temperatures above 20 C. In his findings, the minimum, optimum, and maximum development of the fungus on agar were 5 C, 25.5 C, and 30 C, respectively. He further observed that at 10 and 15 C, the mycelial growth is white and fluffy, and no microsclerotia are produced. Halisky, Garber and Schnathorst (22) recorded that 20 C- and 25 C-soil temperatures were most favorable for disease development in cotton. They noticed the absence of symptom development at a constant soil temperature of 35 C. Culture growth on agar was reported to be maximum at 21 C and 24 C and no measurable growth occurred above 30 C.

Brinkerhoff, Samayoa and Murray (10) reported that the cotton varieties, Seabrook Sea-Island, Stoneville 7A, and Stoneville 62, when inoculated and held for 7 days at diurnal 36 - 18 C before transferring to diurnal 28 - 18 C, reacted as resistant, tolerant, and susceptible, respectively. Furthermore, they stated that plants that remained at diurnal 36 - 18 C showed no symptoms; however, the fungus was readily isolated from the three varieties from the inoculated area of the stem up to 21 days after inoculation.

Selman and Buckly (49) studying factors affecting the invasion of tomato roots by V. albo-atrum reported that sucrose level influenced the ability of the fungus to invade systemically. With 200 ppm of sucrose growth was restricted to the piliferous (hairy) layer; with 2000 ppm the fungus distribution was restricted to the outer layer of cortical cells and lateral root tips; and with 20,000 ppm, the fungus was distributed in all root tissues including the xylem. Malca et al. (33) reported that except for L-sorbose, the fungus utilized most carbohydrates efficiently although a prolonged lag phase occurred when lactose or D-ribose was supplied as a carbon source. They found L-arabinose and maltose to be the best carbon sources for growth, and the utilization of ammonium nitrogen better than nitrate nitrogen.

According to reports of Leyendecker (31), Evans et al. (17), Presley (42) and other workers, under normal conditions V. albo-atrum remains alive in soil over long

periods of time. It is believed to survive as resting microsclerotia until a suitable host is present.

Brandt (9) reported that white, flourescent light delayed or prevented production of microsclerotia and melanin in 14 of 16 microsclerotial isolates on agar, and in 30 of 31 isolates which produced more melanin and microsclerotia on a complex medium than in a defined medium (sucrose-nitrate liquid). Kaiser (26) found that microsclerotia of two isolates of V. albo-atrum formed on colonies exposed continuously for periods up to 10 days to red, orange, yellow, and green light; but not when exposed to blue light. He reported that in all instances, the development of microsclerotia was almost completely inhibited by exposure to blue light for extended periods.

Biotypes of the Fungus

Presley (42) reported that V. albo-atrum consists of numerous biotypes which differ greatly on nutrient media. He put most of the isolates into four main cultural groups designated as X, A, B and C. He described type X to be the normal type usually isolated from diseased plants. It consists of abundant mycelium and microsclerotia which give the colony the typical black and white appearance. Type A consists entirely of microsclerotia which give the colonies a black or dark gray appearance; type B consists of fluffy white mycelium with no microsclerotia; and type C consists of appressed white mycellium with no

microsclerotia. He associated pathogenicity with cultural type in that isolates of type A were very virulent while those of type B and C were weakly pathogenic on a susceptible variety of cotton.

Schnathorst and Mathre (47) reported that two isolates, T-1 and SS-4, which were isolated from the San Joaquin Valley of California, differed in symptom production and disease severity when tested on susceptible, tolerant, and resistant cotton varieties. They reported that virulence of the T-1 isolate was 10-fold greater than that of the SS-4 isolate on some cotton varieties and 100-fold greater on others. They stated that no cotton variety or hybrid tested, representing previously tolerant and highly resistant lines, was resistant to the T-1 isolate in the greenhouse.

Host-Parasite Interactions

Leyendecker (31), reporting on internal symptoms, mentioned that by the time a diseased plant is defoliated its entire vascular system is discolored. From this he reasoned that the fungus was probably preventing water movement from the roots to the leaves. But commenting on leaf symptoms he says that they are not characteristic of typical water deficiency. From his histological studies of the main stem, Leyendecker found that the fungus caused injury only in the xylem tissue while other parts of the stem were healthy. He noted that mycelial development in the main stem was sparse in spite of

extensive browning. He, however, observed that the mycelium was abundant in the xylem of the leaf mid-veins, petioles, and fruiting branches. He did not observe conidia or microsclerotia in the diseased xylem tissue. He contends that since the mycelial strands were most prevalent in the leaf veins, and the leaves were injured more severely than other parts of the plant, the main damage was caused by toxins liberated by the fungus. He further comments that although the mycelium is not really abundant in any part of the plant, its metabolic products are strong enough to discolor the stem, mottle the leaves, and finally defoliate the plant.

Goodman, Kiraly, and Zaitlin (20) state that infectious root diseases and vascular wilts disturb the normal water economy of the host plant in three ways: a) absorption of water by the infected root may be altered, or b) water transport in the invaded xylem may be impaired, or c) the pathogen may exert an effect on the stomatal and osmotic mechanism of leaves. However, they point out that the most important of these is the dysfunction of the water transport system in the xylem.

Beckman (7) states that once vascular infection has been established, resistance to disease may depend upon: a) poor growth of a parasite because of inadequate nutrition or direct inhibition by the host, b) failure of an active parasite to produce specific toxic metabolites, c) an insensitivity of host tissues to the toxic metabolites or an inactivation of the toxic metabolites by

the host, or d) physical localization of the parasite. He reported that systemically-inoculated leaves of broccoli (Brassica oleracea L. var. botrytis L.), tomato (Lycopersicon esculentum Mill.) and stem cuttings of cotton (Gossypium hirstum L.) with various forms of Fusarium oxysporum showed that neither nutritional deficiency nor growth inhibition was a factor in determining resistance to vascular infections of the fungus. Growth of the parasites and foliar symptoms in resistance-type interactions were comparable to or greater than that in comparable susceptible-type interactions. However, he found vascular occlusion was greater in the resistant-type interactions than in susceptible-type interactions. He noted that transpirational water loss dropped markedly within 24 - 48 hours after inoculation. He further observed that transpiration was restored to 80% of that of the noraml plant by recutting the stems to remove affected basal tissues. He believed the rapid occlusion was a result of gel formation rather than from the development of tyloses or mycelial masses. He further states that vascular occlusion appeared sooner and was more pronounced in all of the resistant interactions. He suggests that the small differences in transpirational water loss are not surprising, since occlusion in susceptible reactions differs from the sustained occlusion in resistant reactions only in that occasional intermittent shearing of successive gel plugs and the retardation of tylose

formation would permit a slippage of successive vascular blocks. He postulated that this slippage would permit systemic distribution of the pathogen even though the net increase in water transport would be slight. He believes that vascular occlusion is a primary factor for resistance when occlusion is considered on the basis of a time-space relationship. If a host responds quickly and in depth to an initial infection, that infection will be effectively sealed off and the plant with its reserve of functional capacity will not suffer. This plant is highly responsive to the infection but resistant to the disease. In further discussion, he mentions a sequence of physical barriers. He believes that first, perforation plates and vessel endings would trap spores carried in the transpiration stream; secondly, a gel would temporarily immobilize spores produced beyond the initial barrier; and thirdly, tyloses would permanently seal off the invaded vessel. Thus, he comments that since resistance depends on all of the three factors, there must be an overlapping of these factors with respect to space and time.

Talboys (53), in studies on the invasion of the hop plant by V. albo-atrum, noted that the pathogen's distribution and hence disease tolerance was significantly associated with the capacity of the host to respond to the presence of the pathogen in the vascular system by the formation of tyloses, together with the potential for a compensatory development of additional xylem. Commenting

on the host-parasite interactions he mentions that, once certain specific conditions in the root system after invasion have arisen, the ultimate fate of the individual plant is fully determined, although at that stage symptoms may not have appeared above the soil level. He calls this stage the "determinative" phase and the subsequent process leading to symptom expression as an "expressive" phase.

Page (38), from his observations on the water economy of Fusarium infected banana plants, reported that weight loss due to transpiration, and the volume of aqueous tracheal exudate was less in diseased plants than in healthy plants. He also noted that flow rate was slower through dysfunctional vessels than through healthy vessels. From these observations he came to the conclusion that water shortage resulting from tracheal obstruction was the main cause of an imbalanced water economy and subsequent injury to the plant.

Pierson et al. (39), in their histological studies on the role of pectic enzymes in the development of Fusarium wilt, found that the filtrate from the tomato wilt Fusarium contained pectin-methyl-esterase and depolymerase. When they applied this filtrate to tomato cuttings they got vascular plugging. They obtained similar vascular plugging and discoloration when they treated cuttings with a commercial pectic enzyme preparation containing pectin-methyl-esterase and polygalacturonase. They suggested that the gel in Fusarium infected tomato plants is pectinous on the basis of apparent origin and

staining reaction with Ruthenium Red.

Powers (41), studying the mechanism of wilting in tobacco plants affected by black shank, caused by Pythophthora parasitica (Dastur.) var. nicotinae Tucker, found that tyloses and gums as well as abundant masses of mycelium were found in the vessels of diseased tissue. He concluded that in black shank, tyloses and gums are a primary cause of the obstruction of water movement in diseased plants.

McClure (34) studying Fusarium wilt of sweet potatoes noted the presence of vascular discoloration in advance of the pathogen. He mentioned this as evidence that toxic by-products are produced and are carried in the transpiration stream. He states that low concentration of this toxic product stimulate and high concentration inhibit tylose formation. He further discusses that in the vicinity of the fungus the toxin concentration is high, thus inhibiting tylose formation. But as the toxic products are carried upward in the transpiration stream, the concentration decreases by diffusion to adjacent vessels, and tylose formation is stimulated. When the number of tylose-clogged vessels increases, the concentration of the toxic product increases in the remaining vessels and inhibits further tylose formation, and as a result an unclogged path may be provided to the fungus to by-pass the clogged vessels.

Waggoner and Dimond (54) reported that water flow was reduced by the presence of mycelium in vessels. They

stated that in a tomato vessel containing 10 hyphae of Fusarium whose diameter is 0.1 that of the vessel the water tension must increase 6 times if flow is not decreased. If water tension is not increased, they stated that flow will be reduced to about 17 percent.

Scheffer et al. (46) in their study on physiological aspects of V. albo-atrum of tomato, stated that vascular dysfunction in the form of browning and vessel blockage leads to vascular occlusion, which in turn interfers with water conduction. The culture fluid was found to contain a heat-liable vascular-browning factor. From enzyme assays of the culture fluids, they showed the presence of a polygalacturonase and very little pectin-methyl-esterase. From this, Verticillium is shown to differ from Fusarium in that the latter produced pectin-methyl-esterase and depolymerase.

Porter and Green (40) compared several isolates of V. albo-atrum as to relative pathogenicity and the production of a toxic fraction in a synthetic culture medium. The toxic fraction isolated from the culture filtrate proved to be a complex polysaccharide with extremely heterogenous molecular size.

Bell (8) reported that introduction of V. albo-atrum into boll cavities or xylem vessels of excised stems of G. hirsutum L. or G. barbadense L. induced a marked accumulation of ether-soluble phenolic compounds after 24 - 74 hours. He identified the compound as gossypol. The purified form of gossypol was found to have LD_{50}

values of 20 - 100 ppm (50 - 250 μm) against spore germination of various fungi. The behavior of the gossypol was found to be similar to that described for phytoalexins.

Edgington, Corden and Dimond (15), in their study on the role of pectic substances in chemically induced resistance to Fusarium wilt, found that tomato plants grown on a calcium-deficient nutrient became more susceptible to Fusarium wilt. When they treated tomato plants with the growth regulator naphthalene acetic acid (NAA) the plants became more resistant to the fungus. From this they suggested that both treatments change the nature of the pectic substances laid down in the plant and that this, in turn, affects the resistance of the plant to Fusarium wilt.

Garber (19) gives two views on the nature of resistance: a) the host is resistant if it does not provide a suitable growth medium; or b) the host presents effective defense mechanisms which prevent, inhibit, or restrict the potential multiplication and metabolism of the parasite.

CHAPTER III

MATERIALS AND METHODS

Effect of Media and Temperature on Morphology and Pathogenicity

Six culture media and two temperature regimes were used for growing *V. albo-atrum*. The purpose of the experiment was to determine the effect of different media and temperature regimes on the morphology and pathogenicity of three known isolates of the fungus. Three of the six culture media were Difco products (Difco Laboratories, Detroit, Michigan), and the rest were prepared in our laboratory. These media with their ingredients are given in Table I.

Concentrated prune extract was made by simmering 50 g of chopped-dried prunes in 100 ml of water until the prunes were soft. The mixture was strained through cheesecloth and the liquid portion filtered through Whatman No. 1 filter paper.

For preparing the cotton root medium, 250 g of wet lateral roots were comminuted with equal amounts of water in a high speed Omni mixer. The mixture was centrifuged at 8000 RPM for about 10 minutes. The supernatant was filtered through a sterile Milipore filter.

TABLE I

MEDIUM, INGREDIENT (SOURCE)
AND AMOUNT

Medium	Ingredient or Source	Quantity	
Potato Dextrose Agar	Difco's preparation	39	g
	Distilled water	1000	ml
Nutrient Agar	Difco's preparation	23	g
	Distilled water	1000	ml
Czapek Agar	Difco's preparation	49	g
	Distilled water	1000	ml
Talboy's Prune Agar	Prune extract	100	ml
	Lactose (Bacto)	5	g
	Agar (Bacto)	30	g
	Distilled water to make	1000	ml
Wiltbank's Agar	Sucrose	10	g
	Peptone (Difco Bacto)	5	g
	KH_2PO_4	0.5	g
	$MgSO_4\ 7H_2O$	0.25	g
	Na_2SO_3 (anhydrous)	0.05	g
	Agar	20	g
	Distilled water	1000	ml
Cotton Root Agar	Extract from 250 g wet lateral roots	500	ml
	Agar	34	g
	Distilled water to make	1000	ml

This filtrate was then added to the melted agar to make one liter.

Except for the cotton root medium, the pH of the other media was adjusted to 6.2. This was done after the media had been autoclaved and cooled to 45 C. The pH was adjusted by adding either 1 normal sterile H_2SO_4 or NaOH. About 25 ml of medium was poured into sterilized plates. The pH of the cotton medium was 6.3 and was not adjusted.

The three isolates of V. albo-atrum used for this experiment were Va-S, T-1, and SS-4. The first isolate (Va-S) was a local field isolate. Its abundant production of mycelium and microsclerotia on PDA places it in Presley's (42) X type cultural group. The other two isolates were originally isolated from the San Joaquin Valley in California, by Schnathorst and Mathre (47). The virulence of T-1 was reported to be 10-fold or greater than that of the SS-4 isolate. These two isolates were received from Dr. Mathre in 1966.

Spore suspensions from each of the isolates were made from one square inch of fungus growth (PDA) in 10 ml of sterile tap water. Dilutions 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} from each of the isolate-suspensions were prepared. Then about 1/4 ml from each dilution was streaked on separate PDA plates and left at room temperature for 1 day. On the next day single germinating conidia were isolated under a dissecting microscope. Isolation was done with a small loop that had been made

of flattened and sharpened wire. With this tool it is relatively easy to cut and remove the piece of agar containing a germinating conidium. Four germinating conidia were transferred to each of four plates containing PDA. After 3 days, growing single colonies were transferred to PDA plates and then incubated at 18 C for 12 days. On the 12th day, one monoconidial culture that had the parental characteristics and uniform growth was selected from each of the three isolates. From each of the selected cultures a small circular area of the fungus mycelium was cut with a small sterile cork borer (0.5 cm in diameter) and transferred to the six test media. Each isolate was replicated 10 times on each medium. Plates were covered with polyethylene plastic bags to minimize moisture loss and possible contamination. Half of the plates were placed in an incubator at 18 C and one half at 30 C.

After 15 days, the diameter of each colony was measured and the type of growth on each medium and temperature regime was recorded. The type of culture growth was recorded using Presley's (42) method of indexing but with slight modifications as follows:

X = abundant mycelium and microsclerotia, having both black and white appearance.

A = almost entirely microsclerotial with very little light colored mycelium around the edge of colony or scattered throughout.

B = fluffy white mycelium with no or very few microsclerotia.

C = appressed white mycelium with very few microsclerotia.

The pathogenicity of the three isolates grown at 30 C on PDA, Wiltbank's agar, Czapek agar, and cotton root agar was tested by inoculating 18 plants of three cotton varieties. The cotton varieties used in this test included Seabrook Sea-Island, Stoneville 7A, and Stoneville 62 which are resistant, tolerant, and susceptible to Verticillium wilt, respectively. The seeds were obtained from disease-free parents and had been delinted with sulfuric acid. Seeds were planted in 216, 6-inch clay pots filled with steam sterilized clay loam soil. Seeds of each of the three varieties were planted in each pot. The planted pots were randomized and placed in a greenhouse with a temperature range of 22 - 30 C and a light intensity of about 1000 ft C. Pots were fertilized when the plants were about the two leaf stage and about four leaf stage by adding each time 1 teaspoonful of an organic fertilizer (Milorganite), containing approximately 5.3% N and 3% P_2O_5. The plants were inoculated when they were 42 days old.

Inoculum for each of the fungus isolates was grown for 33 days at 30 C on potato dextrose agar, Wiltbank's agar, Czapek's agar, and cotton root agar. Inoculum was prepared by removing the fungus growth with a sterile scalpel and chopping it in 100 ml of sterile-deionized-distilled water for 30 seconds in a blender. The mixture

was strained through four layers of cheesecloth to remove some of the larger pieces and then counts were made with a hemacytometer to determine the concentration of fungal propagules. Afterwards the inoculum was diluted so that it contained approximately 1×10^6 fungal propagules per ml. Inoculation was done on the same day of preparation.

Evans' (16) stem puncture technique was used as a means of inoculation. This was done by pushing a sterile dissecting needle through the stems of cotton plants about $1\frac{1}{2}$ inches above the soil line into a cellulose sponge saturated with inoculum. As the needle was withdrawn the sponge was squeezed against the stem, allowing fungal propagules to be drawn into the vascular tissue. A total of 198 plants of the three varieties of cotton were inoculated by this method. Eighteen control plants (six of each variety) were treated in the same way using sterile tap water as inoculum.

Temperature was adjusted to 36 C day and 18 C night, 3 days before inoculation. One week after inoculation, the day-time temperature was lowered to 27 C. The night temperature remained the same, 18 C. The purpose of the one week high-temperature treatment was to permit resistance to be expressed when the inoculated plants were removed to temperatures that favor disease development. It was hoped that differences in pathogenicity of the three isolates that might have developed on different media and at different temperatures could be determined.

Symptom development was recorded 20 days after inoculation. Disease development was indexed using the following criteria:

1 = No symptom expression

2 = Epinasty and slight chlorosis of leaves

3 = Chlorosis and one up to 25% leaf defoliation

4 = 25 - 75% defoliation of leaves

5 = 75 - 100% defoliation of leaves

Vascular Plugging in Resistant and Susceptible Cottons

The fungus-host interactions of three cotton varieties were studied to determine whether resistance or susceptibility to V. albo-atrum was related to possible alterations in the vessel cells. Seabrook Sea-Island, Gossypium barbadense L., was used as a representative of a resistant variety. Stoneville 62, G. hirsutum L., was a representative of a very susceptible variety. Another variety, Ok 141-5, G. hirsutum L., which showed resistance under field conditions, was also included in the tests. From each variety, acid delinted seeds were germinated in a bucket filled with moist vermiculite. Five to six day old seedlings (in the early cotyledonary leaf stage) were transplanted to 12 quart buckets containing a modified Hoagland's nutrient solution. Each seedling was held in place by packing cotton lint around the stem of the plant which was inserted through holes in plywood or plastic lids. Eighteen containers were placed in each

of three tanks of circulating water with a temperature of 20 C. Air temperature ranged between 20 and 30 C.

Fifty five day old plants of Seabrook Sea-Island and Stoneville 62, were inoculated with a typical field isolate of V. albo-atrum. Five buckets with four plants each of each variety were transferred to a climate control box under a temperature regime of 36 C day and 18 C nights.

Inoculation was carried out in the following manner. To reduce contamination, stems were wetted with 70% alcohol by daubing with saturated cotton. Each stem was excised from its base and was dipped into a suspension of the fungus with a concentration of 1 X 10^6/ml fungus propagules that had been prepared from a four day old PDA culture. The plants were permitted to take up the inoculum for 30 minutes. Control plants were treated in the same manner except sterile water was used instead of inoculum. Each excised stem was then transferred to a 250 ml Erlenmeyer flask that contained 225 ml of sterile tap water and was plugged with cotton. After inoculation each flask was placed in the climate control chamber, under 2000 ft C of light, and programmed for 12 hours of light and 12 hours of dark at 36 and 18 C, respectively.

Fresh free-hand sections were employed for this test. After inoculation four plants of each variety were immediately moved to the laboratory for sectioning. Longitudinal sections ranging approximately 30 to 100 μ

were prepared from the inoculated area using a sharp single-edged blade. These were floated on distilled water in a petri dish. The floating sections could be transferred fairly easily to a glass slide for staining.

For staining, Gurr's (21) method, using safranin and picro-aniline-blue, was used. Materials required for this method were:

A. Safranin, 1% in distilled water

B. Aniline blue, water soluble, 2.5 g
Distilled water 25 ml
Picric acid, saturated, aqueous, 100 ml

The staining procedure was as follows:

1. Staining one to three minutes in solution A; then washing in water.

2. Flooding sections with solution B and heating over a flame until the liquid begins to simmer; then pouring off excess liquid and allowing the slide to cool before washing with water.

3. Washing with 70% alcohol followed by absolute alcohol; clearing in clove oil; mounting in balsam.

Samples were sectioned and stained at intervals of 24, 48 and 96 hours after inoculation. Stained and mounted sections were examined in detail under the microscope. Black and white photomicrographs of representative samples were made.

Using the three cotton varieties that had been grown in the modified Hoagland's nutrient solution, comparative studies were made on the amount of water flow through the petioles. Two types of inoculating procedures were used for this study. The first procedure was designed to determine whether there was a difference in water

conduction in resistant and susceptible varieties as a result of gel formation following inoculation. Seabrook Sea-Island, Ok 141-5, and Stoneville 62 were used as test varieties. Petioles from two month old plants grown in nutrient solutions were inoculated with the field type isolate of V. albo-atrum. The inoculum contained approximately 1 X 10^6 conidia per ml. The method of Bugbee and Presley (12) was used to inoculate the petioles. A 5 ml capacity syringe with a #27 gauge needle was used for injection. The tip of the needle was just barely pushed into the base of the petiole, and a drop of inoculum was allowed to be drawn into the petiole. The same procedure was repeated on the opposite side of the petiole. This was done so that most of the vessel cells would be exposed to the inoculum. Petioles from four plants of each variety were inoculated using 1 X 10^6/ml of fungus inoculum. Petioles from the other four plants were treated with sterile water as a check.

Water flow through randomly harvested petioles from each variety was determined after 1 day and 2 days of inoculation. Each time 16 petioles from each variety were measured. For measuring water flow, a modified type of Melhus et al. (36) fluometer apparatus was used (Fig. 1). A piece of petiole 4 cm long severed at the point of inoculation was attached to the rubber tube of the fluometer and water was sucked through the piece for 5 minutes applying a constant vacuum. At the end of the 5 minutes, suction was stopped and the amount of water

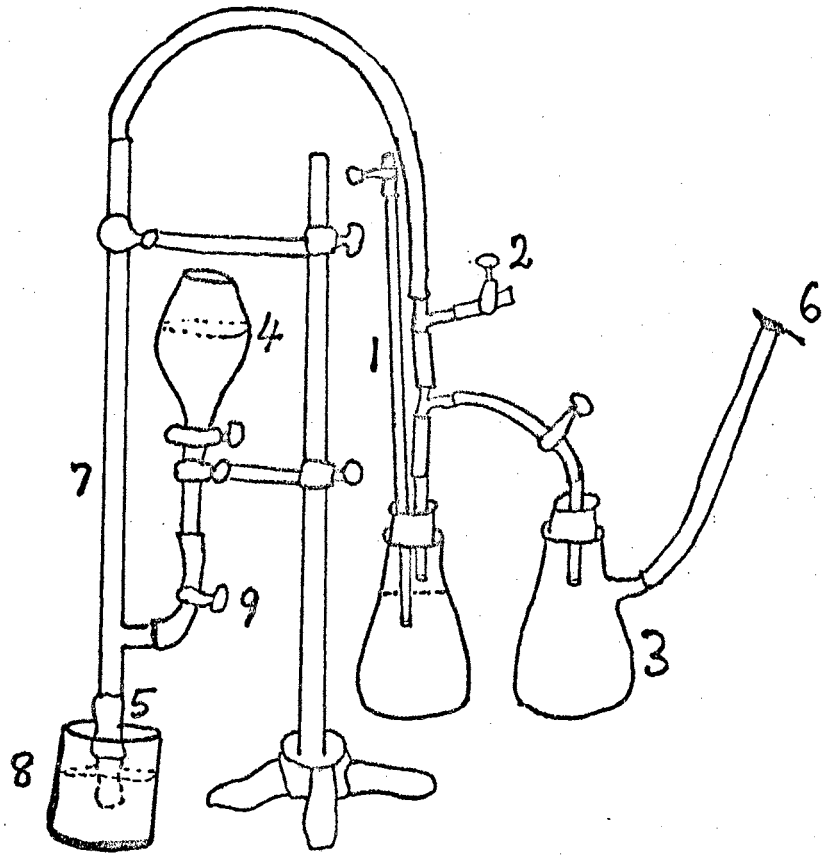

Figure 1. Fluometer. 1 - Dye solution column showing reduced pressure obtained by the vacuum, #6. 2 - Clamp for releasing the vacuum on the burette while changing specimens. 3 - Filter flask which serves to prevent backing water from entering the system after the filter pump is shut off. 4 - Separating funnel reservoir. Water is admitted from this funnel into the burette, #7. 6 - Source of vacuum. 7 - Graduated burette into which the water is pulled through the specimen. 8 - Jar of water into which the end of the specimen is placed. 9 - Clamp for closing the connection between the reservoir, #4 and the burette, #7.

[Modified after Melhus et al. (36)]

raised in the burette was recorded. Average diameter measurement in milimeters was also taken for each of the petioles.

The second procedure was designed to determine the amount of water flow through petioles from the three varieties of cotton that had been inoculated at the base of the stems and kept until symptoms developed. From two month old plants that had been growing in modified Hoagland's nutrient solution, 24 plants from each variety were used. Each stem was inoculated at its base with a hypodermic syringe with 1/4 ml of a conidial suspension containing 1 X 10^6 conidia per ml. Check plants were inoculated with sterile tap water.

After inoculation, half of the number of plants of each variety were moved to a climate control box, programmed for 2000 ft C and 12 hour light and 12 hour dark periods. Temperatures were adjusted for 36 days and 18 C nights. The remaining half of the plants were kept in water tanks at 20 C in the greenhouse. The air temperature of the greenhouse ranged from 20 - 30 C. Seven days after inoculation, the plants from the climate control box were moved to the water tanks in the greenhouse.

Symptoms were observed 9 days after inoculation on Stoneville 62 and Ok 141-5 that had been left at root temperatures of 20 C in the water tank. For plants under both temperature regimes symptoms were very distinctive by 12 days, at which time disease readings were made

using the method described earlier. Also on the 12th day 16 petioles from each variety and each temperature-treatment were harvested at random. These were brought to the laboratory and amount of water flow was determined. The procedures were the same as for the previous measurement.

Stem samples from the above treatments were harvested randomly 21 days after inoculation. Using the same procedures and materials as before, free-hand sections were prepared from the inoculated zone and 3 cm above the inoculated zone. Sections were examined with a compound microscope and representative samples were photomicrographed.

CHAPTER IV

RESULTS

Culture Growth and Type

The three *V. albo-atrum* isolates that had the same type of culture growth, X type, at the beginning of this study, when grown on different media and two temperature regimes, showed differences in colony size and type of culture. In general all the four culture types were represented under the high, 30 C, temperature. The three isolates grown on cotton root agar medium had the same B-type growth (fluffy white mycelium without microscelrotia), at both temperature treatments. The C type (appressed white mycelium with very few microsclerotia) was observed for all three isolates when grown on Czapek agar and for the SS-4 isolate alone on potato dextrose agar at 30 C. More B-type growth was observed for T-1 and SS-4 isolates under 18 C treatment. Va-S had more of X-type growth under the 18 C treatment.

Regarding colony size, all three isolates showed better growth on all media at 18 C than at 30 C. Maximum colony size for all isolates was obtained on cotton root agar (Table II). Colonies of the three isolates after 15 days of growth are shown in Figures 2 and 3.

TABLE II

MEAN OF COLONY SIZE AND CULTURE TYPE FOR EACH OF THE THREE ISOLATES OF *VERTICILLIUM ALBO-ATRUM* AFTER 15 DAYS GROWTH ON DIFFERENT MEDIA AND UNDER TWO TEMPERATURES*

Medium	18 C Treatment					
	Va-S		T-1		SS-4	
	Mean (cm)	Culture Type	Mean (cm)	Culture Type	Mean (cm)	Culture Type
Potato Dextrose Agar	4.8	X	4.1	X	4.1	X
Cotton Root Agar	6.1	B	5.7	B	6.1	B
Czapek Agar	4.5	A	3.3	X	6.0	X
Wiltbank's Agar	4.3	X	5.1	B	6.0	B
Nutrient Agar	3.4	X	4.2	B	4.2	B
Prune Agar	4.7	X	3.5	X	3.6	B
Grand Mean by Isolate	4.6		4.3		5.0	
	30 C Treatment					
Potato Dextrose Agar	3.4	X	2.9	X	2.1	C
Cotton Root Agar	4.3	B	4.9	B	4.8	B
Czapek Agar	3.6	C	2.2	C	2.7	C
Wiltbank's Agar	3.4	X	3.3	A	3.7	A
Nutrient Agar	3.1	X	2.9	X	2.3	X
Prune Agar	3.1	B	2.1	B	1.8	B
Grand Mean by Isolate	3.5		3.1		2.9	

*Actually the colony size that is recorded is 0.5 cm less than each observation in the table because a 0.5 cm plug of the inoclum was used.

TABLE III

RANK OF MEDIUM IN TERMS OF PREFERABILITY FOR THE GROWTH OF THREE ISOLATES UNDER 18 AND 30 C INCUBATION. RANK BASED ON AVERAGE SIZE OF COLONIES ON EACH MEDIUM

	18 C Treatment			30 C Treatment		
	Va-S	T-1	SS-4	Va-S	T-1	SS-4
Potato Dextrose Agar	2	4	5	3	3	5
Cotton Root Agar	1	1	1	1	1	1
Czapek Agar	4	6	2	2	5	3
Wiltbank's Agar	5	2	2	3	2	2
Nutrient Agar	6	3	4	5	3	4
Prune Agar	3	5	6	5	6	6

Figure 2. Colonies of three isolates of Verticillium albo-atrum after 15 days of growth at 18 C. Isolates in columns from left to right: Va-S, T-1 and SS-4. Media in rows from top to bottom: potato dextrose agar, cotton root agar, Czapek agar, and Wiltbank's agar.

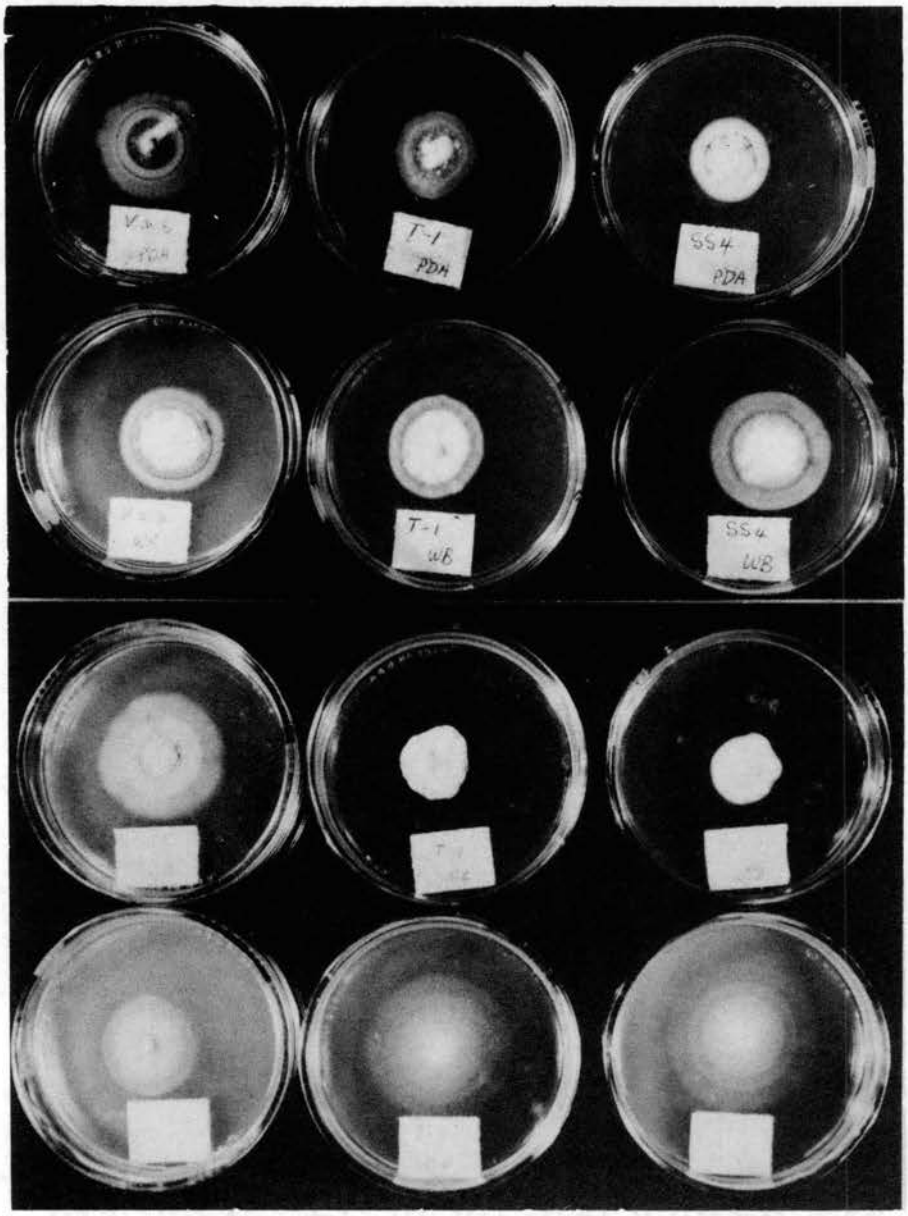

Figure 3. Colonies of three isolates of Verticillium albo-atrum after 15 days of growth at 30 C. Isolates in columns from left to right: Va-S, T-1, and SS-4. Media in rows from top to bottom: potato dextrose agar, Wiltbank's agar, Czapek agar and cotton root agar.

Pathogenicity Test

Twelve days after inoculation, typical Verticillium wilt symptoms on Stoneville 62 and Stoneville 7A varieties were observed. Symptoms included epinasty and slight chlorosis of leaves. No symptoms were observed on Seabrook Sea-Island at this time. Disease readings on symptom developments were taken 20 days after inoculation (Table IV). The data show relatively little difference in pathogenicity for isolates on different media except for the disease condition on Seabrook Sea-Island and Stoneville 7A due to SS-4 which was grown on cotton root agar (Fig. 4).

Pathological Histology

Free-hand sections that were prepared immediately after inoculation did not have any gel or tyloses in the vascular system in either resistant or susceptible varieties. Twenty-four hours after inoculation, sections from the resistant variety, Seabrook Sea-Island, had small gel masses at vessel ends and perforation plates. No gel formation was observed in vessels from the susceptible variety, Stoneville 62, at the same period of time. After 2 days, vessel cells near the point of inoculation of Seabrook Sea-Island were practically filled with big amorphous gel masses. Gel accumulations in Stoneville 62 were small and scattered by the end of 2 days. On the 4th day, very extensive small tyloses were observed above

TABLE IV

MEAN INDEX[a] OF DISEASE DEVELOPMENT GROUPED BY MEDIUM, ISOLATE AND VARIETY[b]

	Va-S			T-1			SS-4		
Medium	SB	Sto 7A	Sto 62	SB	Sto 7A	Sto 62	SB	Sto 7A	Sto 62
Potato Dextrose Agar	2.0	4.7	5.0	1.2	4.0	3.2	1.3	3.2	3.7
Wiltbank's Agar	1.8	4.5	3.7	1.0	2.8	3.3	2.0	3.3	3.8
Czapek Agar	2.0	4.7	4.7	1.2	3.2	3.7	2.2	3.2	4.7
Cotton Root Agar	2.2	3.8	3.8	1.0	3.0	3.2	3.5	4.8	4.7

[a] Key for Disease Index: 1 = No symptom expression, 2 = Epinasty and slight chlorosis of leaves, 3 = chlorosis and up to 25% leaf defoliation, 4 = 25 - 75% defoliation of leaves, 5 = 75 - 100% defoliation of leaves.

[b] Seabrook Sea-Island (SB), Stoneville 7A (Sto 7A), Stoneville 62 (Sto 62).

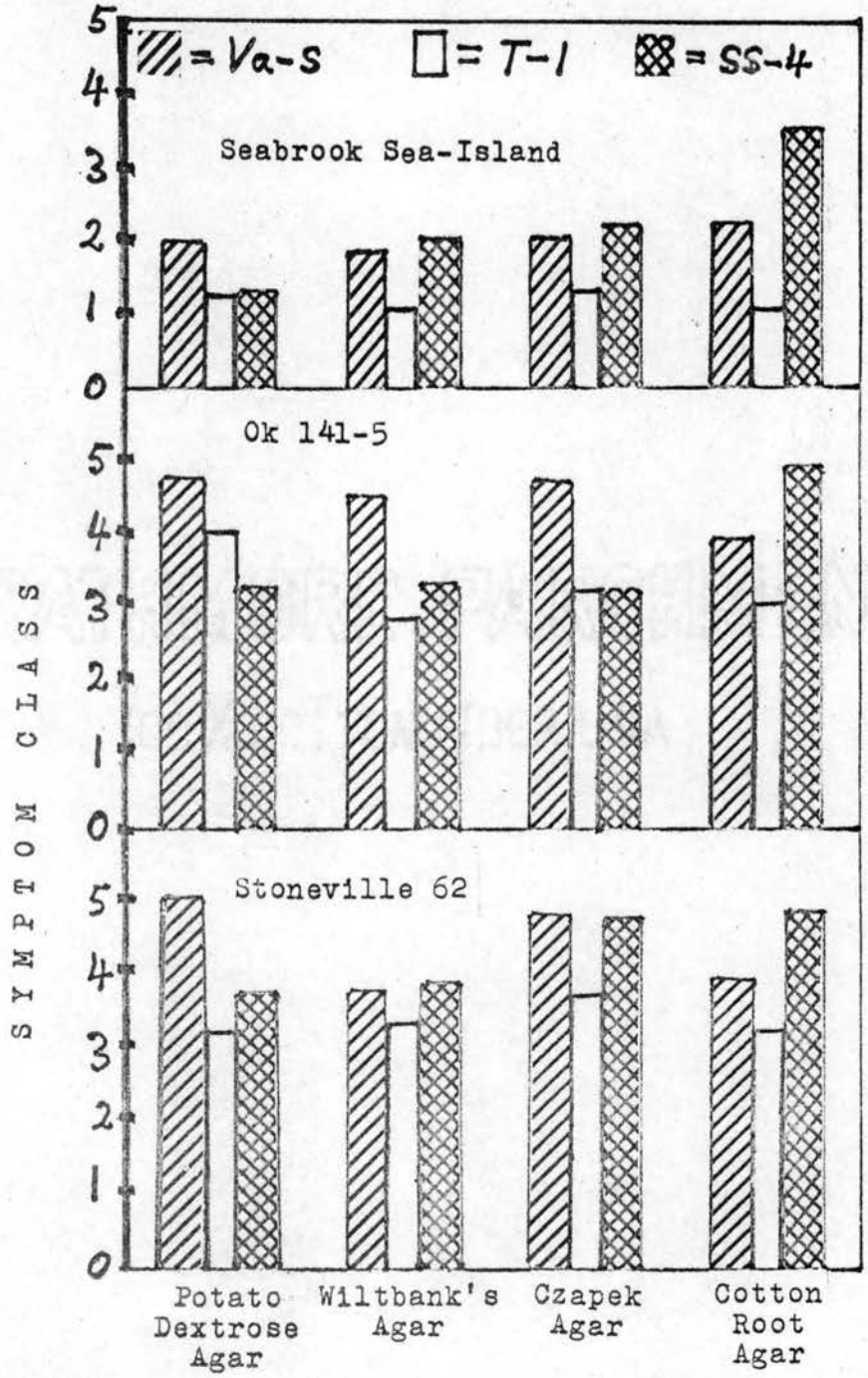

Figure 4. Mean of disease development 20 days after inoculation. Graph shows grouping by medium, variety, and isolate.

the point of inoculation in the resistant variety. By this time, gel formation had become very extensive in the susceptible variety. However, the number of tyloses was fewer than in the resistant variety. During these periods, no mycelial growth was observed in any of the varieties (Figure 5).

Histological sections from stems that had been inoculated by the hypodermic injection method and kept for 21 days showed extensive mycelial growth in both Ok 141-5 and in the susceptible, Stoneville 62, varieties. In both varieties mycelial growth was observed from the area of inoculation and 3 cm above the point of inoculation. No mycelial growth was observed in Seabrook Sea-Island. However, tyloses completely clogging vessels were seen in many areas. Vessel cell walls of Ok 141-5 and Stoneville 62 were thickened, discolored, and disrupted at places. Much of the mycelial growth was linear in relation to the vessel cells. However, some radial growth also was observed through pits and disrupted sites to the adjacent vessel cells (Figure 6).

Water Flow Measurements

This test was designed to determine the reduction of water flow that might result from possible gel or tylose formation. Water flow through inoculated and check petioles after 1 day and 2 days was recorded. The techniques and apparatus used for this test are the ones that are described in Chapter III. Comparing the rate of

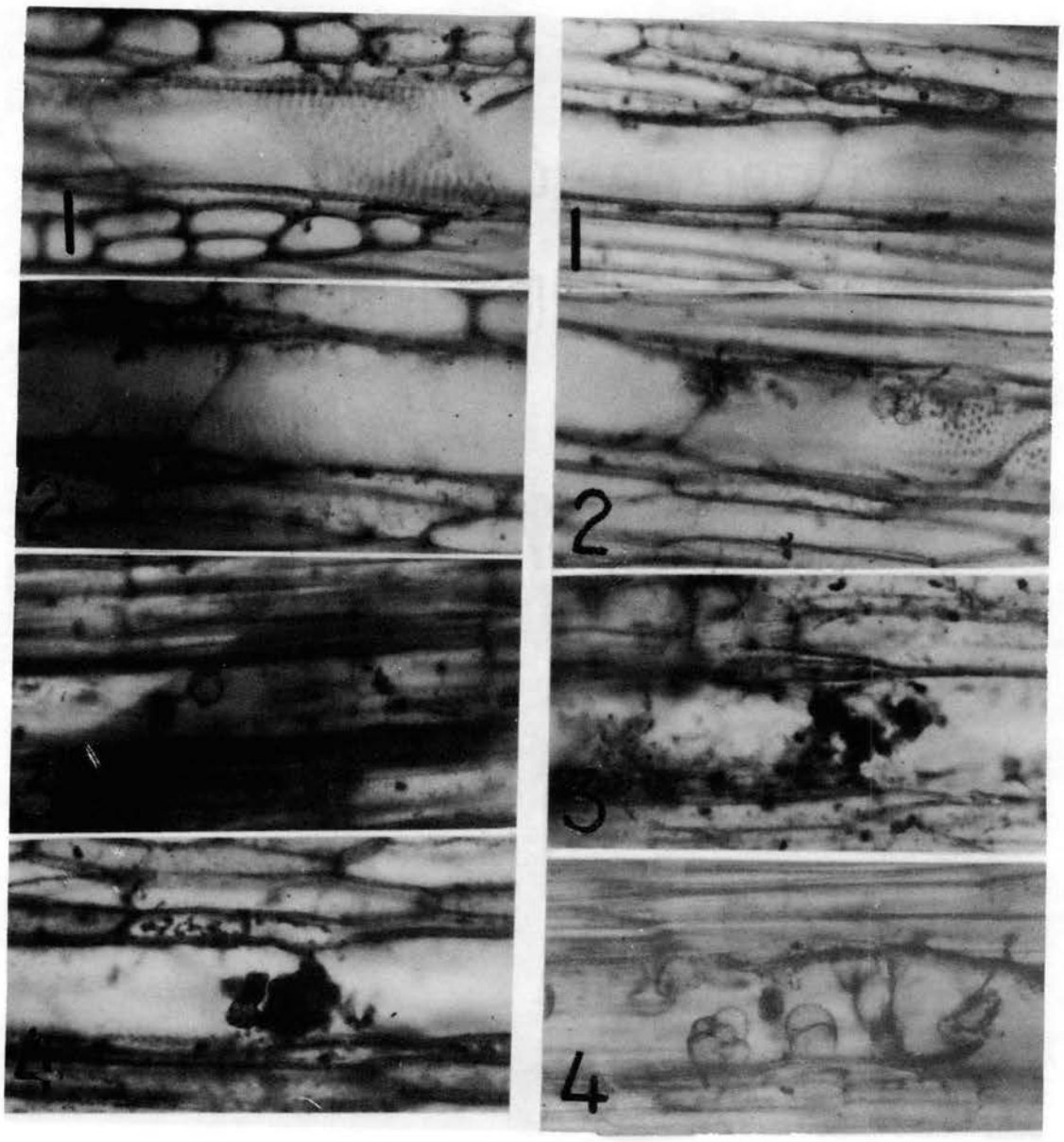

Figure 5. Longitudinal sections of stems showing the physical vascular behavior of Stoneville 62 (left column) and Seabrook Sea-Island (right column). Sections 1, 2, 3, and 4 for both varieties were prepared 0, 24, 48, and 96 hours, respectively, after inoculation (1500 X).

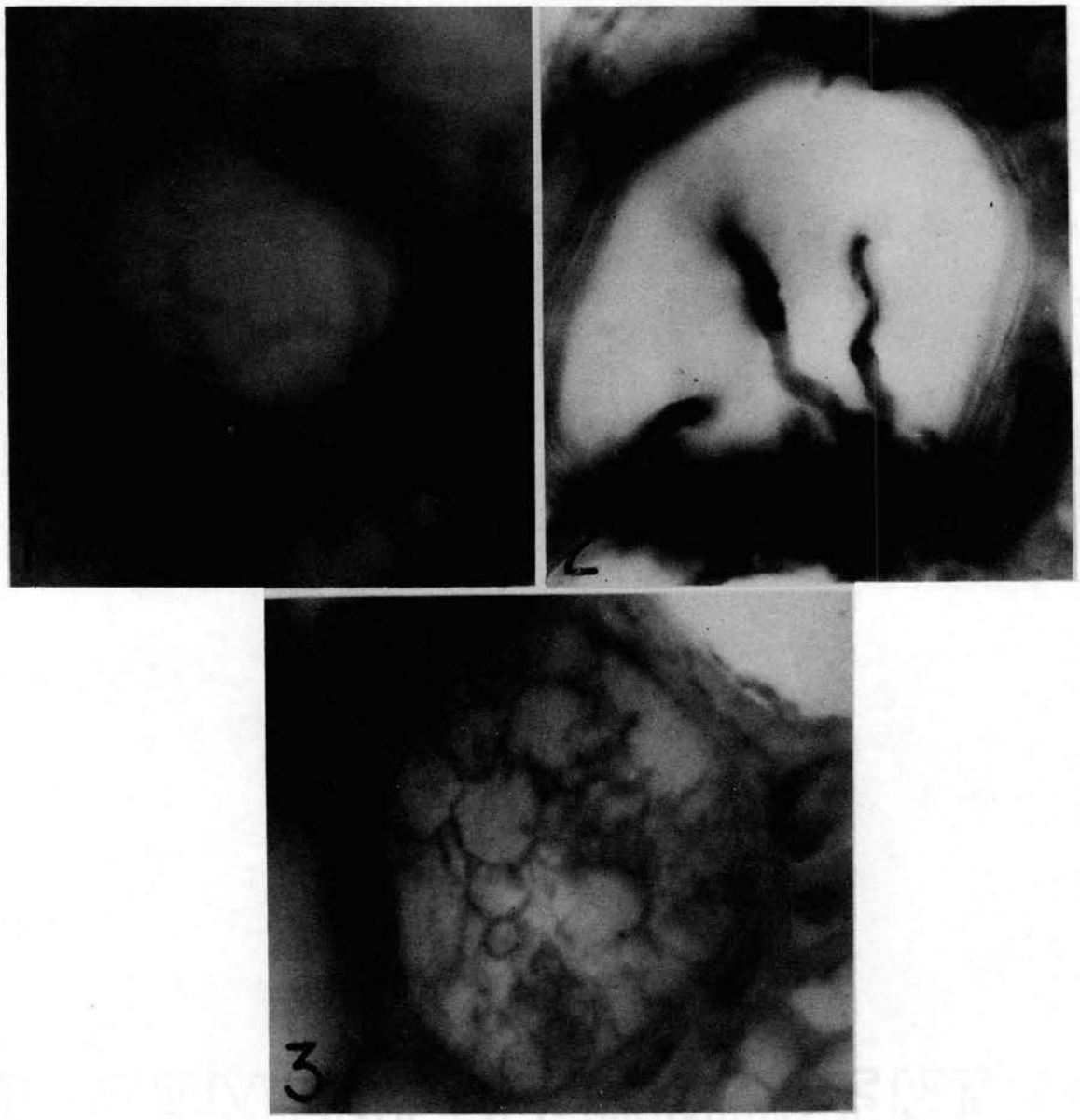

Figure 6. Cross sections of stems of three cotton varieties 21 days after inoculation with Verticillium albo-atrum. 1) Stoneville 62 vessel showing fungus mycelium. 2) Fungus mycelium in Ok 141-5 3) Seabrook Sea-Island with tylose formation (1177 X).

water flow through the same size petioles, it was determined that the three varieties of cotton differ in their capacity to conduct water. This natural variability was thus found to be a limitation in comparing two plants with the same size petioles from two different varieties. To overcome this problem, the average water flow per mm^2 in 5 minutes was found for the check petioles in each variety. Flow per mm^2 of each of the inoculated petioles was calculated from actual flow measurements and petiole sizes. Then each flow from the inoculated petioles was expressed in terms of percentage based on the average check flow. Example: If the check average for a given variety was found to be 1 ml/mm^2 and the flow in one of the inoculated petioles in the same variety was 0.5 ml/mm^2, then the percentage of water flow through the inoculated petiole will be:

$$0.5 \times 100 = 50$$

All measurements in the three varieties were thus expressed in percentages. The rate of water flow through the inoculated petioles, 1 day and 2 days after inoculation, varied with the variety (Table V). Measurements, 1 day after inoculation, indicated that water flow was impeded the most in the resistant variety, somewhat less in the tolerant variety, and least in the susceptible variety. By the second day, water flow in the susceptible variety was impeded as much as in the resistant variety; flow was not impeded in the tolerant variety on the second day. Differences between varieties of cotton were found

TABLE V

AVERAGE RATE OF WATER FLOW THROUGH INOCULATED PETIOLES EXPRESSED AS PERCENTAGE BASED ON THE AVERAGE FLOW OF THE CHECK PETIOLES. MEANS ARE FOR 1 DAY AND 2 DAYS.

Variety	MEAN		GRAND MEAN
	1 Day	2 Days	
Seabrook Sea-Island	59.2	78.6	68.9
Ok 141-5	75.3	102.3	88.8
Stoneville 62	95.2	76.0	85.6

TABLE VI

ANALYSIS OF VARIANCE OF PERCENTAGE OF WATER FLOW FOR DATA SHOWN IN TABLE V AND APPENDIX B

Source of Variation	d.f.	S.S.	M.S.	F
Total	95	87089.625		
Replications	1	35.0417	35.0417	
Days	1	1962.0417	1962.0417	2.57
Varieties	2	7292.2500	3646.1250	4.77 *
Varieties X Days	2	9837.5833	4918.7917	6.44 **
Error	89	67962.7083	763.6259	

* Significant at 5%

** Significant at 1%

to be statistically significant (Table VI).

Water flow was also recorded after the development of symptoms. Petioles from plants that had been inoculated in the hypocotyl region of stems and treated with the high and low temperature regimes were harvested at random after 12 days of inoculation. Sixteen petioles from each variety and each temperature treatment were sampled. Like the previous test, each measurement of the inoculated petiole is expressed as % based on the average flow of the check. Water flow was greatly reduced in all varieties with the low (20 C) temperature treatment. Petioles from Seabrook Sea-Island plants that had been treated with the high (36 X 18 C) temperature regime had an average water flow almost comparable to the check (Table VII). Analysis of variance (Table VIII) indicates differences between the two temperature regimes and varieties to be statistically significant.

Disease Development

Disease readings were very distinctive between the varieties in both temperature regimes. After 12 days, Seabrook Sea-Island plants under the low (20 C) temperature treatment showed epinasty and slight chlorosis; Ok 141-5 developed extensive chlorosis and some leaf defoliation; and leaf defoliation was very extensive in Stoneville 62. When treated with temperature regime (36 X 18 C) and moved to the low temperature (20 C), Seabrook Sea-Island showed high resistance; but disease

TABLE VII

AVERAGE PERCENTAGE OF WATER FLOW THROUGH INOCULATED PETIOLES AT TWO TEMPERATURES 12 DAYS AFTER INOCULATION

Variety	MEANS	
	Low Temperature (20 C)	High Temperature (36 X 18 C)
Seabrook Sea-Island	29.4	98.0
Ok 141-5	43.0	52.5
Stoneville 62	26.9	51.6

TABLE VIII

ANALYSIS OF VARIANCE OF PERCENTAGE OF WATER FLOW FOR DATA SHOWN IN TABLE VII AND APPENDIX C

Source of Variation	d.f.	S.S.	M.S.	F
Total	47	46890.4792		
Temperatures	1	14111.0215	14111.0215	29.17 **
Varieties	2	4925.0417	2462.5209	5.09 *
Varieties X Temperature	2	7537.7910	3768.8955	7.79 **
Error	42	20316.6250	483.7292	

* Significant at 5%

** Significant at 1%

development was severe in both Ok 141-5 and Stoneville 62 after removal from the high temperature treatment. Most of the plants in Stoneville 62 showed similar or comparable disease development to those plants under the low temperature treatment (Table IX).

TABLE IX

DISEASE DEVELOPMENT INDEX[a] AFTER 12 DAYS FOR PLANTS THAT HAVE BEEN GROWN AT 20 C, AND AT 36 X 18 C FOR 7 DAYS BEFORE MOVING THEM TO 20 C

Variety	TREATMENT											
	Low Temperature (20 C)					High Temperature (36 X 18 C)						
	Plant Number					Plant Number						
	1	2	3	4	Mean	1	2	3	4	5	6	Mean
Seabrook Sea-Island	3	2	2	2	2.3	1	1	2	1	1	1	1.2
Ok 141-5	3	3	3	3	3.0	2	2	3	3	2	3	2.5
Stoneville 62	4	4	4	3	3.8	3	4	4	4	4	4	3.8

[a]Key: 1 = No symptom expression, 2 = Epinasty and slight chlorosis of leaves, 3 = Chlorosis and up to 25% leaf defoliation, 4 = 25 - 75% defoliation of leaves, 5 = 75 - 100% defoliation of leaves.

CHAPTER V

DISCUSSION

The results from the effects of media and temperature on the variability of the three isolates of *V. albo-atrum* indicate that environmental factors are very important for growth and the expression of different morphological characters. The greater growth at the lower temperature suggests that the disease might be more severe when the host plants are subjected to low temperature. Conversely, limited growth of the fungus under high temperature can be correlated with less or no symptom development when plants are grown above 30 C. Kendrick and Middleton (28), Garber and Halisky (18), Brinkerhoff, Samayoa, and Murray (10) have reported similar results.

Variation of the same isolate on different media indicates that the presence or absence of certain substances in the nutritional requirement of the fungus may influence the morphological and possibly also the genetical behavior as a result of selective advantage. The growth of the fungus on a wide range of media also may be indicative of the capacity of the fungus to survive under varied conditions in nature.

Considering the effect of the different media and

temperature on the pathogenicity of the three isolates, the differences in pathogenicity in this test are not very great. However, the pathogenicity of the SS-4 isolate that had been grown on cotton root agar is high in all of the three cotton varieties. Schnathorst and Mathre (47) reported the pathogenicity of the T-1 isolate to be 10 - 100 times that of SS-4. But in this test it was not higher than the other two isolates. Probably this might have been due to the repeated cultivation on the artificial media for a long time which influenced its virulence. However, the lower virulence of T-1 was not associated with the loss of ability to produce microsclerotia.

From the size of the colonies and the nature of the vegetative growth, and from the pathogenicity, at least of the SS-4 isolate, the fungus prefers a medium that is prepared from its special host. Changes in morphology from the X culture type to the B type that occurred on cotton root agar at both 18 and 30 C did not significantly change the pathogenicity of the fungus, except possibly SS-4. According to Presley (42) and others, the genetic variants of V. albo-atrum that had the B type of culture growth were found to be less pathogenic.

Finally, the results from this test and the work of others, strongly indicate that growth and morphological characteristics cannot be a reliable means in differentiating isolates unless one is able to reproduce similar environmental conditions.

The observations on the pathological histology and the findings on the uptake of transpirational water in infected plants indicate that vascular occlusion is an important factor for the expression of both resistance and susceptibility. Both the studies on anatomical sections and on the reduction of water flow show that vascular occlusion occurs more rapidly in the resistant variety than in the susceptible variety. This seems to be a very important mechanism for the prevention of the fungal propagules from becoming distributed systemically. Beckman (7), Talboys (53) and other workers have indicated that vascular occlusion is an important factor in the host-parasite relationship. Furthermore, they have indicated the occurrence of resistance when the time-space relationship is promptly met. That is, if the host responds quickly and in depth to an initial infection, that infection will be effectively sealed off and the plant, with its reserve of functional capacity, will not suffer.

Microscopic observation showed the absence of gel in the vessels of the susceptible variety after the first day and only small and scattered gel masses after 2 days. This same relationship was also indicated by the relative amount of water flow in the susceptible variety. Thus it would appear that the eventual, extensive gelation in the susceptible variety can be attributed to the fact that vascular occlusion occurred later in the susceptible than in the resistant variety; consequently, the pathogen

had already become systemic. This systemic gelation and later tylose formation no doubt are important factors in disease development. Abundant gels and tyloses throughout the vascular system would restrict water conduction and this certainly would be a factor in wilting, leaf scorching and other symptoms of the disease. Struckmeyer et al. (51) pointed out that the development of foliage wilt in oak followed extensive vascular plugging and this appeared to result from insufficient water rather than from a toxic substance.

Observations in this study and previous reports by Samayoa (45), and Brinkerhoff, Samayoa and Murray (10) indicated that when the plants were subjected continuously to 36 X 18 C temperatures, the disease did not develop. But when the plants were subjected to the high temperature regime for 4 to 7 days and then transferred to a lower temperature regime (27 X 18 C), the susceptible variety developed the disease. Their studies and the present study showed that growth of the fungus was inhibited by the high temperature. But there must be an additional explanation to this, because the present study shows that vascular occlusion also occurs in the susceptible variety although somewhat later than in the resistant varieties. Thus, the susceptible variety should also remain resistant after 7 days of high temperature because the fungus in its inactive stage should have been sealed by the vascular plugs. Beckman (7) suggests that the small differences found in transpirational water loss

are not surprising. Occlusion in susceptible reactions differs from sustained occlusion in resistant reactions in that shearing of gel plugs and the retardation of tyloses formation would permit systemic distribution of the fungus.

Mycelial growth was readily observed in vessel cells of Stoneville 62 and Ok 141-5 in histological sections from plants that showed disease symptoms. Mycelium was not observed in the resistant, Seabrook Sea-Island, variety; however, disease development did occur under continuous low temperature. These results seem to pose some fundamental questions such as: 1) Why the fungus did not show mycelial growth under the lower temperature in the vessels of Seabrook Sea-Island since it has been found that the fungus grows well at low temperature. 2) If mycelial growth is not observed in the vessels of the resistant variety at both high and low temperatures, why should disease symptoms appear in plants at low temperatures, but almost none at higher temperatures?

No clear explanation is possible from the present studies. Some speculations follow: 1) Because of favorable low temperatures, the fungus might be able to overcome the resistance mechanism of the resistant variety to distribute itself throughout the plant. But still due to the presence of possible inhibition factors, the growth of the fungus might not be so extensive as to be detected by the technique employed in this work.

Samayoa (45) indicated that the fungus was isolated from the inoculated area 28 days after inoculation. 2) Another explanation might be that the ability of the resistant variety to counteract possible toxic products under low temperatures has been altered. Porter and Green (40) have reported the presence of toxic products in culture filtrates of V. albo-atrum. As to the speculation of the inhibiting factor, Bell (8) has reported that cotton plants invaded by V. albo-atrum produce gossypol which inhibits spore germination.

Ok 141-5, which is known to be resistant to the fungus under field conditions, showed mycelial growth and disease development almost comparable to the susceptible variety, Stoneville 62. Possibly, the field environment is more favorable to gel formation or other resistance mechanisms in Ok 141-5. The water flow studies indicated that gels were formed almost as rapidly as in Seabrook Sea-Island, but by the second day the vessels were open again. Further studies on Ok 141-5 are desirable.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Three isolates--Va-S, T-1, and SS-4--of *V. albo-atrum* were cultivated on six culture media at two temperature regimes (18 C and 30 C). Morphological variability of the three isolates was observed on the different media at the two temperature regimes. Colonies were larger at the lower than at the higher temperature treatment. Pathogenicity differences, due to the effect of the media and the temperature treatments, between or within the isolates were not very great.

When inoculated plants were held at high temperature regimes (36 C day and 18 C night), disease development was inhibited.

The results from this test and the work of others strongly indicate that growth and morphology cannot be a reliable means in differentiating isolates unless one is able to reproduce similar environmental interactions every time he makes the test.

Resistant and susceptible cotton varieties were inoculated with the Va-S isolate. Free-hand sections were made 0, 2, 4, and 21 days after inoculation. The sections were stained with safranin and picroaniline blue and examined under a microscope. Within 1 day, gel

masses were observed in the vessels of the resistant variety. No gel was observed in the susceptible variety within the same period. However, 2 days after inoculation, gel formation also started in the susceptible variety. Sections, 21 days after inoculation, showed extensive mycelial growth in the susceptible variety. No mycelial growth was observed in the resistant variety.

Rate of water flow through infected petioles also indicated that vascular occlusion occurred more rapidly in the resistant than in the susceptible variety. This seems to be a very important mechanism for the prevention of the fungal propagules from becoming distributed systemically.

Because in addition to vascular plugging other factors also seem to have a role in the expression of susceptibility or resistance, chemical analysis of inhibitors and toxic products are very desirable.

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APPENDIX A

FREQUENCY AND DEGREE OF DISEASE DEVELOPMENT 20 DAYS AFTER INOCULATION ON THREE COTTON VARIETIES[1] THAT WERE INOCULATED WITH THREE ISOLATES OF VERTICILLIUM ALBO-ATRUM WHICH HAVE BEEN GROWN ON FOUR MEDIA AT 30 C FOR 33 DAYS

Symptom Class	Frequency in each Symptom Class											
	Va-S											
	Potato Dextrose			Wiltbank's Agar			Czapek Agar			Cotton Root Agar		
	SB	S 7A	S 62	SB	S 7A	S 62	SB	S 7A	S 62	SB	S 7A	S 62
1	1	0	0	2	0	0	1	0	0	1	0	0
2	4	0	0	3	0	0	4	0	0	3	0	0
3	1	0	0	1	1	2	1	0	0	2	3	3
4	0	2	0	0	1	4	0	2	2	0	1	1
5	0	4	6	0	4	0	0	4	4	0	2	2
	T-1											
1	5	0	0	6	0	0	5	0	0	6	0	0
2	1	0	0	0	1	0	1	0	0	0	1	0
3	0	2	5	0	5	4	0	5	2	0	4	5
4	0	2	1	0	0	2	0	1	4	0	1	1
5	0	2	0	0		0	0	0	0	0	0	0
	SS-4											
1	4	0	0	2	0	0	0	0	0	0	0	0
2	2	1	0	2	0	0	5	0	0	0	0	0
3	0	3	2	2	4	2	1	5	1	3	0	0
4	0	2	4	0	2	3	0	1	4	3	1	2
5	0	0	0	0	0	1	0	0	1	0	5	4

[1]Seabrook Sea-Island (SB), Stoneville 7A (S 7A), and Stoneville 62 (S 62)

APPENDIX B

PERCENTAGE OF WATER FLOW THROUGH INOCULATED PETIOLES ONE DAY AND TWO DAYS AFTER INOCULATION. PERCENTAGE OF FLOW FOR THE INOCULATED PETIOLES IS BASED ON THE AVERAGE FLOW OF THE CHECK PETIOLES

Variety*	OBSERVATIONS																	Grand Mean
	1	2	3	4	5	6	7	8	Mean	1	2	3	4	5	6	7	8	Mean	
	One Day - Rep I									One Day - Rep II									
SB	65	71	65	47	41	47	71	82	61.1	108	50	67	25	50	92	58	8	57.3	59.2
OK	80	70	70	60	70	60	80	80	71.1	100	33	67	100	100	87	100	67	79.3	75.3
ST	80	80	80	120	120	80	100	120	97.5	86	57	57	143	100	114	100	86	92.9	95.2
	Two Days - Rep I									Two Days - Rep II									
SB	100	79	43	64	57	93	71	100	75.9	100	80	130	70	70	80	60	60	81.3	78.6
OK	186	86	86	86	86	114	143	114	112.6	100	67	67	167	167	67	67	33	91.3	102.3
ST	86	43	57	86	86	57	57	43	64.4	80	120	60	80	120	60	120	60	87.5	76.0

*Seabrook Sea-Island (SB), Ok 141-5 (OK), Stoneville 62 (ST).

APPENDIX C

PERCENTAGE OF WATER FLOW THROUGH INOCULATED PETIOLES 12 DAYS AFTER INOCULATION. SAMPLES WERE TAKEN FROM PLANTS THAT WERE INOCULATED AND KEPT AT 36 X 18 C FOR 7 DAYS AND MOVED TO THE 20 C WATER TANK AND FROM PLANTS THAT WERE KEPT IN THE 20 C WATER TANK THROUGHOUT.

Variety	OBSERVATIONS																
	1	2	3	4	5	6	7	8	Mean	1	2	3	4	5	6	7	8	Mean
	Low Temperature (20 C)									High Temperature (36 X 18 C)								
Seabrook Sea-Island	43	43	21	29	21	21	14	43	29.4	46	123	108	77	92	100	100	138	98.0
Ok 141-5	43	29	43	57	57	29	43	43	43.0	60	60	30	40	60	70	60	40	52.5
Stoneville 62	33	33	0	50	33	33	33	0	26.9	25	38	0	100	75	25	100	50	51.6

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