A QUANTITATIVE GENETIC STUDY OF VERTICILLIUM WILT RESISTANCE AMONG SELECTED LINES OF UPLAND COTTON

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CHAPTER I

INTRODUCTION

Cultivated varieties of upland cotton (Gossypium hirsutum L.) have repeatedly been demonstrated to differ in their ability to withstand certain diseases, insects, and other pests. As a result, the cotton breeder is concerned not only with developing varieties which have high yield levels and acceptable fiber quality, but also with incorporating into those varieties tolerance and/or resistance to those diseases, insects, and pests which are destructive to this crop. In doing so, hazards of production are decreased; and consistency of performance is increased.

Verticillium wilt (Verticillium albo-atrum Reinke and Berth.) has become one of the most serious diseases of cotton in Oklahoma, particularly under irrigated production. The disease is much more widespread throughout the state than it was in the recent past, and it seems to be increasing in severity within areas of previous infestation. Losses in yield can reach 50%, and average losses of 10 to 15% over large areas are common (56). The disease reduces fiber length, strength, and grade as well as other factors of fiber quality (24).

Losses due to verticillium wilt can be reduced by certain cultural practices such as utilizing rotations with crops like corn, sorghum, small grains, sweet clover, lespedeza, alfalfa, or soybeans; avoiding deep cultivation; using skip-row planting; and applying adequate but not

excessive applications of water and nitrogen fertilizer to mention only a few (22, 32, 57). Still, as with other plant diseases, the most effective and economical control of this disease should be obtained through the use of tolerant and/or resistant varieties (57) provided that such varieties can be developed. Cotton breeders have been working toward this objective for many years, and considerable progress has been made in creating varieties possessing a fair degree of tolerance to the disease (24, 32, 57, 69). However, commercial upland varieties resistant to verticillium wilt have yet to be developed. Progress has been made in spite of the fact that little is actually known about the inheritance of resistance to this disease. Should this genetic basis be defined, it is probable that much more rapid progress toward varietal resistance would be achieved.

The purpose of this study was to investigate the inheritance of resistance to verticillium wilt among 10 selected lines of cotton. A diallel cross experiment involving parents, F_1's, and F_2's over multiple years and/or locations was employed. The information derived therefrom should prove useful in increasing the efficiency and effectiveness of breeding programs to develop varieties of cotton more tolerant, and perhaps even resistant, to this disease.

CHAPTER II

REVIEW OF LITERATURE

Identification of the Pathogen

Within the genus Verticillium, V. albo-atrum was first described as the causal pathogen of potato wilt in Germany by Reinke and Berthold in 1879. Klebahn isolated V. dahliae from wilting dahlia in Germany in 1913. He claimed that the difference between the two species was that his isolate formed microsclerotia from septation and budding of cells from hypha; whereas, V. albo-atrum formed only resting mycelia (41).

Because of the different interpretation of the original description by Reinke and Berthold, the relationship between V. albo-atrum and V. dahliae has been a subject of much controversy. The microsclerotial and resting mycelial types can be easily distinguished on a prune extract medium (41). Some investigators have considered that the fungi which form either microsclerotia or resting mycleia are members of a large variable species (54, 60) and classify them under V. albo-atrum. Others maintain them as separate species (41). In the United States the microsclerotial form is the common one on cotton, and it is universally referred to as V. albo-atrum by U. S. cotton pathologists (Brinkerhoff, personal communication).

Relationship of Pathogen to Environmental Factors

Environmental conditions are very important in determining verticillium wilt resistance. Environment may affect the resistance of the host plant or the infectivity of the pathogen. Cotton varieties have been demonstrated to have different reactions under different environmental conditions while V. albo-atrum cultural types have also differed in pathogenicity under different environmental conditions (55).

Temperature

Temperature plays a critical role in the incidence and control of verticillium wilt. For the disease, an optimum temperature of 22.5-25° C and a maximum of 32-35° C have been reported (26). For the fungus, V. albo-atrum, the optimum temperature is listed as 22.5° C, and the growing range appears to cover the temperatures from 10 to 31° C (26). Leyendecker, with isolates he studied (45), found the minimum, optimum, and maximum development of the fungus on agar were 5° C, 25.5° C, and 30° C, respectively. At temperatures above 20° C, microsclerotia were formed. Wilhelm (76) in a study with other isolates found that a temperature difference of 3-6° C within the growing range of V. albo-atrum caused differences in cultural appearance and in morphological characters, particularly of the resting stages. For instance, at low temperatures of 10-20° C, colonies were jet black and were composed almost entirely of microsclerotial crusts. At higher temperatures of 25-31° C, colonies were creamy white and had only a sparse development of microsclerotia.

Samayoa (61) showed that no symptoms developed while inoculated plants were held continuously under a 36-18° C regime, but when the inoculated plants were first held at the 36-18° C regime for four days

and then transferred to a 29-18° C temperature regime, resistant and susceptible plants could be distinguished. Brinkerhoff et al (12) who studied verticillium wilt reactions in cotton as affected by post-inoculation temperatures found that plants held at diurnal 36-18° C for seven days and then transferred to 28-18° C could be distinguished as resistant or susceptible. However, if plants were reinoculated just prior to being placed at 28-18° C, the resistant plants became susceptible. Abdel-Raheem and Bird (1) studied three cotton strains and found that resistance to both fusarium wilt and verticillium wilt could be distinguished at a soil temperature of 28° C. Bell and Presley (8) reported "Seabrook Sea Island 12B2," "Acala 4-42-77," and "Stardel" which were resistant, tolerant, and susceptible varieties, respectively were all susceptible at 22° C. All these varieties were resistant at 32° C. The tolerant varieties were susceptible at 25° C, tolerant at 27° C, and resistant at 29° C. Ashagari (5) also reported that the disease did not develop at a 36-18° C temperature regime. He found that after 12 days inoculation at 20° C, Seabrook Sea Island plants showed slight chlorosis; "OK 141-5" (G. hirsutum) exhibited extensive chlorosis and some leaf defoliation; and "Stoneville 62" leaves were defoliated heavily. After treatment at a 36-18° C temperature regime, and moved to 20° C, Seabrook Sea Island showed high resistance; OK 141-5 and Stoneville 62 were susceptible.

Moisture

It is difficult to determine the influence of moisture in relation to the development of disease as it is usually confounded with temperature. Nelson (53) in a study of verticillium wilt of peppermint

reported the optimum temperature for infection was 24-28° C with moisture at 70 or 100 percent field capacity. However, maximum wilt infection also occurred in soil with low moisture content. Rudolph (60) reported in California, the disease was observed in both droughty and well watered soils. In tomato fields the disease was serious when subjected to drought conditions. In prune, peach, and apricot orchards, diseases have been observed in widest extremes of soil moisture. Probably soil moisture not only affects the activity of V. albo-atrum, but also the physiological responses of the host which condition susceptibility or resistance.

Soil Type and Fertility

Rudolph (60) stated that many workers agree soils with high manure, compost, humus, and other types of organic matter are conducive to the spread and reproduction of the disease. Loams and sandy loams are favorable to the disease, but the disease is more severe on sandy clay loams. Sedimentary soils tend to be favorable to disease development. Haenseler (34) found in acid soils, the disease is less severe at least in the egg-plant. Rudolph and Harrison (59) reported in clay soils, the disease spread more rapidly. Presley (55) found the amount and kind of fertilizer has some influence on the development of the disease. Fertilizers with high percentage of nitrogen promoted the development of the disease, while the amount of potash was increased, the percentage of the disease decreased.

History, Distribution, and Host Range

Cotton wilt as caused by V. albo-atrum was first demonstrated in

the United States at Arlington, Virginia, in 1914 by Carpenter (18). In 1918, using a culture of V. albo-atrum designated 3156, he (19) inoculated cotton plants through wounds in the hypocotyl, and obtained 80% infection in 14 days. In 1928 Sherbakoff (63) reported that cotton wilt caused by V. albo-atrum also occurred in Lake County, Tennessee. He found the pathogen of wilted cotton to be indistinguishable from the V. albo-atrum cultures which he obtained from wilt-infected maple at Knoxville, Tennessee. The following year, Sherbakoff (64) observed the disease in other places along both banks of the Mississippi River. Miles and Persons (51) also detected the presence of verticillium wilt in Mississippi in the fall of 1930 in plots which were used to test for varietal resistance to fusarium wilt. Brown (16) reported verticillium wilt of cotton in all cotton districts in Arizona in 1937. In a recent article, Cotton et al (23) stated the first verticillium wilt attacked commercial cotton planting was in western Tennessee. Shortly thereafter, the disease was found in Arkansas, Texas, and California.

Humphrey (40) first reported verticillium wilt in Oklahoma in 1932. In 1942, McLaughlin (48) made isolations of V. albo-atrum from diseased plants from Geary and Mangum, Oklahoma. At the present time, verticillium wilt in Oklahoma is widespread and destructive in nearly all irrigated soils of central and southwestern Oklahoma (Brinkerhoff, personal communication). Cotton (24) reported severe losses occurred in the Mesilla Valley of New Mexico in 1949. In the Deming area of New Mexico, the disease has become a serious problem each year. In the Animas area, and Lea County eastern New Mexico, the disease also is responsible for extensive losses. In California, losses caused by wilt occur year. The South Plains of Texas is one of the largest areas

infested by severe wilt in the entire Cotton Belt.

At present, verticillium wilt occurs in most of the cotton growing areas of the world. In the United States, it is found over most of the Cotton Belt (24, 75).

Verticillium wilt is also common on hosts other than cotton in the northern United States, Canada, Holland, New Zealand, Australia, and England where the summers are relatively cool (46, 66).

Bewley (10) showed that V. albo-atrum isolated from tomato could successfully induce wilt in cotton and several other crops. Miles (50) studied field inoculations of three sources of V. albo-atrum in 1933 and reported that a strain from cotton at Stoneville, Mississippi caused infection within 10 days; a strain from Irish potatoes in St. Catherines, Canada produced no infection; and a strain from cotton in California caused 16% infection among inoculated plants.

Verticillium wilt attacks many plant species, and appears to be the major cotton disease in South America. In the United States and Europe, it commonly attacks elm, maple, and many other ornamentals, stone fruits, bush fruits, strawberries, potato, tomato, cucumber, cotton, alfalfa, okra, peppers, egg-plant, and many weeds (20).

Dissemination and Survival of the Fungus

Brown (16) believed the disease to be seed-borne because the disease affected cotton on new land. Presley (55) investigated the possibility of seed dissemination of the pathogen from 1938 to 1940. He was unable to demonstrate that the pathogen was transmitted in the seed. However, Chilton (21) found that cotton seed fuzz infested with dry crushed leaves and bracts from verticillium wilt-infected cotton

plants disseminated the disease. In 1951, Blank and Leyendecker (11) showed that wilt-infected stalks can function as a means of spreading the disease to areas previously free of the pathogen and suggested that removal of diseased plants during the growing season could reduce the spread of the disease. Evans et al (30) reported previously infected senescent leaf tissues on moist soil may serve as a means to spread conidia of the fungus throughout the growing season. They also found that tremendous numbers of microsclerotia build up in infested plant debris and exist in soil either as free units or in the decaying plant tissues. Brinkerhoff (14) found that microsclerotia formed in previously infected and dried leaves within 2 to 5 days at 18-30° C in plastic bags with moisture approaching saturation. He also found that microsclerotia developed at 5° C but were inhibited at 32° C. When the soil moisture level was near the water-holding capacity, abundant microsclerotia developed in both steamed and nonsteamed soil. At lower soil moisture levels, microsclerotia developed in greater numbers in steamed soil than in nonsteamed soil. The difference was attributed to competition by the microflora in the nonsteamed soil.

Symptoms in Upland Cotton

G. hirsutum is susceptible to verticillium wilt at all stages of plant growth, but is not usually attacked in the field until plants are three or four weeks old. The disease may cause stunting of plants at any intermediate stage of growth. In older plants, symptoms usually appear first on the lower leaves. The leaves then become dull looking with the leaf tissue between the veins and on the margins becoming mottled and later necrotic. As the disease progresses upward from the

bottom of the plant towards the top, usually many of the leaves are shed from the plant. Plants may be killed immediately after attack by the disease, although generally they survive until the end of the season. The bolls of diseased plants may open prematurely, and thus produce a poor quality fiber. In tolerant varieties, new growth may form at the base of the stems. The leaves of the new growth are usually stunted to a greater or lesser extent and very poorly developed (22, 60). The roots of the diseased plants usually show no external lesions or other kinds of obvious symptoms. The main roots and rootlets are normal in size and development (60).

The xylem of leaves, stems, and roots of the diseased plants show discoloration. The discoloration is quite variable with a brownish black or brownish red color developing soon after the fungus invades the xylem (60).

Hyphal Invasion and Nature of the Disease

Rudolph (60) in reviewing the work of Reinke and Berthold pointed out that they were unable to obtain infection of the uninjured roots of potato in humidity chambers by means of spores which had not germinated. When hyphae were used, infection quickly took place. This last was convincing proof that the fungus could penetrate healthy uninjured root tissue. Leyendecker (45) found that hyphae developed most abundantly in the xylem of the leaf midribs, petioles, and fruiting branches. Hyphae grew mostly longitudinally, and he observed no conidia nor microsclerotia in the diseased xylem tissue. However, Chilton (21) and Garber and Houston (33) have observed conidia in vessels, and microsclerotia develop abundantly in dead plant parts as soon as moisture and tem-

perature are favorable. Garber and Houston (33) pointed out that the fungus could penetrate directly through the root tip. The fungus also attacked other areas of the root and the hypocotyl of seedlings. When the fungus penetrated into the epidermis, it moved intercellularly and intracellularly to the vascular tissue. Presley et al (58) found the vessels of one-month-old cotton plants to be sufficiently large and continuous to permit conidia to move freely throughout the plant and exhibit symptoms at multiple sites.

Ashagari (5) reported that in inoculated resistant cotton plants gel masses were observed in the vessels within one day. No gel was observed in the susceptible variety within the same period, but gel formed after two days.

Metliskii and Ozeretskoskaya (49) in a recent book discuss two theories, "Plug" and "Toxin," in relation to the cause of wilt and the nature of resistance. Those who advocate the plug theory reason that the dysfunction of the xylem vessels is due to plugging with hyphae, polysaccharides, fungus secretions, gum and resin compounds, gels, and tyloses. Resistant plants react rapidly and prevent the movement of the fungus in the xylem. The principle of the toxin theory is that the pathogen secretes substances in the xylem vessels that disturb the osmotic function in the cells, especially in leaves. These two theories cannot fully explain the nature of the disease and the resistance of plants.

Bell (9) found that the synthesis of wilt-resistant gossypol-related compounds was greater in resistance than in susceptible tissues of stem sections, and of plants, especially when 10^{-3} m $CuCl_2$ was used as an inducer. Bell and Presley (8) found that the wilt-resistant

Seabrook Sea Island 12B2 produced gossypol-related compounds in response to conidia of V. albo-atrum at 35-40° C much more readily than wilt-susceptible Stardel.

Inoculation Techniques

Inoculation techniques are highly important in breeding cotton for resistance to V. albo-atrum. Inoculation aids in differentiation of degrees of resistance, and by reducing the number of escapes saves much time in screening.

Evans (31) described a needle-and-sponge method in which a sterile dissecting needle was used to puncture the stem about two inches above the soil level. A cellulose sponge saturated with inoculum was squeezed until inoculum oozed through the puncture into the stem. Banfield (6) introduced the spores of three elm wilt-inducing fungi through chisel cuts into the trunks and into the tops of tall elm trees. Brinkerhoff (13) described the effectiveness of a hypodermic injection method. Inoculum was injected into the hypocotyl of young cotton plants in the field just below the soil level. Erwin et al (29) employed a variation of Brinkerhoff's method in which a hypodermic needle that had been dipped in a suspension containing spores was used to puncture the center of the cotyledonary node of the cotton plant. Wiles (73) inoculated seedlings in the four-leaf stage by dipping the roots in a blended suspension of the pathogen and then transplanting the seedlings. Schnathorst and Mathre (62) used an aerosol pressurized spray can to spray conidial suspensions onto the root ball. Bewley (10) put conidia of V. albo-atrum in sterilized soil and then transplanted tomato plants into the potted soil. Bugbee and Presley (17) in further modifying the

stem-puncture technique inoculated into the hypocotyl of the stem above the soil line. The needle was inserted into the lower stem at approximately a 45 degree angle to the stem, and a single drop of inoculum was permitted to be taken up.

Breeding for and Inheritance of Resistance to Verticillium Wilt

Most of the commercial varieties of upland cotton (Gossypium hirsutum L.) are susceptible to verticillium wilt. Many varieties derived from G. barbadense types (Egyptian, American-Egyptian, Sea Island, and some South American cottons) appear to have a relatively high degree of resistance to the disease (56). Herbert and Hubbard (39) in a screening test containing both G. barbadense ("Pima," an American-Egyptian type) and G. hirsutum ("Acala," "Mebane," "Delfos," and many of the more popular upland varieties of the Cotton Belt at that time) observed that Pima appeared highly resistant to the disease. At the end of the growing season, most of the Pima plants showed characteristic discoloration of the vascular system even though they exhibited no external symptoms. At present, the search for resistant breeding stocks among all known sources of material is being actively pursued.

There is actually very little known about the inheritance of resistance to verticillium wilt in upland cotton. It is generally thought that resistance within G. hirsutum is not inherited in a simple manner. Different levels of resistance may be transmitted to progenies by crossing the parents which possess the character themselves, but there is no definite pattern to its inheritance. The transfer of resistance in some cases can be scarcely observed in the progenies or it may even

involve transgressive segregation. In some cases, resistance may be increased by crossing two resistant strains; whereas, in other combinations, the progenies demonstrate less resistance than either parent (24, 32).

The primary objective of breeding work involving verticillium wilt resistance is to transmit this character into commercially acceptable varieties, but this objective may be difficult to accomplish because of association of this trait with undesirable agronomic and fiber characters. Cotton (24) described wilt-resistant breeding stocks as usually being linked with one or more undesirable characters including late vegetative and low fruiting plant types; low lint percentage; inferior fiber length, strength, and micronaire; and excessive pubescence of the leaves.

In a review article, Sherbakoff (65) discussed the development of verticillium wilt-resistant cotton. The wilt resistance of "Tanguis" cotton is associated with high lint yields and high lint percentage, long staple, and a high market value per pound of lint. Verticillium wilt resistant Tanguis constituted 91% of the Peruvian crop in 1933. This variety is a form of G. barbadense, and selections were based specifically on those economic characters including resistance to verticillium wilt (65). In 1946 in Uganda, crosses between "KP28 X B181" (resistant) and "K40 X BP50" (susceptible) were made. Jameson found the resistance of the F_1 progenies was close to the geometric means of the two parents, and he concluded that the resistance was due to either blending inheritance or to qualitative inheritance (65). In the same year, verticillium wilt-tolerant "Acala 1517 WR" (selected from within "Acala 1517") was released by the U. S. Cotton Field Station, State

College, New Mexico (65). Soon it was grown extensively in certain counties of New Mexico, Arizona, and Texas where verticillium wilt was a serious problem, but it became susceptible only a few years after it was released (Brinkerhoff, personal communication).

Wiles (74) in studies of the reaction of cotton varieties to verticillium wilt reported that G. barbadense and G. arboreum were more resistant to verticillium wilt than G. hirsutum. The highest degree of resistance in the G. hirsutum varieties studied were the varieties "Smith 81-14," "Auburn 56," "Alabama Hybrid 257-202," and "Hartsville." These varieties were selfed, and subsequent progenies of individual plants were found to be more uniform in their resistance than their parent varieties.

Wilhelm, Sagen, and Tietz (77) found that selections from G. barbadense varieties and from G. barbadense X G. hirsutum hybrids (Seabrook, Coastland, Ashmouni, Wild Argentina, Montserrat, Russian, St. Kitts Superfine, and a selection from the Bonn, Germany Botanical Garden) were nearly true breeding and highly resistant to verticillium wilt in greenhouse inoculations and field tests. No high levels of resistance to verticillium wilt were found in upland cottons. Fourteen crosses among verticillium wilt resistant G. barbadense lines in general gave all resistant F_1 progenies. A few crosses involving one particular Sea Island line did appear slightly less resistant than their resistant parents. This particular line probably was an old G. barbadense X G. hirsutum hybrid. Fifty-six crosses between resistant (G. barbadense) and susceptible (G. hirsutum) lines gave predominantly resistant F_1 progenies, but some intermediate plants also appeared. First generation backcrosses of seven F_1's to their resistant parents gave resistant

progenies with an occasional slightly less resistant individual. Twelve F_1 backcrosses to their susceptible parents gave resistant, intermediate, and susceptible plants. The authors concluded that resistance to verticillium wilt was inherited as a dominant or as a partially dominant factor because there were gradations between resistance and susceptibility in progenies of most of the crosses. High yield and early maturity of the crop were noted for first generation G. barbadense X G. hirsutum individuals which were resistant to verticillium wilt under environmental conditions favorable for disease development. Subsequent research (78) in a cross between Seabrook (G. barbadense) and "Rex" (G. hirsutum) gave F_1 progenies which were intermediate in resistance. In the F_2, the segregation ratio approximated three resistant plants to one susceptible. Again, there was variation within the resistant and susceptible classes. The backcross between the F_1 and Rex was 0:1 while the backcross between the F_1 and Seabrook was 1:0. These results suggest a single dominant gene as having major control over resistance between these two lines. The backcross ratios suggest a genetic background effect. The crosses between Seabrook and Rex gave fertile, predominantly upland-type progenies.

Bell and Presley (8) reported that crosses between resistant Seabrook Sea Island and seven susceptible G. hirsutum varieties produced two F_1 progenies that were less resistant than the resistant parent while the other five progenies were equal in resistance to that parent. They concluded that resistance was transferred as a dominant character. Screening was accomplished using the D (defoliating) strain of the pathogen and was effective only under particular temperature regimes.

Barrow (7), also using a specific temperature regime but using

the less virulent SS-4 isolate of the pathogen, found in a cross between "Acala 9519" (a tolerant G. hirsutum) and "Acala 227" (a susceptible G. hirsutum) that the F_2 segregation ratio was approximately 3:1 of tolerant to susceptible plants. The first backcross of the F_1 to its susceptible parent segregated 1:1. This suggested that a single dominant gene was determining resistance to SS-4 between these two lines. The more virulent T-1 isolate produced symptoms too severe for accurate differentiation between these two parental types.

Stevenson and Jones (68) reported the inheritance of verticillium wilt resistance in both G. barbadense and G. hirsutum to be polygenic. Stith (69) concluded from theoretical considerations that the inheritance of resistance to verticillium wilt within G. hirsutum was a quantitative, rather than a qualitative, character.

CHAPTER III

MATERIALS AND METHODS

Description of the Host Population

Ten populations of upland cotton, four of which were released varieties, were used as parents in this experiment. One of the 10, "Stoneville 62," was included as a complete susceptible while the other nine were selected to sample different degrees of tolerance to the disease. The parents used in the study, their pedigrees, and mean disease grades over the test period are presented in Table I. The 10 populations do not constitute a random sample of all possible upland varieties. Therefore, inferences derived from the data apply only to the material studied. The extent to which they apply to Gossypium hirsutum as a whole is unknown.

Experimental Procedure

Diallel crosses among the 10 parents, ignoring reciprocals, were made at Iguala, Mexico, in the winter of 1967-68. These experiments were conducted at Altus and Stillwater, Oklahoma, in 1968 and 1969. Both test locations were on clay soils which had continuous histories of heavy verticillium wilt infestation over several years, and both received supplemental irrigation. A randomized complete-block design with four replications was employed in these studies. In both years, the 10 parents and 45 F_1 crosses among them were planted. In the second

TABLE I

PARENTS USED IN THE STUDY; THEIR PEDIGREES AND MEAN VERTICILLIUM WILT GRADES*

Parents	Pedigrees	Mean Verticillium Wilt Grades Over Locations+	
		1968	1969
M66-068	[($B_2B_3B_6$ X L.57) X L.57] X DPL 5540 F_9	6.4	6.0
M66-067	(L.611 X Fox 42-5) X Fox 42-5 F_8	5.9	5.3
M66-076	[(3060 X L.57) X L.57] X DPL 5540 F_9	6.0	5.2
OK 141-5	Sel. from 62 NM 8060-3 (2503 X Coquette $F_?$)	2.6	2.9
Mo-Del	---------	5.9	6.0
Stoneville 7A	---------	6.4	5.4
Westburn	---------	6.6	7.3
67-40A-409	Im2-1-6 X Acala 44 F_5 411-10	5.6	5.2
832-B	Sel. from Lankart 57	6.4	5.6
Stoneville 62	---------	7.5	8.2

*See Table II for verticillium wilt grades and their corresponding adult plant symptoms.

+Since a significant years X parents interaction was obtained for this trait (see Table VI), the means for each year over the Stillwater and Altus locations are listed separately.

year, the 45 F_2 progenies were also included. Plots were single rows 7.6 m long, and rows were 1.0 m apart. Plants were spaced at a distance of 0.3 m except at Altus in 1968 where they were 0.2-0.3 m apart with one to four plants/hill. In each test, a highly susceptible variety "Kemp" and a fairly tolerant variety "Stoneville 7A" were planted in separate rows between each fourth and fifth plots as checks.

Juvenile plants were tested by inoculating alternate plants in the row during the latter part of June and early July each year, but high temperatures following the inoculations apparently inhibited disease development. All plants were then reinoculated in early September except in the 1968 Altus test where natural infection was of such severity that reinoculation was judged unnecessary. A hypodermic needle inoculation technique (13, 17) was used in 1968, and a needle-and-sponge puncture method (31) was employed in 1969. For both methods, a small drop of a suspension of Verticillium albo-atrum containing approximately 1×10^6 conidia/ml was placed in the cambial region of the main stem of each plant.

Plots were heavily irrigated about the middle of September, some two to three weeks later than irrigation is normally applied in these areas. Comparative disease development in adjacent irrigated versus non-irrigated plots strongly suggested that the late irrigation materially increased wilt severity.

Plants were graded by visual inspection of gross external symptoms and of vascular discoloration in cut stems of those plants without external symptoms. The plants were graded on a scale ranging from one to 10. Descriptions of the grades may be found in Table II. Since this work was completed, a question has arisen regarding the use of vascular

TABLE II

VERTICILLIUM WILT GRADES AND THEIR CORRESPONDING ADULT PLANT SYMPTOMS

Grades	Adult Plant Symptoms
1	No visible leaf symptoms; no vascular discoloration in stems
2	Very mild leaf symptoms or vascular discoloration in stems
3	Moderate leaf symptoms
4	Severe leaf symptoms; little defoliation
5	Approximately 50% defoliated
6	Approximately 75% defoliated; often plants dwarfed
7	Approximately 90% defoliated; terminals of side branches last to shed
8	Nearly defoliated; often some regrowth from lower part of plant
9	Defoliated; stems dying back but alive at ground level
10	Defoliated; stems dead down to ground level

discoloration as one of the criteria in the differentiation between grades one and two. Fisher and Blank (W. D. Fisher, personal communication, 1970) in Arizona have found nearly 100% stalk discoloration under moderate-to-severe wilt infection. Plants with no discoloration in those circumstances have invariably been escapes. The degree of discoloration has been variable, and its correlation with external wilt symptom ratings has been erratic. Under mild wilt expression where leaf symptoms were inconspicuous or absent, 75-100% of discolored stalks were found in tolerant lines whereas 0-30% were found in lines considered susceptible. One point that may be of consequence here is that many of their tolerant lines were derived from similar genetic materials. J. R. Barrow (personal communication, 1970) in New Mexico has found severe stem discoloration in some of his most tolerant as well as most susceptible strains. He cites unpublished data of C. Roberts which only rarely gave significant correlations between stem and foliage grades. As a consequence, some ambiguity may well exist between grades one and two. However, since approximately 98% of the plants in this experiment were assigned grades from two through 10, the bias this ambiguity (if present) introduces into the data was considered of negligible magnitude.

Disease readings were taken on an individual plant basis each year in the first two weeks of October on 10 consecutive plants within each plot. The first plant in each plot was not graded because of possible border effects. One of the replications at Stillwater in 1969 was discarded because infection was not present in the susceptible check rows over much of that replication.

The data were then analyzed using the diallel analysis proposed by Jinks and Hayman (35, 42, 43). By conducting this sort of analysis,

quantitative, rather than qualitative, inheritance was assumed for wilt resistance in this material. In the interest of saving space and avoiding repetition, the steps in the diallel analysis procedure will be described in the next chapter as the results of those analyses are presented.

CHAPTER IV

RESULTS AND DISCUSSIONS

Analyses of Variance

Analyses of variance were conducted on a plot mean basis for the F_1 and parental data from each location in each year. Analyses were also conducted for the F_2 and parental data from each location in 1969. The results of those analyses are presented in Table III. Significant differences among entries at the 0.01 probability level were detected in every case. Since significant differences were obtained, diallel analyses could then be and were conducted at each location on three sets of data (i.e., the parents and their F_1's in 1968; the parents and their F_1's in 1969; and the parents and their F_2's in 1969).

The Diallel Analysis and Its Assumptions

Crumpacker and Allard (25) defined a diallel crossing system as one in which p genotypes are chosen and intercrossed. If all possible crosses are made, it is termed a complete diallel cross which can then be divided into three groups:

A. The p parental combinations $p_1 \times p_2$, $p_2 \times p_2$, ..., $p_n \times p_n$;

B. One set of $\frac{1}{2}$ p (p-1) F_1 combinations; and

C. The set of $\frac{1}{2}$ p (p-1) reciprocal F_1 combinations.

The parental genotypes are usually inbred lines. However, individuals, clones, open-pollinated varieties, or other genetic entities can

TABLE III

ANALYSES OF VARIANCE OF INDIVIDUAL EXPERIMENTS

Sources	df[+]	Mean Squares					
		Altus			Stillwater		
		F_1(1968)	F_1(1969)	F_2(1969)	F_1(1968)	F_1(1969)	F_2(1969)
Replications	3 (2)	3.8667**	0.1997	0.5477	1.9647	1.9260	7.5360**
Arrays	54 (54)	3.2011**	2.9276**	3.7551**	3.0327**	3.2696**	2.6438**
Error	162 (108)	0.6784	0.4689	0.4188	0.8769	0.9052	0.6810

*,**Significant at the 0.05 and 0.01 levels of probability, respectively.

[+]Numbers in parentheses denote the degrees of freedom in the Stillwater 1969 F_1 and F_2 analyses.

also be used. The diallel crossing system can be easily expanded to include more parents; and as a result, it permits the sampling of a wide range of germplasm. This systematic method leads to a rapid evaluation and identification of superior parents and/or hydrids.

Assumptions of the diallel analysis (25) are as follows:

A. No genotype-environment interaction within locations and years (except within certain prescribed limits),

B. Homozygous parents,

C. Diploid segregation,

D. No reciprocal differences,

E. No epistasis (that is, no nonallelic gene interactions),

F. No multiple alleles, and

G. Uncorrelated gene distributions.

General Tests of the Assumptions

The validity of the estimates obtained by means of the diallel analysis is dependent, at least to some extent, on the degree to which the assumptions of the analysis are fulfilled. Failure of any one or any combination of those assumptions invalidates to some degree the inferences derived by means of the analysis. To determine whether verticillium wilt fulfills the assumptions of the analysis, three broad, general tests of the assumptions (70, 71) were employed:

A. Analysis of variance of the quantity $(W_r - V_r)$,

B. Analysis of the (W_r, W_r') regression, and

C. Analysis of the (V_r, W_r) regression.

W_r is the covariance of the members of the rth array with their non-recurrent parents, W_r' is the covariance of the members of the rth

array with the array means of their non-recurrent parents, and V_r is the variance of the members of the rth array where the rth array includes the rth parent as well as the crosses in which the rth parent is involved.

Analysis of Variance of the Quantity $(W_r - V_r)$

If the assumptions are valid, the quantity $(W_r - V_r)$ is expected to be constant over arrays. Heterogeneity of this difference indicates that the character in question does not fulfill one or more of the assumptions of the analysis (35, 38, 43).

The quantity $(W_r - V_r)$ was obtained for each array in each replication, and then analyses of variance were conducted on the 40 values obtained with each set of data. Since there were only three replications at Stillwater, in 1969, there were only 30 values available in each of those sets. The results of this analysis for the F_1 populations in 1968 and for the F_1 and F_2 populations in 1969 are presented in Table IV. The arrays means squares were not significant at the 0.05 probability level in any set of data. This lack of significance suggests that verticillium wilt resistance in this material fulfills the assumptions of the analysis.

Analysis of the (W_r, W_r') Regression

In the (W_r, W_r') analysis, the regression coefficients are expected to be significantly different from zero but not from 0.5 if the assumptions are valid (2).

The (W_r, W_r') regression was estimated within each set of data using the means of W_r and W_r' for each array over replications. The coeffi-

TABLE IV

ANALYSES OF VARIANCE OF $(W_r - V_r)$ VALUES

Sources	df[+]	Mean Squares					
		Altus			Stillwater		
		F_1(1968)	F_1(1969)	F_2(1969)	F_1(1968)	F_1(1969)	F_2(1969)
Arrays	9 (9)	.3421	.0900	.1075	.2287	.2515	.1992
Replications	3 (2)	.3919	.5018**	.0402	.0810	.0940	1.0449
Error	27 (18)	.3782	.1060	.0866	.1480	.3438	.9641

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

[+] Numbers in parentheses denote the degrees of freedom in the Stillwater 1969 F_1 and F_2 analyses.

cients and their 95% confidence limits were calculated using the methods described by Steel and Torrie (67) and are presented in Table V. The coefficient for only one set of data, the F_2 population at Altus in 1969, was significantly different from zero but not from 0.5. The coefficient for the F_1 population at Altus in 1968 failed both criteria of the test. The remaining analyses either included zero in their confidence intervals or did not include 0.5. This test suggests at least a partial failure of the assumptions.

Analysis of the (V_r, W_r) Regression

In the (V_r, W_r) analysis, the regression coefficients should be significantly different from zero but not from 1.0 if the assumptions are valid (43).

Mean estimates of V_r and W_r, their regressions, and confidence intervals were calculated in the same manner as in the previous test. The coefficients and their 95% confidence limits are also presented in Table V. The coefficients for the F_1 and F_2 populations at Altus in 1969 and both F_1 populations at Stillwater in 1968 and 1969 were significantly different from zero but not from 1.0, thus, complying with expectations. The interval for the F_1 population at Altus in 1968 included zero while the interval for the F_2 population at Stillwater in 1969 failed to include 1.0.

In summarizing the general tests of the assumptions, three tests were conducted on three sets of data from two locations to check compliance of wilt resistance in this material with the assumptions of the diallel analysis. Of those 18 analyses, there was only one complete failure of the assumptions while six partial failures were noted.

TABLE V

(W_r, W'_r) AND (V_r, W_r) REGRESSION COEFFICIENTS AND THEIR 95% CONFIDENCE LIMITS

Locations	Generations	(W_r, W'_r)		(V_r, W_r)	
		Coefficients	95% Confidence Limits	Coefficients	95% Confidence Limits
Altus	F_1(1968)	.171	(-.087)-.429	.511	(-.131)-1.153
	F_1(1969)	.199	(-.106)-.504	.749	.222 -1.276
	F_2(1969)	.565	.355 -.775	.631	.222 -1.040
Stillwater	F_1(1968)	.328	.248 -.408	.727	.149 -1.305
	F_1(1969)	.285	.184 -.386	.740	.223 -1.257
	F_2(1969)	.350	(-.224)-.924	.536	.200 -0.872

Therefore, one cannot say that verticillium wilt resistance in this material fulfills all assumptions of the diallel analysis. However, the much greater frequency of successes leads one to conclude that such failures as were present were relatively minor.

Specific Tests of the Assumptions

Though the three general tests appeared to only partially fulfill the assumptions of the analysis, it is not possible to pinpoint which particular assumptions have failed using the results of those tests. However, certain assumptions may be considered valid while a few of the remainder can be tested using specific tests of the assumptions.

Assumptions Not Tested

The assumption of diploid segregation was not tested. The different chromosome sizes between the A and D genomes of the amphidiploid G. hirsutum can be used to help recognize the univalency of chromosomes at meiosis in the haploids of this tetraploid species (27). Kimber (44) and Endrizzi (28) in studies of the cytological chromosome behavior of G. hirsutum during meiosis reported that it formed bivalents, rather than multivalents. It is probable that a process of diploidization, similar to that in wheat, has occurred so that only homologous chromosomes within a genome can pair.

Generally, reciprocal crosses are not significantly different within G. hirsutum. Recently, White and Richmond (72) reported no significant reciprocal differences for the agronomic and fiber characters they studied. However, no specific information concerning the results of reciprocal crosses in regard to verticillium wilt resistance

in cotton are known to the author. Whether this accounts for the partial failure of the assumptions is therefore unknown.

It is understood that cotton is predominantly a self-pollinated plant. The parents in this study were selfed prior to crossing and testing and can be assumed to be relatively homozygous, though not necessarily homogeneous. However, some heterozygosity may remain even after many generations of self-pollination (4, 15). Therefore, the assumption of homozygous parents is probably not correct. This may account for part of the non-compliance with assumptions noted earlier.

The assumptions of no multiple alleles and of uncorrelated gene distributions were not tested because no such testing method or methods are known to the author at present. Either or both could have been involved in the partial failure of the assumptions detected herein.

Test for Epistasis

The assumption of no epistasis (no nonallelic gene interaction) was tested using the chi-square test formulated by Hayman (36). To conduct this test, F_2 data are required. Therefore, only the 1969 data could be subjected to this test. In the test, a $(2L_2 - L_1)$ table is used where the term L_1 is employed to denote a diallel table containing F_1 and parental means and the term L_2 denotes a diallel table containing F_2 and parental means. From the $(2L_2 - L_1)$ table, V_{OLO}, V_{OLX}, V_{1LX}, and W_{OLOX} can be estimated. These estimates are analogous to V_{OLO}, V_{OL1}, V_{1L1}, and W_{OLO1} calculated from the L_1 table. V_{OLO} is the variance of the parents, V_{OL1} is the variance of array means, V_{1L1} is the mean variance of arrays, and W_{OLO1} is the mean covariance of arrays. The formulas for calculating the chi-square value are as follows:

$$\text{Chi-square}_{(cal)} = k_2 [(n - 1)(V_{1LX} - V_{OLX}) + n(\bar{p} - \bar{x})^2/(1 + k) + (n - 1)(V_{OLO} - 4W_{OLOX} + 4V_{OLX})/(2 + k)]$$

with $\frac{1}{2}n(n - 1)$ degrees of freedom and

where $k = (nE_0)/(8E_2 + 2E_1 - E_0)$ and

$k_2 = n/(8E_2 + 2E_1)$.

E_0, E_1, and E_2 are estimates of the parental, F_1, and F_2 environmental variances, respectively; n is the number of parents; $\bar{p}$ is the mean of the parents; and $\bar{x}$ is the overall mean of the experiment.

The observed chi-square values with 45 degrees of freedom were 21.0 at Altus and 30.0 at Stillwater. Neither was significant at the 0.05 probability level. Therefore, epistasis is either absent in or made a negligible contribution to the expression of verticillium wilt resistance in this material.

Tests for Genotype-Environment Interaction

The assumption of no genotype-environment interaction within locations and years may be tested using the analysis proposed by Allard (3). The general method for this test is to analyze separately the additive and dominance components of variation.

The test for the additive components is based on the assumption that, in the absence of epistasis, heritable differences among homozygous parents are determined by the additive effects of genes. The parental lines which differ significantly from each other carry genes with different additive effects. From the interaction between parents and environments, the constancy over environments of the additive components can be tested. When epistasis exists in the genetic system, the

situation is unambiguous (3) only if the parents by environments interaction is not significant. Since epistasis has already been ruled out as a complicating factor, the complications resulting from its presence need not be considered further.

Since the 1969 Stillwater test had reliable data from only three replications whereas the other tests each had four, a random process was used to equalize the number of replications/test at three, thereby permitting a balanced design and analysis. The analysis of variance for the additive components was conducted on the 120 plot mean estimates of the 10 parents, two years, two locations, and three replications/test. The results of this analysis are presented in Table VI.

The significance of the locations mean square suggested that the total additive effects differed between Stillwater and Altus. This is expressed additive effects probably in relation to the severity of the disease at the respective locations rather than to inherent differences, since the same parents were grown at both locations. Inspection of location means showed the disease to be more severe each year at Stillwater. This was probably due to slightly lower mean air temperature from the middle of August to the middle of October at Stillwater for both years. The total additive effects exhibited did not vary significantly, from year to year nor was the interaction between years and locations significant. The parents mean square was significant which suggested that these parents did have genes with different additive effects for verticillium wilt resistance. Examination of the first-order interactions revealed that relative performance among the parents for these effects was constant from location to location but not from year to year. The second-order interaction was not significant which

TABLE VI

GENOTYPE BY ENVIRONMENT ANALYSIS OF THE ADDITIVE COMPONENTS OF VARIATION

Sources	df	Mean Squares
Years	1	.9700
Locations	1	6.6200*
Years X Locations	1	3.3400
Reps Within Years and Locations	8	.9238
Parents	9	21.6044**
Years X Parents	9	1.9444**
Locations X Parents	9	.6689
Years X Locations X Parents	9	.6333
Error	72	.4126

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

in turn suggested that only a negligible portion of the interactions could not be associated with years.

The analysis of variance for dominance components was conducted by a combined analysis of variance of the 120 V_r and 120 W_r adjusted estimates obtained from the 10 arrays, two years, two locations, three replications/test from the L_1 table. The same replications were employed in this analysis as were used in the analysis of the additive components. The values of V_r and W_r were adjusted by dividing those estimates by the variance of the parents within that replication before conducting the analysis. This adjustment was performed in order to minimize the variation of the additive components in the test while increasing the prospects of detecting interactions of dominance effects with environments; it also tends to reduce the fluctuation of basic variability between environments which can mask between-environment comparisons in genetic systems. The dominance component of variation is expected to be significant except when mean dominance is complete or zero. The interaction between environments and dominance is an estimate of the constancy of mean dominance over environments. The arrays component of variation detects differences in the dominance order of parents. The interaction between environments and the array component tests the constancy of the dominance relationships among parents over environments. The interaction between dominance and arrays and the higher-order interactions between dominance, arrays, and environments provides a test for non-allelic interaction and for the constancy of such effects over environments, respectively (3). The results of this analysis are presented in Table VII.

The lack of significance of the years, locations, and interaction

TABLE VII

GENOTYPE BY ENVIRONMENT ANALYSIS OF THE DOMINANCE COMPONENTS OF VARIATION

Sources	df	Mean Squares
Years	1	.0487
Locations	1	.0005
Years X Locations	1	1.1986
Reps Within Years and Locations	8	.3587
Dominance	1	1.5974**
Years X Dominance	1	.0001
Locations X Dominance	1	.0024
Years X Locations X Dominance	1	.7889**
Arrays	9	.2103**
Years X Arrays	9	.0870*
Locations X Arrays	9	.0405
Years X Locations X Arrays	9	.0823*
Dominance X Arrays	9	.0214
Years X Dominance X Arrays	9	.0383
Locations X Dominance X Arrays	9	.0236
Years X Locations X Dominance X Arrays	9	.0153
Error	152	.0391

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

between years and locations mean squares indicated that the rescaling was effective in accomplishing the reduction of basic variability fluctuation between environments. The significance of the dominance mean square suggests that mean degree of dominance was either in the partial or overdominance ranges. The lack of significance of the interaction between the years and dominance and between the locations and dominance mean squares and the significance for the second-order interaction indicated that interactions were present but that they could not be attributed to effects consistent over years or locations. The significance of the arrays mean square indicated that the parents differed among themselves in the relative dominance effects of their genes. The significance of the interaction between years and arrays indicated that the relative dominance of the parents changed from year to year. Such changes could not be attributed to locations. The significance of years by locations by arrays mean square suggested that some relative dominance changes among parents could not be traced to either year or location effects. The remaining four interactions were not significant which provides additional evidence that epistasis was of negligible importance in the inheritance of verticillium wilt resistance in this material.

Estimates of Population Parameters

Even though verticillium wilt resistance appeared not to completely fulfill the assumptions in this study, the population parameters for such a character may still be estimated (35). However, estimates made under such circumstances are admittedly not as accurate as they would have been had all the assumptions been fulfilled.

Estimates were calculated in each replication with each replicate

being treated as a separate experiment with its own estimate of environmental variance. The standard errors of the mean, used in the tests of significance, were estimated by the variation of the block values around the overall mean (52).

The population parameters estimated were E_0, E_1, E_2, D, F, H_1, and H_2. E_0, E_1, and E_2 are the estimates of parental, F_1, and F_2 environmental variances, respectively. Estimates of E_0 were obtained from a between plot-within plot analysis of variance of the parental entries within each replication. Since the other parameters were estimated on a plot mean basis, it was necessary to convert E_0 to equivalent basis by dividing the within plot mean square by the average number of plants/plot for the parental entries in that replication. E_1 and E_2 were calculated in an identical manner using F_1 and F_2 entries, respectively. The parameters D, F, H_1, and H_2 are defined by Jinks and Hayman (43) using the notation of Mather (47). D is the additive genetic variance which may include additive by additive epistatic variance. H_1 and H_2 are dominance genetic variances which may include dominance by dominance, additive by dominance, and a portion of the additive by additive epistatic variance not included within D. F serves as an indicator of the relative frequency of dominant and recessive alleles in the parents. If F equals zero, either the dominant and recessive alleles in the parents are equally distributed or there is no dominance. If F is negative, an excess of recessive alleles is suggested while a positive value indicates an excess of dominant alleles in the parents (25).

The above population parameters were estimated by the use of Hayman's (35, 37) formulas. Estimates were obtained for the F_1 and parental data where n equals the number of parents using the equations which

follow:

A. Variance of the parents = $V_{OLO} = D + E_0$,

B. Mean covariance of arrays = $W_{OLO1} = \frac{1}{2}D - \frac{1}{4}F + E_0/n$,

C. Mean variance of the arrays = $V_{1L1} = \frac{1}{4}D + \frac{1}{4}H_1 - \frac{1}{4}F + [E_0 + (n - 1)E_1]/n$, and

D. Variance of array means = $V_{OL1} = \frac{1}{4}D + \frac{1}{4}H_1 - \frac{1}{4}H_2 - \frac{1}{4}F + [E_0 + (n - 2)E_1]/n^2$.

Estimates of F, H_1, and H_2 in the F_2 were obtained using F_2 and parental data where n again equals the number of parents. Those equations are as follows:

E. Mean covariance of arrays = $W_{OLO2} = \frac{1}{2}D - 1/8F + E_0/n$,

F. Mean variance of arrays = $V_{2L2} = \frac{1}{4}D + 1/16H_1 - 1/8F + [E_0 + (n - 1)E_2]/n$, and

G. Variance of array means = $V_{OL2} = \frac{1}{4}D + 1/16H_1 - 1/16H_2 - 1/8F + [E_0 + (n - 2)E_2]/n^2$.

The means of these estimates and their significance levels are presented in Table VIII.

It is obvious that environment influences the expression of verticillium wilt resistance since the estimates of environmental variation were in every case significantly different from zero. E_1 was larger than E_0 in three out of four cases while E_2 was larger than E_0 and E_1 at both locations in 1969.

The estimates of D were significantly different in every instance and were larger than the other parameters estimated in the same test

TABLE VIII

MEAN PARAMETER ESTIMATES AND THEIR LEVELS OF SIGNIFICANCE

Parameters	Altus			Stillwater		
	F_1(1968)	F_1(1969)	F_2(1969)	F_1(1968)	F_1(1969)	F_2(1969)
E_0	.72**	.29**	---	.37**	.18*	---
E_1	.55**	.34**	---	.44**	.21**	---
E_2	---	---	.49**	---	---	.44**
D	1.18*	1.92**	---	2.34*	2.00*	---
F	-.12	1.07*	1.07	1.42	.94	1.69
H_1	.69	.91*	1.47	1.68*	3.63*	4.68*
H_2	.97	.64*	1.14	1.30*	3.01*	4.09

*,**Significantly different from zero at the 0.05 and 0.01 levels of probability, respectively.

except for the H_1 and H_2 estimates at Stillwater in 1969. Therefore, additive genetic variance does exist for verticillium wilt resistance and was generally the most important source of variation in this material.

The calculated F values were inconsistent in sign. Only one estimate out of six was significantly different from zero, and it was significant only at the 0.05 probability level. As a result, firm conclusions regarding the relative frequency of dominant and recessive alleles in this material could not be reached.

Most estimates of H_1 and H_2 were significantly different from zero. In general, they were second to D in relative magnitude. Therefore, dominance does exist in this material for verticillium wilt resistance, and it is probably second in importance to the additive variance.

Investigation of Genetic Systems

Several estimators were calculated, using the parameters estimated in Table VIII, to provide further information about the genetic system of wilt resistance in this material. Each estimator was obtained in each replication, and standard errors of the mean were calculated as before. The means of those estimators and their 95% confidence limits are presented in Table IX.

Dominance

Dominance estimators one, two, and three were estimated in the F_1 by the ratios H_1/D, $(H_1/D)^{\frac{1}{2}}$, and $(V_{1L1} - E)/(W_{OLO1} - E/n)$, respectively, and in the F_2 by $\frac{1}{4}H_1/D$, $(\frac{1}{4}H_1/D)^{\frac{1}{2}}$, and $(V_{2L2} - E)/(W_{OLO2} - E/n)$, respectively. Each of the estimators is a weighted overall measure of the

TABLE IX

MEAN ESTIMATORS AND THEIR 95% CONFIDENCE LIMITS

	Altus					
Estimators	F_1(1968)	95% Confidence Limits	F_1(1969)	95% Confidence Limits	F_2(1969)	95% Confidence Limits
Dominance 1*	.88	(-.54)-2.30	.51	.18 -0.84	.17	(-.01)-0.35
Dominance 2*	.82	(-.02)-1.66	.70	.44 -0.96	.36	(-.17)-0.87
Dominance 3*	.68	.15 -1.21	.70	.48 -0.92	.57	.36 -0.78
$(\bar{F}_1 - \bar{P})^+$	.27	(-.24)-0.78	.03	(-.11)-0.17	.06	(-.22)-0.34
1/4 (H_2/H_1)	.26	(-.08)-0.60	.17	.12 -0.22	.15	(-.02)-0.32
Heritability	.27	.12 -0.42	.64	.36 -0.92	.58	.29 -0.87

*Dominance estimators one, two, and three in the F_1 were obtained using the formulas H_1/D, $(H_1/D)^{\frac{1}{2}}$, and $(V_{1L1} - E)/(W_{OLO1} - E/n)$, respectively. These formulas in the F_2 were modified into 1/4 (H_1/D), $[1/4\ (H_1/D)]^{\frac{1}{2}}$, and $(V_{2L2} - E)/(W_{OLO2} - E/n)$, respectively. Interpretations of the symbols, V_{1L1}, V_{2L2}, W_{OLO1}, W_{OLO2}, and n, may be found in Jinks and Hayman's papers (35, 37, 42, 43).

+Mean of the 45 F_1 (or F_2) versus midparent comparisons within each replication.

TABLE XI (Continued)

Estimators	Stillwater					
	F_1(1968)	95% Confidence Limits	F_1(1969)	95% Confidence Limits	F_2(1969)	95% Confidence Limits
Dominance 1*	.85	.32 -1.38	1.86	1.30 -2.42	.67	(-.30)-1.64
Dominance 2*	.91	.64 -1.18	1.36	1.16 -1.56	.79	.15 -1.43
Dominance 3*	.83	.43 -1.23	1.56	1.42 -1.70	.93	.30 -1.56
$(\bar{F}_1 - \bar{P})^+$	.11	(-.24)-0.46	.17	(-.66)-1.00	.12	(-.77)-1.01
1/4 (H_2/H_1)	.21	.08 -0.34	.21	.18 -0.24	.21	.03 -0.39
Heritability	.54	.10 -0.98	.36	.17 -0.55	.57	.02 -1.12

*Dominance estimators one, two, and three in the F_1 were obtained using the formulas H_1/D, $(H_1/D)^{\frac{1}{2}}$, and $(V_{1L1} - E)/(W_{0L01} - E/n)$, respectively. These formulas in the F_2 were modified into 1/4 (H_1/D), $[1/4\ (H_1/D)]^{\frac{1}{2}}$, and $(V_{2L2} - E)/(W_{0L02} - E/n)$, respectively. Interpretations of the symbols, V_{1L1}, V_{2L2}, W_{0L01}, W_{0L02}, and n, may be found in Jinks and Hayman's papers (35, 37, 42, 43).

+Mean of the 45 F_1 (or F_2) versus midparent comparisons within each replication.

degree of dominance. With no dominance, the estimates should equal zero; with partial dominance, the estimates should fall in the range from zero to one; if there is complete dominance, the estimates should equal one; and estimates greater than one indicate overdominance (25).

All the estimates were between zero and one, except for those obtained in the Stillwater 1969 F_1 where all were greater than one. Therefore, in five out of six tests, verticillium wilt resistance in this material appeared to be in the partial dominance range. However, none of the overdominance estimates included one (i.e., complete dominance) within their confidence intervals. This adds reassurance that the overdominance observed was a real phenomenon. This observation coupled with that in the next paragraph could have rather important breeding implications. If the trait truly exhibits overdominance for susceptibility in an occasional environment, this sporadic occurrence would tend to delay the eventual fixation of desirable homozygous recessive genotypes by selection since at those times the breeder would select against the more heterozygous genotypes. He could thereby nullify, at least in part, previous selection progress. It also suggests that the breeder should not be so ruthless that he discards all lines except the very best ones since to do so over a period of time would perpetuate homozygosity of intermediate genotypes in his materials.

The direction of dominance could be estimated by $(\bar{F}_1 - \bar{P})$. This estimator revealed a rather consistent trend for the F_1 to be more susceptible than its midparent value. However, the variation was such that in no set of data was this difference significantly different from zero. If this trend is a real phenomenon, then selection within early generation materials should often be effective in increasing the mean

level of tolerance.

Distribution of Alleles

The quantity $\frac{1}{4}(H_2/H_1)$ is an estimator of the average frequency of negative versus positive alleles in the parents (25). If the distribution is equal, the ratio should equal 0.25; if unequal, it should be smaller. Of the six estimates, only one was close to 0.25 while two were significantly different from it. Apparently, the frequency of negative versus positive alleles in the parents was unequal. An examination of the means in Table I would suggest that the majority of genes in this population are for greater susceptibility.

Number of Effective Factors

The number of effective factors, K, as defined by Mather (47) were highly erratic ranging from 112 to less than one. Only one estimate was significantly different from zero, and it was 0.13. As a result, reasonable doubt existed as to the validity of those estimates; and they were omitted from this paper.

Heritability

Narrow-sense heritabilities were estimated on a plot mean basis in the F_1 according to the formula given by Crumpacker and Allard (25) which follows:

$$\text{Heritability} = (\tfrac{1}{4}D)/(\tfrac{1}{4}D + \tfrac{1}{4}H_1 - \tfrac{1}{4}F + E)$$

In the F_2, heritabilities were estimated using the modified formula given by Verhalen and Murray (70) and listed below:

$$\text{Heritability} = (\tfrac{1}{4}D)/(\tfrac{1}{4}D + 1/16H_1 - 1/8F + E)$$

All estimates were significantly different from zero. Four of the six estimates ranged from 0.54 to 0.64 indicating rapid selection progress would be possible in this material in most environments. This is provided of course that the techniques used in this experiment were utilized in those selections. The techniques considered most critical are spaced plantings, inoculation and late irrigation in the fall, use on a plant basis of the grading scheme devised herein (or a similar one), and selection on a row mean basis rather than on individual plants. It is noteworthy that the two lower heritabilities were obtained under circumstances peculiar to those individual tests. The estimate of 0.27 was obtained at Altus in 1968 where plants/hill varied from one to four and where inoculations in the fall were not made (i.e., natural infection was depended upon to produce symptoms). The estimate of 0.36 was obtained at Stillwater in the 1969 F_1 where overdominance was shown to be present. The cause for the overdominance, presumably some environmental factor, cannot be determined; but it is well known that overdominance itself reduces narrow-sense heritabilities rather drastically when compared to similar models of no, partial, or complete dominance.

CHAPTER V

SUMMARY AND CONCLUSIONS

Ten selected lines of upland cotton (Gossypium hirsutum L.) and the 45 possible F_1 crosses among them (ignoring reciprocals) were studied in replicated, randomized tests at Altus and Stillwater, Oklahoma, in 1968 and 1969. Forty-five F_2 populations were included at both locations in 1969. The objective of the study was to determine the inheritance of resistance to verticillium wilt in this material. The extent to which the inferences derived from this study apply to G. hirsutum as a whole is unknown.

Analyses of variance were conducted on plot mean data from each location in each year. Significant differences among entries at the 0.01 probability level were obtained in every case. A diallel analysis was then conducted at each location on three sets of data (i.e., parents and F_1's in 1968; parents and F_1's in 1969; and parents and F_2's in 1969).

Three broad, general tests of the diallel assumptions were applied to the three sets of data at each location to determine degree of compliance of verticillium wilt resistance in this material with the assumptions of the analysis. One test completely failed the assumptions while six partial failures were recorded among the 18 tests conducted. Thus, one may conclude that verticillium wilt resistance does not fulfill all the assumptions but such failures as were noted were probably relatively minor.

In specific tests of the assumptions, five of the assumptions were not tested because such tests were considered unnecessary or because of the lack of appropriate tests. Those assumptions were diploid segregation, homozygous parents, no reciprocal differences, no multiple alleles, and uncorrelated gene distribution. Epistasis could be tested only in 1969 because F_2 data are required for the test. Epistasis was found to be either absent in or to make a negligible contribution to the expression of verticillium wilt resistance in this material. The assumption of no genotype-environment interaction within locations and years was conducted by separate analyses of the additive and dominance components of variation. The parents included in the experiment had genes with different additive effects for verticillium wilt resistance. Such effects were constant from location to location but not over years. Significant differences in dominance among the parents were also found. These differences among parents were again constant relative to one another from location to location, but not over years. A significant portion of the dominance interactions among parents could not be attributed to locations or to years.

All estimates of parental, F_1, and F_2 environmental variances were significantly different from zero. Thus, it is obvious that environmental factors do influence the expression of verticillium wilt resistance. All estimates of additive genetic variance were significant and, except in one set of data, were the largest component of variation in each set. These results verify the presence of additive genetic variance and imply that it is the most important source of variation in this material. Estimates of dominance genetic variance were, in general, significant and second to the additive genetic variance in relative

magnitude. Therefore, dominance does exist in this material for verticillium wilt resistance, and it is second in importance to the additive source of variation.

Firm conclusion regarding the frequency of dominant versus recessive alleles in the parents could not be drawn since the F values were inconsistent in sign and only in one instance was a value obtained which was significantly different from zero.

In the investigation of genetic systems, it was noted that the degree of dominance for verticillium wilt resistance fell in the partial dominance range, except one case where overdominance was observed. The direction of dominance rather consistently tended toward greater susceptibility for the F_1 than for its midparent value. However, the variation was such that in no set of data was this difference significantly different from zero. If this trend is a real phenomenon, then selection within early generation materials should often be effective in increasing the mean level of tolerance. The occasional exhibition of overdominance for greater susceptibility also has rather important breeding implications. This sporadic occurrence would tend to delay eventual fixation of desirable homozygous recessive genotypes by selection since at those times the breeder would tend to select against the more heterozygous genotypes and select for the more homozygous intermediate genotypes.

The average frequency of negative versus positive alleles in the parents is apparently unequal and biased towards greater susceptibility. Reasonable doubt existed as to the validity of the number of effective factors, and those estimates were omitted from this paper.

Narrow-sense heritabilities on a plot mean basis were estimated in

each set of data at each location. Four of the six estimates ranged from 0.54 to 0.64. Therefore, mass selection should be effective as a breeding method for verticillium wilt resistance in this material in most environments provided that the techniques used in these experiments (or similar ones) were followed in those selections. One of the lower heritability estimates was obtained where spaced plantings and artificial inoculations were not used. The other was associated with the overdominance estimate for degree of dominance.

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