THE EFFECT OF DIFFERENT DAY AND NIGHT TEMPERATURE REGIMES ON THE DEVELOPMENT OF VERTICILLIUM WILT OF COTTON

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CHAPTER I

INTRODUCTION

Verticillium wilt has become the most serious disease of cotton in the United States. Since it is favored by cool temperatures and high soil moisture, the disease tends to be more destructive along the northern side of the Cotton Belt and under irrigation. Besides the United States, it is known to occur in many other cotton growing regions of the world.

Since the fungus is soil borne and has not been successfully controlled by chemical or cultural methods, resistant varieties seem to be the most promising method of controlling the disease.

Plant breeders searching for genetic resistance to Verticillium wilt of cotton are handicapped by the fact that disease severity is highly influenced by the environment and often in a population of susceptible plants it does not develop uniformly.

Temperature is generally recognized as one of the critical factors in the epiphytology of the disease. Contrary to expectations based on field observations, previous studies under constant soil temperatures in the greenhouse have not given precise temperature requirements for the development of the disease. However, plant growth chambers today offer better control of the environment than those available to previous investigators. Thus, a suitable inoculation technique paired with proper manipulation of the environment to give a uniform and severe infection

during the early stages of plant growth might enable the breeder to distinguish resistant and susceptible populations.

Since the findings on genotype-temperature interactions have proven to be useful in evaluating segregating populations for resistance and immunity to bacterial blight (6), we wondered whether similar studies might not prove to be equally useful for breeding and inheritance studies on Verticillium wilt of cotton.

The purpose of this study was to determine the effect of different day and night temperature regimes on the expression of genetic resistance in cotton.

CHAPTER II

REVIEW OF LITERATURE

History and Distribution of the Fungus

The fungus, Verticillium albo-atrum Reinke & Berth., has an extensive host range which includes at least 160 species of plants (27), including many important crop plants, and is one of the most widely distributed wilt fungi found in agricultural soils.

V. albo-atrum was first isolated from diseased potato plants in Germany by Reinke and Berthold who described and named it in 1879 (27). Its pathogenecity to cotton, Gossypium spp., was unknown within the United States until Carpenter (8) in 1914 discovered two diseased cotton plants at Arlington, Virginia, which yielded pure cultures of the fungus.

Verticillium wilt of cotton did not attract much attention until 1927 when Shapovalov and Rudolph (29) reported it in California in a potato field which had been plowed because of crop failure and replanted to cotton.

In 1928, Sherbakoff (30, 31) isolated V. albo-atrum from diseased cotton plants from the Mississippi Delta areas of Tennessee, Arkansas, and adjacent states. Young (36) also isolated Verticillium in 1928 from the vascular tissues of cotton plants grown in a greenhouse at Fayetteville, Arkansas.

In 1932, Herbert and Hubbard (16) reported that the disease

attacked all 52 cotton varieties grown in a test in the San Joaquin Valley except Pima, a variety of Gossypium barbadense L.

In 1937, Brown (7) reported that Verticillium wilt was present in all of the cotton growing districts of Arizona. A survey made in 1944 by the Technical Cotton Committee in New Mexico (1) stated that 75 to 80% of the cotton fields examined were infested with Verticillium wilt. By 1946, Harrison and Brinkerhoff (15) reported the disease widely distributed throughout the cotton growing area of central California.

Verticillium wilt of cotton was first recorded in Oklahoma by Humphrey (17) in 1932. McLaughlin (24) isolated V. albo-atrum in 1942 from diseased plants from Geary and Mangum, Oklahoma.

A report by Lehman and Garriss (19) in 1948 identifying plants infected with Verticillium wilt in North Carolina completed the known occurrence of the disease across the entire United States Cotton Belt.

Effect of Temperature on Verticillium albo-atrum

The influence of temperature on growth and distribution of the pathogen is significant. Edgington and Walker (10) reported that the fungus causes marked symptoms on tomato plants at soil temperatures of 20°C or 24°C combined with air temperatures of 16°C, 20°C, and 24°C. Symptoms were less severe when the air temperature was 28°C. When the soil temperature was 28°C, the disease was slight at air temperatures of 16°C, 20°C, and 24°C. When soil and air temperatures were both at 28°C, no symptoms appeared.

Ludbrook (21) and McKeen (23) found that the microsclerotium-producing fungus designated as V. albo-atrum incited the maximum disease development in tomatoes at a soil temperature of 24°C, with 28°C -

30°C being the upper limit for disease expression.

Nelson (25) noted severe wilt of mint at 24°C and 28°C. He also reported that the optimum temperature for growth on agar media was at 24°C and 26°C with the minimum being 8°C to 10°C and the maximum being approximately 34°C. Thus, the mint strain of the fungus differs in that it is favored by higher temperatures.

Kendrick and Middleton (18) reported that the disease was severe on peppers at soil temperatures of 15°C - 30°C with air temperature of 24°C but decreased markedly at 35°C soil temperature.

Schneider (28) found with guayule that the fungus becomes more active at soil temperatures of 18°C - 22°C and that its activity is nil between 27°C and 31°C. Zentmyer (37) found that avocado seedlings inoculated by dipping the roots in a suspension of spores and mycelium showed disease symptoms in 12 days at soil temperatures of 20°C, 25°C, and 30°C.

Boza Barducci (4) in Peru reported that the optimum temperature for the development of the fungus in cotton is 22°C. Leyendecker (20) in New Mexico found that the disease in cotton was decreased in the field when soil temperatures at the six-inch depth were increased from 21°C to 25°C or above by changing the profile of seed beds. He also reported that the minimum, optimum, and maximum temperatures for the development of the fungus on agar were 5°C, 25.5°C, and 30°C, respectively.

Halisky et al. (14) found in cotton that the soil temperatures most favorable for disease development were 20°C and 25°C. No wilt symptoms developed at a constant soil temperature of 35°C. Studies with the fungus on agar showed that maximum growth was at 21°C and 24°C and

that no measurable growth occurred above 30°C.

Greenhouse studies in New Mexico on cotton (2) showed that the pathogenecity of V. albo-atrum was affected by alternating soil temperatures from 33°C during the day to 26°C at night. Wilt severity was appreciably decreased for plants under the altered cycle as compared to plants grown at a constant soil temperature of 26°C.

Other than the New Mexico greenhouse test (2), the control of different day and night temperature regimes has not been reported in the literature which has been reviewed.

Symptoms

Under normal conditions V. albo-atrum may remain alive in soil over long periods of time (35). Microsclerotia survive in the soil until a suitable host plant is present.

The fungus enters the cotton plant through the roots and becomes established in the water conducting tissues of the stem, branches, petioles, and leaves. Once in the plant, the fungus persists even after the cotton plant dies and begins to dry up.

Cotton plants are susceptible to Verticillium wilt at all stages of growth, but in the field they normally do not show symptoms until they are about 21 days old. In the seedling stage, the cotyledons become yellowish followed by rapid desiccation. Vascular discoloration is evident particularly at the base of the hypocotyl, and the infected seedlings usually die. In young plants with three to five leaves there is considerable stunting. The outstanding symptom of the disease is the distinctly mottled appearance of the leaves with yellow areas developing between the veins and on the leaf margins.

Although plants may be killed almost immediately, the majority usually survive and make a certain amount of regrowth from the lower parts of the main stalk. In older plants the symptoms occur in the lower leaves first and then gradually spread to the middle and upper leaves of the plant. The chlorotic areas gradually become larger and paler and finally become necrotic, and many of the leaves are shed. Bolls on affected plants may open prematurely, producing fiber of poor quality.

CHAPTER III

MATERIALS AND METHODS

In order to have rather precise control of the temperature the experiments were established in growth chambers. Twelve-hour periods of dark were alternated with twelve-hour light periods with a light intensity of 2,000 ft. c. A room with the same environmental conditions except that the diurnal light intensity was 1,000 ft. c. was used in conjunction with the growth chambers.

Four upland varieties of cotton were used in the early tests. One of these was susceptible, and the other three possessed some tolerance to Verticillium wilt under field conditions. The tolerant varieties were Stoneville 204 WR, Stoneville 213, and Paymaster 62-M-29, and the susceptible one was Kemp. Further along in the studies a resistant variety of Gossypium barbadense L., Seabrook Sea-Island, was also included.

Seed of these varieties were planted in six-inch pots filled with steamed-clay soil previously fertilized with four grams of an organic fertilizer (Milorganite) containing 5.3% N and 3% P_2O_5. The plants were thinned to three per pot. Two plants were inoculated, and one served as an untreated check. Pots were assigned to the growth chambers in a completely randomized design. The number of pots included for each treatment varied from three to twelve. Two inoculation methods were used in establishing the disease.

The first method of inoculation employed was as outlined by

Brinkerhoff (5) and Evans (12). By this procedure, a sterile slender dissecting needle is pushed through the hypocotyls of 28- and 45-day old seedlings about one inch above the soil level and into a cellulose sponge saturated with a suspension of inoculum. As the needle is withdrawn the sponge is squeezed gently against the hypocotyl, allowing spores to be drawn into the hypocotyl (Figure 1).

The second method of inoculation was patterned after the one devised by Wiles (33), except that much younger seedlings were used. Eight-day old seedlings that had been grown in vermiculite were dipped in a suspension of inoculum for two minutes after about three inches of the tap root had been clipped off. The seedlings were transplanted to steamed clay soil after inoculation.

Control plants with the first method were punctured with a disinfected needle and with the second method seedlings were dipped in tap water which had been previously sterilized. These control plants were inoculated first to avoid possible contamination with the Verticillium albo-atrum inoculum.

The inoculum was grown from a monoconidial culture obtained locally and identified as the defoliating strain of V. albo-atrum by Mathre et al. (22). The culture falls in the type X described by Presley (26), and it produces appressed mycelium and abundant microsclerotia on potato-dextrose agar (PDA). One-month old PDA cultures grown at 18°C were homogenized in a blender with 100 ml. sterile tap water and then washed by centrifugation. The inoculum was prepared the day of inoculation.

Inoculum concentration was calculated from direct counts of conidia, hyphal fragments, and microsclerotia, in an hemocytometer and also on dilution plates inoculated with aliquots of the same inoculum.

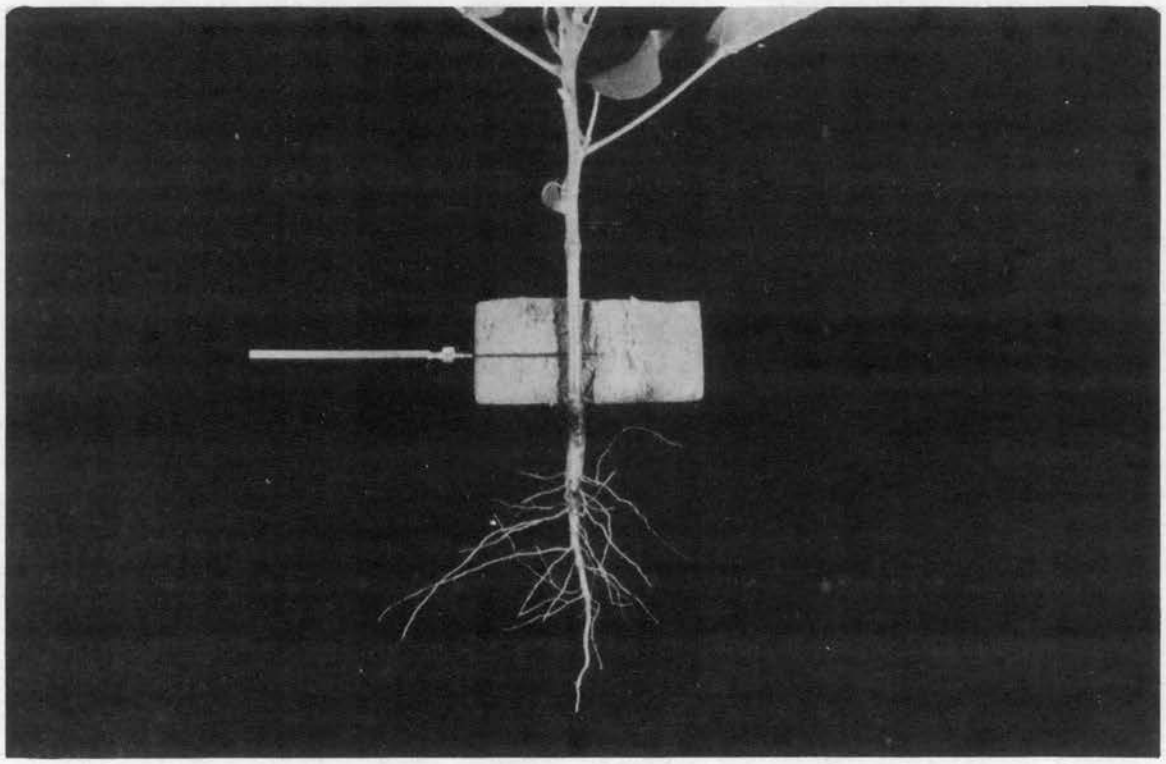

Figure 1. Needle Puncture Technique of Inoculating a Cotton Plant with Verticillium albo-atrum

A concentration of approximately one million viable conidia per ml., was used for both inoculation methods.

Temperature regimes were patterned after the temperatures found to be the most favorable for the development of the fungus by Boza Barducci (4).

The first temperature regimes studied were 22°C day temperature and 22°C night temperature, 27°C X 18°C, 31°C X 18°C, and 36°C X 18°C where plants were grown for 28 days and then inoculated by needle puncture.

To further evaluate the effect of these temperature regimes on the severity of infection, two tests were conducted in which the plants were grown under the 36°C X 18°C regime for 45 days, and then inoculated by the needle puncture technique. The plants were transferred to a temperature regime of 29°C X 18°C on the day of inoculation, two days after inoculation, and four days after inoculation. A check treatment was left under the 36°C X 18°C regime.

In order to determine whether resistance is expressed in very young seedlings, plants were started in vermiculite and inoculated when eight-days old by the root dipping method and then transplanted to steamed-clay soil under the temperature regimes of 27°C X 18°C and 31°C X 18°C.

The effect of different levels of inoculum was also observed at the 29°C X 18°C regime using eight-day old seedlings and inoculating them by the root dipping technique.

Temperature was measured by thermographs and standard mercury bulb thermometers. Temperatures for any given setting normally fluctuated within $\pm$ 1°C.

Studies were conducted to relate the growth activity of the causal

fungus to the different temperature regimes. Growth was determined by observing colony development of dilution plates in the plant growth chambers during the incubation period of the inoculated plants. Dilution plates were seeded with the same inoculum used to inoculate the cotton plants.

Since no disease symptoms appeared on the inoculated plants of the susceptible, tolerant, and resistant varieties grown under the 36°C X 18°C temperature regime, isolations were made on potato-dextrose agar from different parts of the plants in order to determine whether the inoculum was still viable in the plants or not.

The effect of temperature regimes upon the severity of infection was determined by placing inoculated plants in one of a series of classes outlined by Evans (12) as follows:

1 - No symptom expression.

2 - Wilting, mottling, and yellowing of the leaves.

3 - Scorching of the leaves.

4 - Abscission of the leaves.

5 - Plants dead.

Data were analyzed by the analysis of variance method of Steel and Torrie (32). The F test was then applied to the mean squares obtained.

CHAPTER IV

RESULTS

The first symptoms of Verticillium wilt in all the experiments developed within seven to twelve days after inoculation. Disease severity for all experiments is reported in the appendix.

The mean Verticillium wilt grades of the four upland cotton varieties 28 days after inoculation under four different temperature regimes are reported in Table I.

The analysis of variance of disease grades is presented in Table II. The analyses do not indicate that genetic differences exist among varieties for Verticillium wilt resistance under the conditions of this experiment. However, the disease level ranged from no symptoms to severe injury under the different temperature regimes, and temperature differences were significant at the 1% probability level. Under the $36^{\circ}C$ X $18^{\circ}C$ regime no disease symptoms appeared. Symptoms were more severe under the $27^{\circ}C$ X $18^{\circ}C$ temperature regime than at the $22^{\circ}C$ X $22^{\circ}C$ or $31^{\circ}C$ X $18^{\circ}C$ regimes, as can be seen in Figure 2.

Since no disease symptoms appeared on the inoculated plants grown under the $36^{\circ}C$ X $18^{\circ}C$ temperature regime, isolations were made from the inoculated area, one inch above and below the punctured area, the node at the cotyledon leaves, and from the root two inches below the soil level. The causal fungus was still viable after 28 days in the area of the needle puncture, but had not spread in the plant as would be

TABLE I

VERTICILLIUM WILT REACTIONS 28 DAYS AFTER INOCULATION OF 56-DAY OLD PLANTS INOCULATED BY THE NEEDLE PUNCTURE TECHNIQUE

Variety	Mean grade			
	Temperature regimes			
	$27^{\circ}C$ X $18^{\circ}C$	$22^{\circ}C$ X $22^{\circ}C$	$31^{\circ}C$ X $18^{\circ}C$	$36^{\circ}C$ X $18^{\circ}C$
Stoneville 204 WR	3.61	2.83	2.00	1.00
Stoneville 213	3.76	2.83	2.56	1.00
Paymaster 62-M-29	3.35	2.73	2.50	1.00
Kemp	3.50	2.94	2.78	1.00

TABLE II

ANALYSIS OF VARIANCE OF VERTICILLIUM WILT GRADES FOR DATA SHOWN IN TABLE I AND APPENDIX A

Source of Variation	S. S.	d.f.	M. S.	F
Total	332.106	282		
Temperatures	247.714	3	82.5713	256.83**
Varieties	2.190	3	0.7300	2.27
Varieties X Temp.	5.550	9	0.6167	1.92
Experimental Error	41.152	128	0.3215	
Sampling Error	35.500	139		

** Significant at 1% probability level.

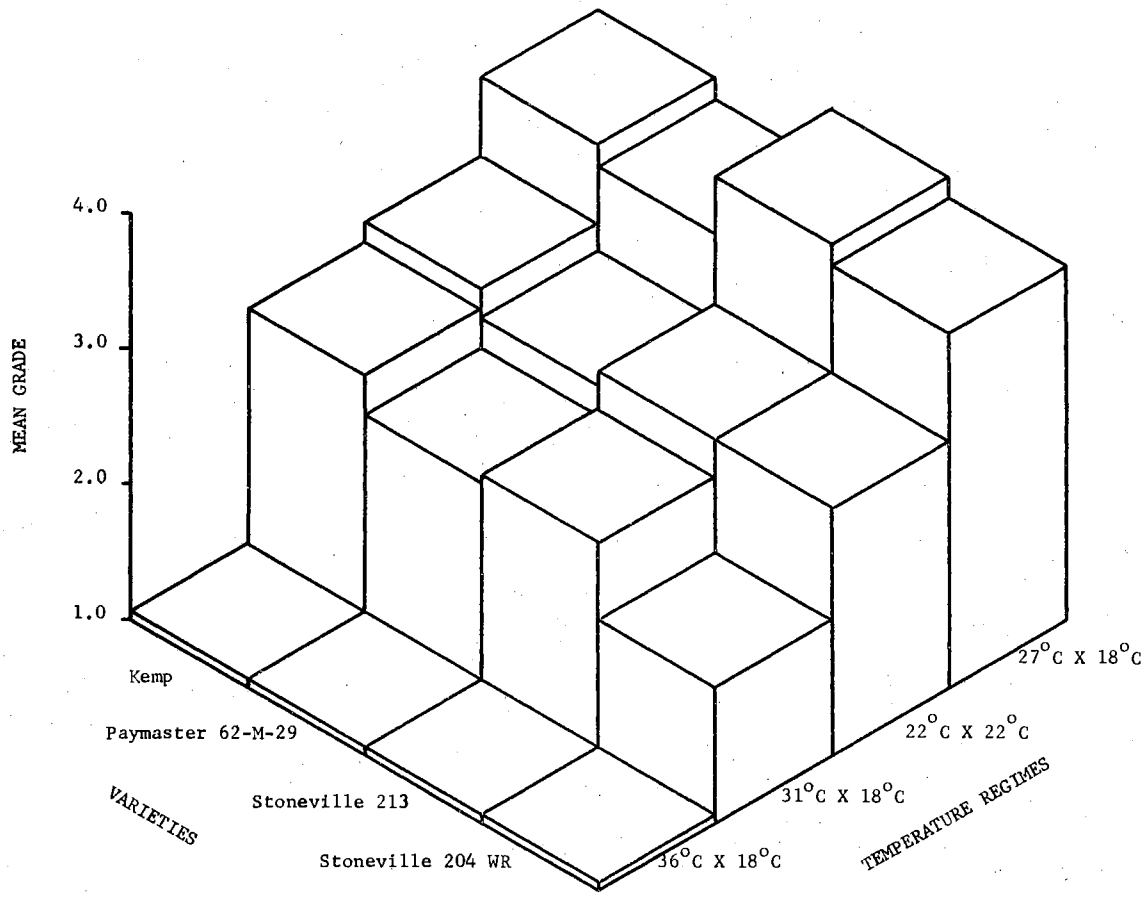

Figure 2. Verticillium Wilt Mean Grades of Four Cotton Varieties under Four Different Temperature Regimes 28 Days after Inoculation by Needle Puncture

expected under more favorable conditions (13).

When these inoculated, but symptomless, plants were transferred to a 27°C X 18°C temperature regime, wilt developed within seven to twelve days, and genetic differences among varieties were apparent.

In order to evaluate these temperature-genotype interactions, two experiments were conducted in which the plants were grown under a 36°C X 18°C regime for 45 days and then inoculated by the needle puncture method. The treatments consisted of growing the plants at the 36°C X 18°C regime for various lengths of time after inoculation before transferring them to the lower temperature. The first sample was transferred to the 29°C X 18°C regime on the day of inoculation, the second two days after inoculation, the third four days after inoculation, and a check treatment was left at the high day temperature regime throughout the experiment.

Calculated mean grades taken 28 days after inoculation for each of the cotton varieties under the different treatments are reported in Tables III and IV.

The effect on the Verticillium wilt reactions of the five varieties for the different incubation periods is shown graphically in Figures 3 and 4.

In the first experiment, a significant genetic difference in wilt severity was evident among the cotton varieties for the four day treatment (Table III). In this treatment, the Seabrook wilt reaction was significantly different from the upland varieties, and also Stoneville 204 WR was significantly different from the other three varieties. However, in the latter variety, the differences were not striking in appearance.

TABLE III

VERTICILLIUM WILT REACTIONS 25 DAYS AFTER INOCULATION OF PLANTS GROWN FOR 45 DAYS UNDER A 36°C X 18°C TEMPERATURE REGIME AND THEN TRANSFERRED TO A 29°C X 18°C REGIME AT DIFFERENT TIME INTERVALS FOLLOWING INOCULATION BY THE NEEDLE PUNCTURE TECHNIQUE

Variety	Mean grade			
	Days to transfer			
	0	2	4	High temp.
Seabrook	3.67	1.67	1.00	1.00
Stoneville 204 WR	5.00	3.00	2.17	1.00
Stoneville 213	4.83	4.50	3.50	1.00
Paymaster 62-M-29	4.83	3.67	3.17	1.00
Kemp	5.00	3.83	3.83	1.00

TABLE IV

VERTICILLIUM WILT REACTIONS 25 DAYS AFTER INOCULATION OF PLANTS GROWN FOR 45 DAYS UNDER A 36°C X 18°C TEMPERATURE REGIME AND THEN TRANSFERRED TO A 29°C X 18°C REGIME AT DIFFERENT TIME INTERVALS FOLLOWING INOCULATION BY THE NEEDLE PUNCTURE TECHNIQUE

Variety	Mean grade			
	Days to transfer			
	0	2	4	High temp.
Seabrook	4.00	1.83	1.00	1.00
Stoneville 204 WR	4.00	3.17	3.83	1.83
Stoneville 213	4.50	3.67	3.33	1.33
Paymaster 62-M-29	4.00	4.33	4.33	1.50
Kemp	5.00	4.33	3.50	1.67

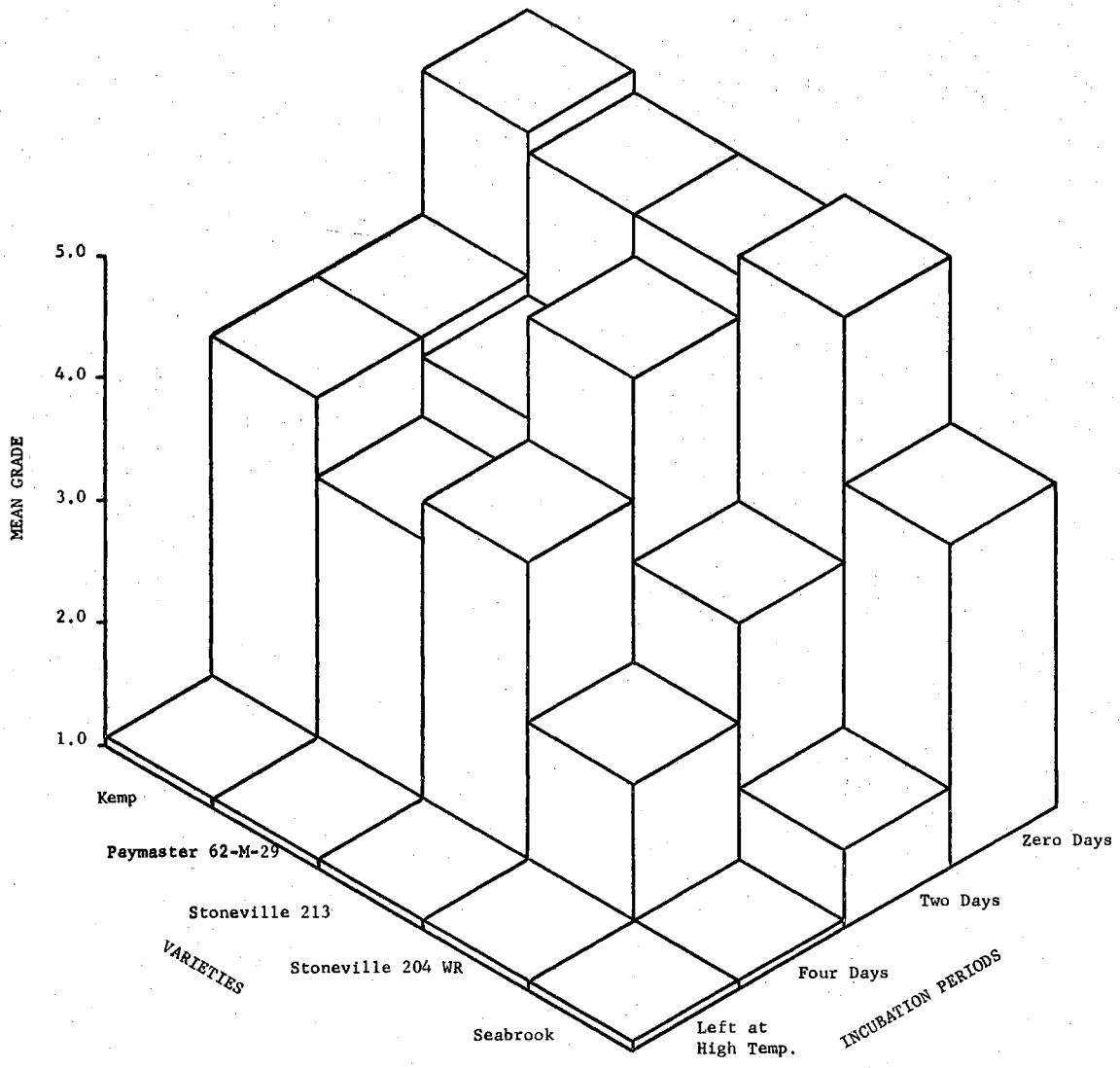

Figure 3. Verticillium Wilt Mean Grades of Five Cotton Varieties under Four Different Incubation Periods 25 Days after Inoculation by Needle Puncture

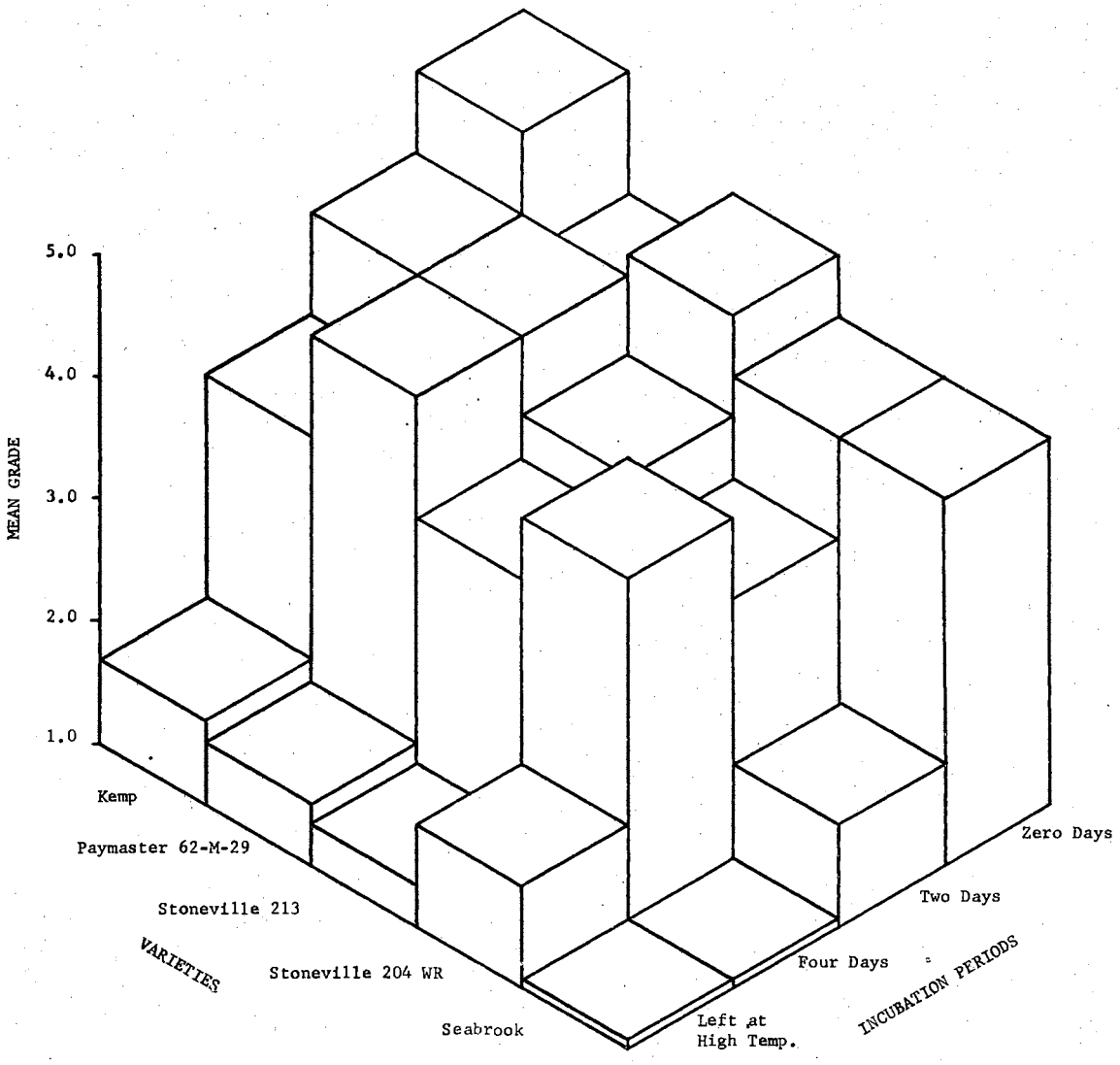

Figure 4. Verticillium Wilt Mean Grades of Five Cotton Varieties under Four Different Incubation Periods 25 Days after Inoculation by Needle Puncture

The difference between Seabrook and the other varieties was less pronounced in the two day treatment than the four day treatment.

Plants left at the high temperature regime did not develop disease symptoms in the first test. However, in the second experiment very mild wilt symptoms developed in all varieties except Seabrook (Table IV). The mild infection was probably due to the temperature being essentially borderline for the growth of the fungus.

The genetic differences among the varieties in the second experiment had essentially the same pattern as those in the first experiment except Stoneville 204 WR did not show a significant degree of resistance.

In both experiments all plants transferred to the $29^{o}C$ X $18^{o}C$ regime the day of the inoculation developed severe wilt symptoms.

The analysis of variance of the wilt reaction grades for the two tests (Tables V and VI) indicate that the varieties X treatments interaction is significant at the 5% and 1% probability levels, respectively.

A comparison of Seabrook plants transferred the day of the inoculation and four days after the inoculation are shown in Figure 5.

Results to determine the effect of different temperature regimes on very young seedlings are presented in Table VII.

As shown by the analysis of variance of the disease grades (Table VIII) the genetic differences among the varieties were not significant under either of the temperature regimes, nor was the interaction, varieties X temperatures, significant.

Figure 6 shows the mean wilt grades of the different varieties under the two temperature regimes.

TABLE V

ANALYSIS OF VARIANCE OF VERTICILLIUM WILT GRADES FOR DATA SHOWN IN TABLE III AND APPENDIX B

Source of Variation	S. S.	d.f.	M. S.	F
Total	347.467	119		
Varieties	43.050	4	10.763	12.30**
Transfers	208.267	3	69.422	79.34**
Varieties X Transfers	24.150	12	2.013	2.30*
Experimental Error	35.000	40	0.875	
Sampling Error	37.000	60		

* Significant at 5% probability level.
** Significant at 1% probability level.

TABLE VI

ANALYSIS OF VARIANCE OF VERTICILLIUM WILT GRADES FOR DATA SHOWN IN TABLE IV AND APPENDIX C

Source of Variation	S. S.	d.f.	M. S.	F
Total	261.592	119		
Varieties	43.134	4	10.7835	12.44**
Transfers	127.559	3	42.5197	49.06**
Varieties X Transfers	29.732	12	2.4777	2.86**
Experimental Error	34.667	40	0.8667	
Sampling Error	26.500	60		

** Significant at 1% probability level.

Figure 5. Seabrook Plants Showing the Symptoms for the Zero Days (Left Pot) Treatment and for the Four Days (Right Pot) Treatment. The Third Plant at the Back of Each Pot Served as a Non-inoculated Check

TABLE VII

VERTICILLIUM WILT REACTIONS UNDER TWO TEMPERATURE REGIMES OF 38-DAY OLD SEEDLINGS INOCULATED BY THE ROOT-DIP METHOD EIGHT DAYS AFTER PLANTING

Variety	Mean grade	
	Temperature regimes	
	$27^{o}C$ X $18^{o}C$	$31^{o}C$ X $18^{o}C$
Seabrook	2.53	2.58
Stoneville 204 WR	2.86	2.57
Kemp	2.90	2.70

TABLE VIII

ANALYSIS OF VARIANCE OF VERTICILLIUM WILT GRADES FOR DATA SHOWN IN TABLE VII AND APPENDIX D

Source of Variation	S.S.	d.f.	M.S.	F
Total	181.686	120		
Temperatures	0.716	1	0.7160	0.44
Varieties	1.281	2	0.6405	0.39
Varieties X Temp.	0.597	2	0.2985	0.18
Experimental Error	105.092	64	1.6421	
Sampling Error	74.000	51		

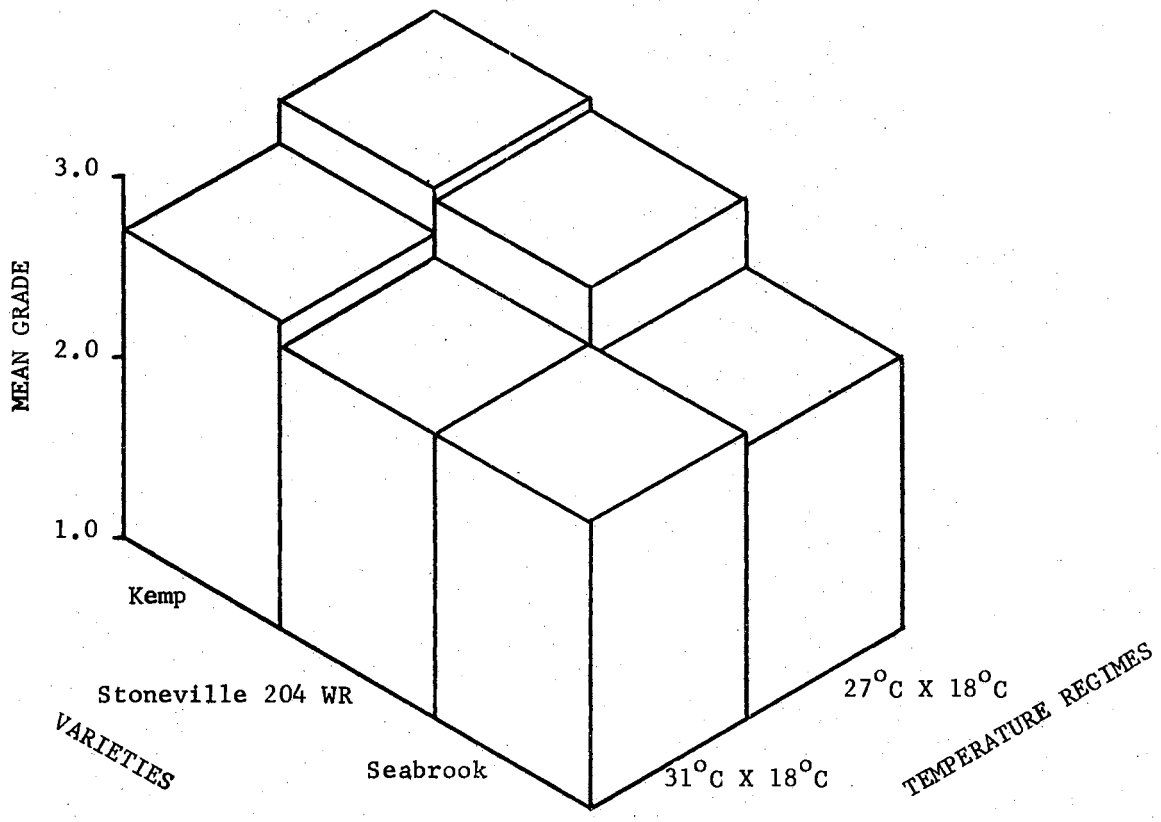

Figure 6. Verticillium Wilt Mean Grades of Three Cotton Varieties under Two Temperature Regimes 30 Days after Inoculation by the Root-Dip Method

Even though no significant differences were apparent 30 days after inoculation, Seabrook plants grown under the 31°C X 18°C temperature regime showed considerable recovery 60 days after inoculation.

Results of a test to determine the effect of inoculum levels for the root-dip method are presented in Table IX and graphically in Figure 7. The temperature regime for this test was 29°C X 18°C.

The analysis of variance of the Verticillium wilt grades (Table X) shows that the concentration of inoculum had no effect on the severity of the wilt symptoms developed by any of the varieties.

Whether genetic differences can be expressed under high day temperatures in the very young inoculated seedlings has not yet been determined.

To determine the effect of some temperature regimes on the growth of the fungus on PDA, dilution plates seeded with inoculum were held at a 36°C X 18°C temperature regime for different periods before being transferred to a 29°C X 18°C regime.

Growth of the fungus on dilution plates showed that colony initiation was retarded 10 to 12 days under the 36°C X 18°C regime. Except for a pronounced lag in colony initiation at the high day temperature, growth of the colonies showed little difference for the zero, two, and four day treatments. However, on plates held continuously at 36°C X 18°C the fungus produced very few sclerotia.

Figure 8 shows the growth of the fungus on the plates 10 days and 30 days after inoculation.

TABLE IX

VERTICILLIUM WILT REACTIONS FOR THREE INOCULUM LEVELS OF 38-DAY OLD SEEDLINGS INOCULATED BY THE ROOT-DIP METHOD EIGHT DAYS AFTER PLANTING

Variety	Mean grade		
	Levels of inoculum		
	300,000	600,000	1,000,000
Seabrook	3.70	4.20	4.20
Stoneville 204 WR	3.00	4.50	4.40
Kemp	4.60	4.00	4.60

TABLE X

ANALYSIS OF VARIANCE OF VERTICILLIUM WILT GRADES FOR DATA SHOWN IN TABLE IX AND APPENDIX E

Source of Variation	S.S.	d.f.	M.S.	F
Total	144.400	89		
Varieties	3.267	2	1.6335	1.18
Levels	6.467	2	3.2335	2.33
Varieties X Levels	11.666	4	2.9165	2.10
Experimental Error	50.000	36	1.3889	
Sampling Error	73.000	45		

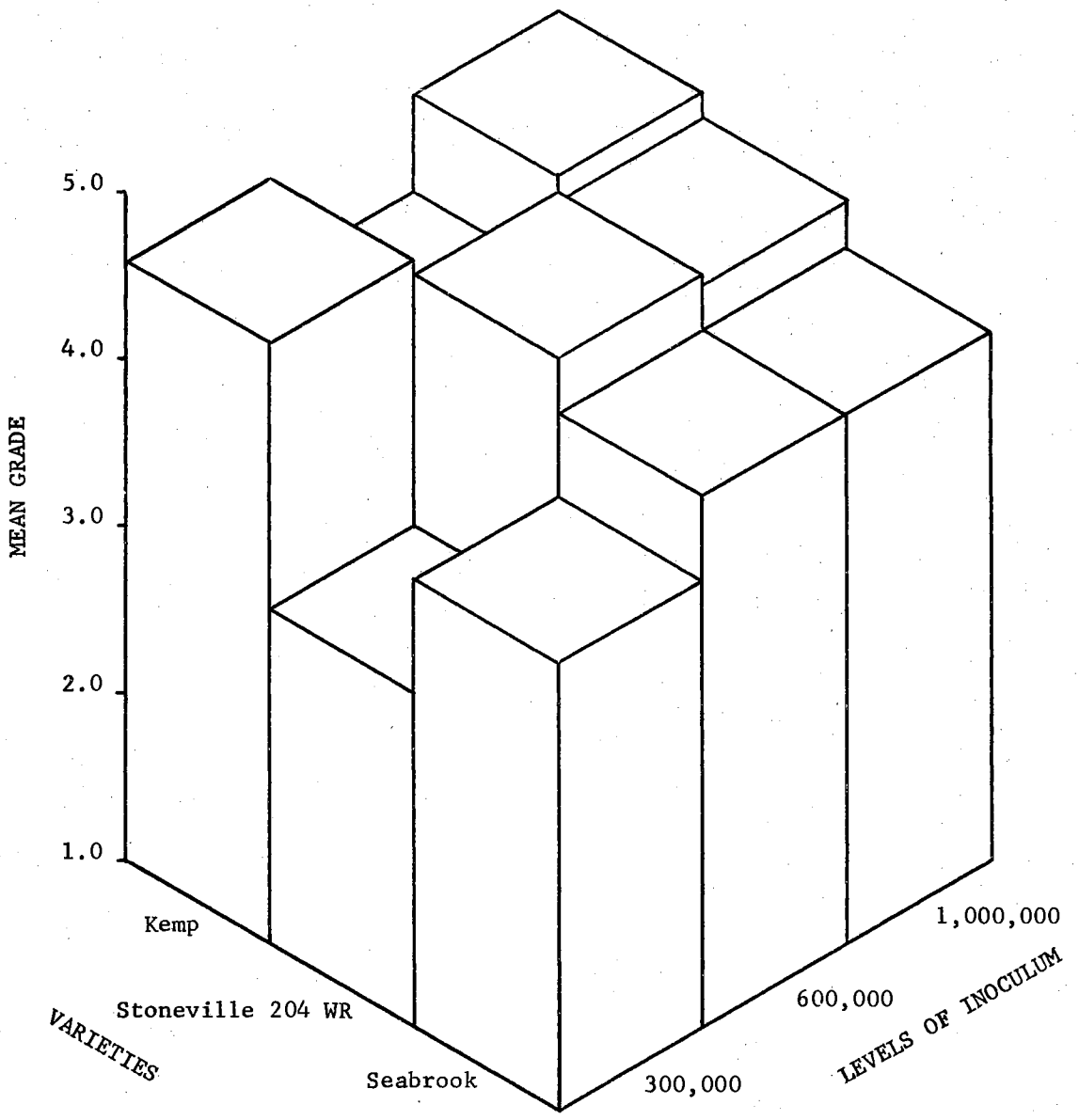

Figure 7. Verticillium Wilt Mean Grades for Three Levels of Inoculum of 38-Day Old Seedlings Inoculated by the Root-Dip Method Eight Days after Planting

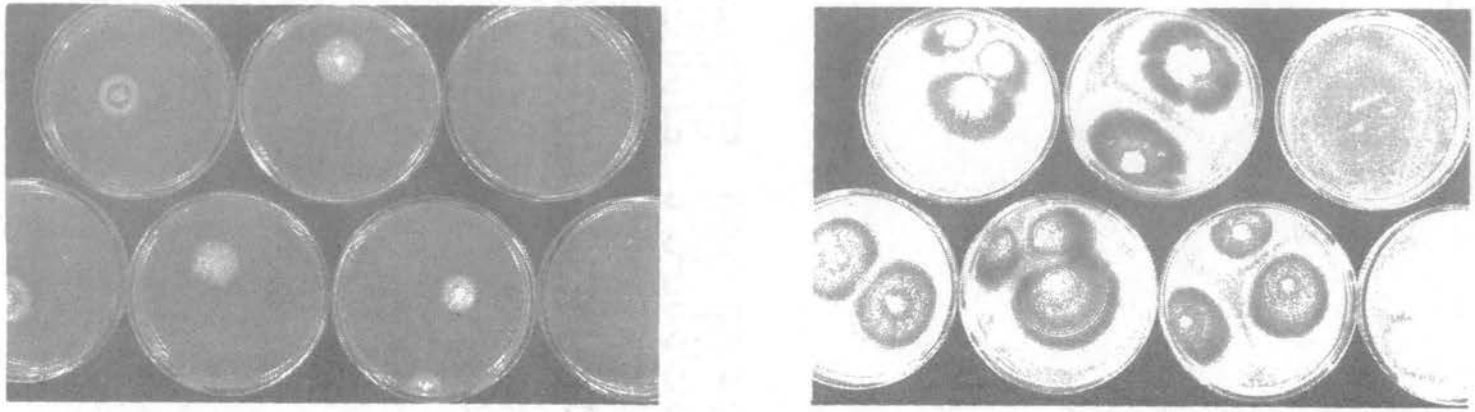

Figure 8. Colonies of Verticillium albo-atrum from Diluted Inoculum Grown on PDA and Incubated under Different Temperature Regimes. Left Photograph 10 Days after Seeding. Left to Right: top row 18°C, 28°C, and 36°C Constant Temperatures. Bottom row 0 Days at 36°C X 18°C and 10 Days at 29°C X 18°C; same Regime 2 Days/8 Days; same Regime 4 Days/6 Days; and same Regime 10 Days/0 Days. Right Photograph 30 Days after Seeding. Temperature Regimes same as before

CHAPTER V

DISCUSSION

Differences in genetic resistance among varieties were not evident when the inoculum was placed within the vascular system by either the needle puncture technique or the root dipping method, although all varieties tended to react alike, disease severity varied very significantly under the different temperature regimes. The differences obtained in disease severity agree with the results found under different temperatures in New Mexico (2).

When plants grown at a high day temperature were inoculated and held four days before transferring to a low day temperature, genetic resistance of the Seabrook variety became evident. Studies of the fungus growth in PDA revealed that growth was retarded by high day temperatures. Isolations made from different parts of the plants held at high day temperature throughout the experiment indicated that the distribution of the fungus within the plant was restricted to the punctured area. However, when the susceptible varieties were moved to lower day temperatures the disease developed, indicating that the fungus remained alive.

Whether the expression of genetic resistance of Seabrook is due to the effect of the incubation environment on the host, on the fungus, or on both, is not known. However, since some varieties remained susceptible, the temperature effects are probably on the host or on the

host-fungus interaction. In addition, the presence of the fungus appeared to be necessary to develop the resistance since plants transferred to the cooler temperatures immediately after inoculation are susceptible.

Nevertheless, the results suggest that proper genotype-temperature interactions may be useful in screening breeding material for Verticillium wilt tolerance.

Early investigations of the severity of the disease on plants grown at constant soil and air temperatures (10, 14, 18, 20, 21, 23, 28) reveal that there is quite a fluctuation of maximum temperature requirements for the fungus. Even the temperature effects reported disagree with some reports. These differences in results might be due to the great range of temperature requirements for the different strains of the fungus.

Even though no differences were observed when using either of the inoculation techniques, Erwin et al. (11) reported that a lower incidence of the disease occurred when using the root dipping method as compared with the needle puncture method.

Although, the studies indicate that a simple inoculation screening technique can be developed, more information is needed on the behavior of plants grown under lower day temperatures before inoculation, and also what effect extending the incubation period at high day temperatures would have on the expression of resistance. Research should be carried on to see if the young seedlings express genetic resistance when held at high day temperatures after inoculation. Also, studies should be made to find out if differences of light intensity would affect the incidence of the disease in plants grown at high day temperatures.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Temperature has been recognized by most investigators as one of the critical factors in the epiphytology of Verticillium albo-atrum.

In order to determine the effect of different day and night temperature regimes on the development of Verticillium wilt of cotton a series of experiments were conducted in plant growth chambers where seedlings of resistant, tolerant, and susceptible cotton varieties were grown under different temperature regimes and then inoculated as 28- and 45-day old seedlings in the hypocotyls by a needle puncture technique or as eight-day old seedlings by a root dipping technique.

Inoculated plants were also subjected to different incubation periods before being transferred from a high day temperature regime to a low day temperature regime. Studies were also conducted to relate the growth activity of the causal fungus to the different temperature regimes.

Verticillium wilt became apparent in the inoculated plants within seven to twelve days after inoculation.

These studies indicate that when inoculum is placed within the vascular system disease expression is altered drastically by different temperature regimes. The disease was most severe under a $27^{o}C$ X $18^{o}C$ regime. Inoculated plants held continuously at a $36^{o}C$ X $18^{o}C$ temperature regime did not develop symptoms of Verticillium wilt.

An analysis of variance of disease severity (recorded as wilt grades) indicated that the differences in disease expression under the different temperature regimes were statistically significant.

Genetic resistance was not expressed unless plants inoculated by the needle puncture method were first held at high day temperature for four days and then transferred to a $29^{o}C$ X $18^{o}C$ temperature regime.

Whether the effect of the different incubation periods in the host is dependent only on restricted growth of the fungus at the higher day temperatures or is dependent on an interaction between the host and the growth of the fungus at high day temperature was not determined. Growth of the fungus on potato-dextrose agar was definitely retarded at the high-day temperature, but the fungus eventually grew and produced viable conidia.

Both the needle puncture method and the root dipping method were effective inoculation techniques. However, the needle puncture method is faster than the root dipping method.

These studies as yet are preliminary but indicate that certain genotype-temperature interactions may be useful in developing a relatively simple screening technique that would aid in breeding and genetic studies on Verticillium wilt of cotton.

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APPENDIX A

VERTICILLIUM WILT GRADES OF 56-DAY OLD PLANTS 28 DAYS AFTER INOCULATION BY THE NEEDLE PUNCTURE TECHNIQUE

Temperature regime: 27°C X 18°C

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Stoneville 204 WR	0	0	7	11	0	3.61
Stoneville 213	0	2	3	9	3	3.76
Paymaster 62-M-29	0	0	11	6	0	3.35
Kemp	0	0	11	5	2	3.50

Temperature regime: 22°C X 22°C

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Stoneville 204 WR	0	6	9	3	0	2.83
Stoneville 213	0	5	11	2	0	2.83
Paymaster 62-M-29	0	7	5	3	0	2.73
Kemp	0	5	10	2	1	2.94

APPENDIX A (Continued)

Temperature regime: 31°C X 18°C

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Stoneville 204 WR	0	18	0	0	0	2.00
Stoneville 213	0	8	10	0	0	2.56
Paymaster 62-M-29	0	9	9	0	0	2.50
Kemp	0	4	14	0	0	2.78

Temperature regime: 36°C X 18°C

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Stoneville 204 WR	18	0	0	0	0	1.00
Stoneville 213	18	0	0	0	0	1.00
Paymaster 62-M-29	18	0	0	0	0	1.00
Kemp	18	0	0	0	0	1.00

APPENDIX B

VERTICILLIUM WILT GRADES 25 DAYS AFTER INOCULATION OF PLANTS GROWN FOR 45 DAYS UNDER A 36°C X 18°C TEMPERATURE REGIME AND THEN TRANSFERRED TO A 29°C X 18°C REGIME AT DIFFERENT TIME INTERVALS FOLLOWING INOCULATION BY THE NEEDLE PUNCTURE TECHNIQUE

Days to transfer: 0

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	1	0	0	4	1	3.67
Stoneville 204 WR	0	0	0	0	6	5.00
Stoneville 213	0	0	0	1	5	4.83
Paymaster 62-M-29	0	0	0	1	5	4.83
Kemp	0	0	0	0	6	5.00

Days to transfer: 2

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	4	0	2	0	0	1.67
Stoneville 204 WR	1	0	3	2	0	3.00
Stoneville 213	0	0	0	3	3	4.50
Paymaster 62-M-29	1	0	1	2	2	3.67
Kemp	0	0	2	3	1	3.83

APPENDIX B (Continued)

Days to transfer: 4

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	6	0	0	0	0	1.00
Stoneville 204 WR	3	0	2	1	0	2.17
Stoneville 213	1	0	2	1	2	3.50
Paymaster 62-M-29	1	0	3	1	1	3.17
Kemp	0	0	3	1	2	3.83

Days to transfer: left at high temperature

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	6	0	0	0	0	1.00
Stoneville 204 WR	6	0	0	0	0	1.00
Stoneville 213	6	0	0	0	0	1.00
Paymaster 62-M-29	6	0	0	0	0	1.00
Kemp	6	0	0	0	0	1.00

APPENDIX C

VERTICILLIUM WILT GRADES 25 DAYS AFTER INOCULATION OF PLANTS GROWN FOR 45 DAYS UNDER A 36°C X 18°C TEMPERATURE REGIME AND THEN TRANSFERRED TO A 29°C X 18°C REGIME AT DIFFERENT TIME INTERVALS FOLLOWING INOCULATION BY THE NEEDLE PUNCTURE TECHNIQUE

Days to transfer: 0

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	0	0	2	2	2	4.00
Stoneville 204 WR	0	0	2	2	2	4.00
Stoneville 213	0	0	0	3	3	4.50
Paymaster 62-M-29	0	0	2	2	2	4.00
Kemp	0	0	0	0	6	5.00

Days to transfer: 2

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	2	3	1	0	0	1.83
Stoneville 204 WR	0	1	4	0	1	3.17
Stoneville 213	0	0	4	0	2	3.67
Paymaster 62-M-29	0	0	2	0	4	4.33
Kemp	0	0	1	2	3	4.33

APPENDIX C (Continued)

Days to transfer: 4

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	6	0	0	0	0	1.00
Stoneville 204 WR	0	1	1	2	2	3.83
Stoneville 213	0	0	4	2	0	3.33
Paymaster 62-M-29	0	0	1	2	3	4.33
Kemp	0	0	3	3	0	3.50

Days to transfer: left at high temperature

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	6	0	0	0	0	1.00
Stoneville 204 WR	3	1	2	0	0	1.83
Stoneville 213	4	2	0	0	0	1.33
Paymaster 62-M-29	4	1	1	0	0	1.50
Kemp	3	2	1	0	0	1.67

APPENDIX D

VERTICILLIUM WILT GRADES OF 38-DAY OLD SEEDLINGS GROWN UNDER TWO TEMPERATURE REGIMES AND INOCULATED BY THE ROOT-DIP METHOD EIGHT DAYS AFTER PLANTING

Temperature regime: 27°C X 18°C

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	2	8	6	3	0	2.53
Stoneville 204 WR	4	7	2	4	4	2.86
Kemp	6	3	3	5	4	2.90

Temperature regime: 31°C X 18°C

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	2	5	11	1	0	2.58
Stoneville 204 WR	4	3	13	0	1	2.57
Kemp	5	6	4	0	5	2.70

APPENDIX E

VERTICILLIUM WILT GRADES FOR THREE INOCULUM LEVELS OF 38-DAY OLD SEEDLINGS INOCULATED BY THE ROOT-DIP METHOD EIGHT DAYS AFTER PLANTING

Level of inoculum: 300,000 conidia per ml.

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	0	2	1	5	2	3.70
Stoneville 204 WR	3	2	1	0	4	3.00
Kemp	0	0	1	2	7	4.60

Level of inoculum: 600,000 conidia per ml.

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	1	0	0	4	5	4.20
Stoneville 204 WR	0	1	0	2	7	4.50
Kemp	2	0	0	2	6	4.00

Level of inoculum: 1,000,000 conidia per ml.

Variety	Number of plants in grade					Mean grade
	1	2	3	4	5	
Seabrook	0	0	1	6	3	4.20
Stoneville 204 WR	1	0	0	2	7	4.40
Kemp	1	0	0	0	9	4.60

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