BREEDING PEANUTS (*ARACHIS HYPOGAEA* L.) FOR RESISTANCE TO VERTICILLIUM WILT

By

BAZ MOHAMMAD KHAN

Bachelor of Science (Hons) Agriculture
University of Peshawar
Peshawar, Pakistan
1960

Master of Science
American University of Beirut
Beirut, Lebanon
1964

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
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DOCTOR OF PHILOSOPHY
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Thesis Approved:

James S. Kirby
Thesis Adviser

Ralph S. Matlock

J. Q. Lynd

D. F. Wadsworth

D. D. Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

Peanut kernels are high (57) in protein (25-30%) and oil (45-50%). The kernels are important as a good source of protein and edible oil for many people throughout the world. As a result peanuts are one of the leading crops of the world, and considered a major crop in the United States of America. It is an important cash crop in Oklahoma and other southern states.

Production and uses of peanuts are increasing, and will continue to increase as the need for food increases and new varieties are produced to meet these needs.

Verticillium wilt of peanuts caused by species of *Verticillium*, has been reported from Asia, Australia and the United States of America (14, 32, 41). The disease was reported in Oklahoma in 1970. At that time it was found only in Caddo county where the disease was associated with losses in yield of approximately 63% (48). Verticillium wilt is now known to occur in at least 5 counties of Oklahoma. Because of the apparent spread of the disease, the severe losses caused, persistence of the pathogen in the soil and its wide host range, Verticillium wilt is regarded as a major disease of peanuts in Oklahoma.

Attempts have been made to control Verticillium wilt in crops such as peppermint, potatoes and tomatoes. Treating infested soil with fumigants (40), chemical seed treatment and crop rotation have not given any practical control (18, 32). Resistant varieties have been developed in other crops such as cotton, tomatoes and eggplant (9, 36, 22), therefore, a possible control of the disease in peanuts may also be the development of resistant varieties.

Genetic resistance is a heritable capacity of plants to escape or to withstand disease damage to a great extent. The use of a resistant variety is an ideal method of disease control for the grower. It costs him no more in money, time or effort, and once resistance is obtained, it is relatively permanent. However, developing resistant varieties requires long periods of time. The first step in developing varieties resistant to a disease organism is to locate germ plasm that carries the desired resistance and initiate a breeding program. The inheritance pattern of resistance should be determined to develop an efficient breeding program for incorporating the resistance into agronomically desirable varieties.

The initial purpose of this study was to screen peanut germ plasm for possible sources of resistance to Verticillium wilt. The secondary purpose was to determine the mode of inheritance of Verticillium wilt resistance in peanuts to facilitate incorporation of resistance into agronomically desirable varieties.

CHAPTER II

LITERATURE REVIEW

Occurrence and Symptoms of Verticillium Wilt of Peanuts

Verticillium wilt of peanuts (Arachis hypogaea L.) has been reported from Asia, Australia and the United States of America (14, 32, 41). Purss (32) reported that Verticillium wilt of peanuts was widely recognized in Australia and generally thought to be of little importance even though in certain fields a loss in peanut yield of 14 to 64 percent was estimated. In Israel (14), Verticillium wilt was found as a common disease of potatoes, tomatoes, cotton and peanuts. Verticillium wilt of peanuts is not widely distributed in the United States of America but in some areas a large portion of the peanut crop becomes affected. Smith (41) reported the occurrence of Verticillium wilt of peanuts in Roosevelle County, New Mexico in 1960 in various fields; and since then the disease has become endemic in those areas of New Mexico. Recently, in Oklahoma, Wadsworth and Sturgeon (48) identified Verticillium wilt of peanuts in the late summer of 1970 under irrigated field conditions. They showed an average loss in yield of 63 percent from 2 fields.

The disease usually appears about flowering time (32, 48) in irregular patches and in low areas of a field (Fig. 1). Seldom does it occur uniformly over large areas. The Verticillium fungus invades

Figure 1. Verticillium Wilt of Peanuts Under Field Conditions

plant roots and initiates a systemic infection through the vascular tissues. Usually disease symptoms appear as dull green or chlorotic discoloration of portions of the lower leaflets (Fig. 2). As the disease progresses, many leaflets over the entire plant turn yellow, wither, become brown, and fall from the plant. Infected plants may progressively lose foliage until they die, but unless unusually dry weather prevails, the infected plants do not die rapidly. If adequate moisture is present, the infected plants remain alive but are stunted with sparse foliage and are relatively unproductive. Brown to black vascular discoloration can be seen in the roots and lower parts of the stem. In advanced stages of disease development, the vascular discoloration extends into the upper petioles (Fig. 3).

Taxonomy and Morphology of the Fungus

The causal organism of Verticillium wilt is a soil-borne fungus, belonging to the class "Fungi Imperfecti" with a specific growth characteristic of verticilliate conidiophores. Isaac (19), reviewing literature, reported that the Verticillium fungus was first identified as the causal agent of a wilt disease of potatoes in Germany by Reinke and Berth in 1879. They stated that the dieseased plant tissues became black or dark brown due to a blackening of the septate hyphae of the pathogen. The short cells so-formed increased in size and became spherical with brown to black coloration. These cells and cellular masses constituted the resting mycelium for the overwintering of the fungus. They named the fungus "Verticillium albo-atrum Reinke & Berth." Isaac (19) further reported that in 1913 Klebahn isolated another form of Verticillium from a diseased Dahlia plant. Klebahn

Figure 2. Verticillium Wilt Infected Peanut Seedling on the Right and Healthy on the Left

Figure 3. Verticillium Wilt Infected Peanut Stem Showing Vascular Discoloration on the Right and a Healthy Stem on the Left

considered it sufficiently different from the organism described by Reinke and Berth to be named Verticillium dahliae Kleb. The principle point of difference was that his isolate formed sclerotia from irregular multilateral septation and budding of the cells of a single hypha and not from the anastomosis of several hyphae.

In 1919 Pethybridge (31) discovered two more species of Verticillium on the surface of potato tubers. These were named Verticillium nigrescens Pethybr. and Verticillium nubilum Pethybr.

Although taxonomically the genus Verticillium has been classified into five known species, only Verticillium albo-atrum and V. dahliae are well recognized as plant pathogens. Even these two species are hardly separable; and considerable controversy exists on the validity of separate taxa for them. Since cogent opinions support the separation or synonomy of the two species, in this study only the genus name was considered for the pathogen.

Physiological Studies of the Pathogen

The commonly known resting bodies of Verticillium spp. are black resting mycelia, microsclerotia and chlamydospores (20). One or more types of these resting bodies are usually found in the so-called different species of Verticillium. On culturing these resting bodies on artificial media (Fig. 4), growth started within 24 hours at room temperature (20°C). An optimum temperature of 22.5°C for growth of Verticillium albo-atrum and V. dahliae and 22.5 - 25°C for V. nigrescens was recorded, but at 30°C only V. dahliae and V. nigrescens could develop (19). The optimum pH of potato-dextrose-agar medium reported for V. albo-atrum was 8.1 to 8.6, whereas for V. dahliae and V.

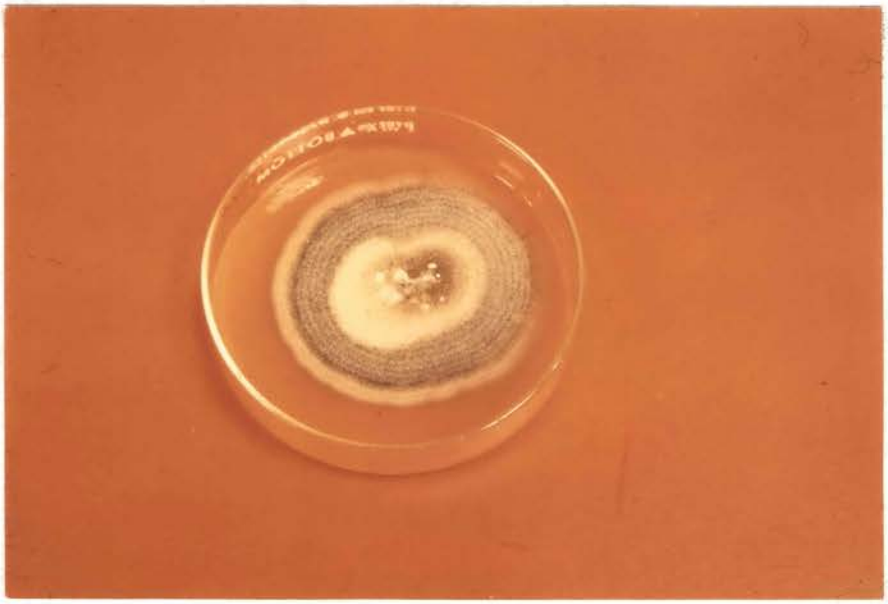

Figure 4. Verticillium Fungus in Culture on Potato-Dextrose-Agar

nigrescens a pH of 5.3 to 7.2 was the best (19). The microsclerotial form (V. dahliae) and chlamydospore form (V. nigrescens) grew well on sucrose, dextrose and maltose media, but poorly on glycerine, whereas the dark mycelium strain (V. albo-atrum) developed fairly well on glycerine and also produced maximum growth on maltose. The best source of nitrogen for all strains of Verticillium was ammonium-ions (19). The microsclerotial form (Verticillium dahliae) was pathogenic on tomato at 25°C to 27°C, but Verticillium albo-atrum was most pathogenic at 21°C (19).

Nelson and Wilhelm (30) tested Verticillium isolates from diseased rose and tomato plants in hot water and dry heat for the minimal lethal temperature. After 10 minutes of exposure in hot water, 47°C was lethal to both hyphae and conidia, whereas for microsclerotia 50°C was lethal. Forty minutes exposure at 47°C also killed the microsclerotia. In a dry atmosphere at 40°C, microsclerotia survived for 2½ years while the conidia succumbed within 3 days.

Basu (5) reported that 3-year-old dried cultures of Verticillium isolates grew when transferred to fresh media and retained pathogenicity on tomato plants. Schnathorst (38) found that isolates of Verticillium produced three structures that gave rise to new growth after a one-year dormant period. These were clusters of hyaline microsclerotial cells, hyaline chains of chlamydospore-like cells and hyaline inter-twined hyphae in microsclerotial masses. He suggested these as the principal means of survival of Verticillium albo-atrum. Schnathorst (37) reported that new growth originated from three different resting forms of Verticillium species associated with microsclerotia but never from melanized thick-walled cells. Gordee and Porter (17) distinguished two types of microsclerotial cells: thick-walled melanized cells and thin-walled hyaline cells. The latter were attached to the former ones. Nadakavukaren (27) found two kinds of cells in a microsclerotium. One had a non-organized nucleus and the other had one or more intact nuclei and accounted for previously reported differences in viability of microsclerotia.

Alteration in Virulence of *Verticillium* spp.

Alteration in pathogenicity of *Verticillium* spp. through serial host passage has been reported. Under greenhouse conditions, Fordyce and Green (13) tested 46 isolates of *V. albo-atrum* obtained from peppermint (*Mentha* spp.) for stability of pathogenicity. Five of the most virulent isolates were selected for serial host passage studies. All the isolates were weakly-pathogenic initially to tomatoes but two isolates became more pathogenic after one passage and one isolate after 5 passages. Simultaneously these 3 isolates, with an increase in pathogenicity to tomatoes, ultimately became nonpathogenic to their original host, *Mentha* spp.

Effect of Inoculum Potential and Environmental Factors on Verticillium Wilt Severity

Inoculum potential in relation to Verticillium wilt development and severity is a very important factor. In a study conducted by Tolmsff and Young (46), it was revealed that treatment of *Verticillium* infested soil with Vapam (sodium N-methyl-dithio-carbonate dihydrate) resulted in disease control as measured by yield and severity of foliage symptoms on potatoes. The incidence of infection in treated plants remained similar to that in untreated plants. From a study using 15 levels of inoculum, they found that severity of symptoms, time of plant death and loss of tuber yield were related to the amount of inoculum. Amendment materials for soil such as cotton seed meal, fish meal, blood meal, Dithane Z-78 and Fermate, all reduced the *Verticillium* inoculum potential (52). Somewhat similar results were obtained by Young *et al*. (58) who showed that alfalfa meal at the rate of 2000 lbs per acre plus 100 lbs of ammonium nitrate produced significant yield increases of cotton in Verticillium wilt tests.

In a direct-plate count assaying method for determining the changes in natural uncropped soil following inoculation with pseudosclerotia, Menzies and Griebel (26) reported that, in uncropped soil, populations consistently increased to a peak of 3 to 5 times the initial inoculum concentration in the first 10 days, then gradually subsided, eventually remaining relatively constant. The increased population was due to the growth and conidial production by the pseudosclerotia.

Soil pH seems to have little influence on the development of Verticillium wilt. Wilhelm (50) revealed that generally Verticillium wilt is a problem in alkaline soils in California, but severe outbreaks also occurred repeatedly on soils well in the acid pH range. It was concluded that the occurrence and severity of Verticillium wilt is not greatly affected by soil reaction within the range in which susceptible crops are commonly grown.

Soil moisture level has a significant effect on the severity of Verticillium wilt. Longenecker and Henfner (23) reported from one test on cotton that the incidence of Verticillium wilt was 32% for four irrigations, compared with a doubling of the incidence with more and frequent irrigations in the El Paso valley of Texas.

The importance of nonhost plants in maintaining the inoculum of Verticillium has been reported by Martison and Horne (24), when known host and nonhost plants were grown in soil infested with microsclerotia of the peppermint strain of Verticillium albo-atrum. A field infested with the potato strain of Verticillium albo-atrum and monocropped to nonhost cereal crops for 8 to 10 years retained a high inoculum potential. Roots of nonhost weeds and cereals growing in the infested field were heavily infected with Verticillium. Potatoes grown in this field showed 100% infection and severe wilting. This gave evidence that infected nonhosts maintained the inoculum potential of both strains of Verticillium in the absence of susceptible hosts. Conversely the reports of Wilhelm (53) suggested that survival by nonpathogenic root invasions in immune plants probably does not play an important part in the persistence of Verticillium in soil. Verticillium cultures were retained in original tubes at room temperature and persisted for 13 years in culture by means of microsclerotia; persistence for a similar period of 14 years in field soil with no hosts present was thought also to reflect the longevity of the resting structure. Evans et al. (11) reported that Verticillium exists in soil as microsclerotia, either as free units or embedded in decaying plant debris. The population of free microsclerotia in a cultivated soil was counted. Individual counts up to 2000 microsclerotia/gram of soil were recorded in some samples.

Wilhelm (51) further reported that, once land is infested with

Verticillium, the inoculum potential is a reflection largely of the crop history and thus increasing rapidly in the presence of susceptible crops but not by the independent saprophytic growth in soil. He suggested that Verticillium is a soil invader and not a soil inhabitant.

Quantitative information on the vertical distribution of Verticillium albo-atrum was obtained (49) for a variety of soils from different regions of California, and with different crop histories. In general, the 0-6 inch and 6-12 inch depths contained 3-4 times the degree of infestation of the deeper soil layers. No relation between soil type, climatic environment, or crop history to the vertical distribution of Verticillium in soil was apparently found.

Rudolph (35), summarizing the early results, reported that opinions were sharply divided as to which soil types were most conducive to the development of Verticillium wilt. The disease had been reported to be severe on loams, sandy loams, clays and soils very rich in organic matter. On cotton, the disease generally causes the most striking damage on clay soils. Verticillium wilt was more damaging to cotton on sedimentary and alluvial soils than on sandier types (28). The disease has been reported to be severe in sandy soils on tomato in Florida and was severe on eggplant, tomato, potato and peanuts in sandy and loess soils in Israel, especially when temperatures were high (21, 14). In Oklahoma sandy loams are becoming infested rapidly where cotton and peanuts are in rotation (48).

To develop a rapid inoculation technique that will give reproducible results with critical differentiation of degrees of susceptibility, Bugbee and Presley (8) ran greenhouse and field tests to evaluate resistance of cotton varieties to Verticillium albo-atrum. Inoculation methods of root dipping in conidial suspensions, spraying spores on the root balls and injection of conidial suspensions directly into the vascular system by stem puncture were compared. The stem puncture technique was the most rapid and satisfactory.

Host Range

Since 1879 when Reinke and Berth isolated and identified *Verticillium* as a causal agent of a wilt disease of potatoes, as reported by Isaac (19), the host range for Verticillium wilt has been determined to a large extent. Baker *et al.* (2) isolated *Verticillium albo-atrum* from many vegetable and garden plants in California. Snyder *et al.* (44) extended the list of hosts for Verticillium wilt by isolating *Verticillium* from many plants of cruciferous, chenopodiaceous, solanaceous and malvaceous families.

Some isolates were cross-infective and produced the disease upon inoculation whereas some strains did not have cross-infection characteristics which corroborates the evidence of existence of strains of *Verticillium albo-atrum* differing in pathogenicity.

Schnathorst and Mathre (39) obtained two isolates of *Verticillium albo-atrum* from a diseased cotton plant and evaluated them for virulence at various inoculum levels of either conidial or microsclerotial inoculum on the following hosts: cotton (*Gossypium hirsutum*), (*G. barbadense*), tomato (*Lycopersicon esculentum*), snapdragon (*Antirrhinum majus*), celery (*Apium gravedense*), safflower (*Carthamus tinctorius* L.), cowpeas (*Vigna sinensis*. Torner Savi), peppers (*Capsicum frutenses* L.), watermelon (*Citrullus lapatus* Thumb.) and okra (*Hibiscus esculentus*). With the exception of watermelons and cowpeas, all other hosts were differentially susceptible to each isolate with either inoculum type but there were significant differences in virulence of the isolates. In general most broad leaved plants are susceptible hosts to Verticillium spp. whereas grasses are considered nonhost plants.

Control Methods

Chemical and Cultural Methods

Since the Verticillium wilt pathogen has an extremely wide host range (2, 39, 44), persists for long periods of time in soil (26, 53) and usually occurs irregularly over large areas, control methods such as chemical spot treatment of infested soil or crop rotation would be impractical. Treatment of entire areas would be expensive and uncertain. However, on a small scale in nursery soils, adequate chemical control of Verticillium in infested soil has been reported (40) in a few crops (peppermint, potato) with Methyl-isocynate, chloropicrin, and similar volatile fumigants. Nonvolatile and relatively insoluble fungicides have not proven of much value (40). Another report has been published by Wilhelm and Ferguson (55) on the effectiveness of 21 soil fumigants for the control of Verticillium in soil. At very high dosages only chloropicrin, chlorobromopropene and allyl bromide were effective against Verticillium. The other fumigants were ineffective.

Rotation schedules have some benefit. Purss (32) suggested that severely infested land be cropped to grasses for an extended period because the longevity of the fungus (Verticillium) made the maize-peanut rotation relatively ineffective. Hsi (18) found that peanuts following cotton, okra or peanuts developed severe Verticillium wilt, whereas following grain sorghum or alfalfa, less wilt occurred. From a report by Menzies and Griebel (26) one might infer that clean fallowing with occasional plowing during dry periods would lead to a significant depletion and death of soil-borne inocula. Long term rotations employing clean fallowing and a series of nonhosts might possibly be helpful to control *Verticillium*. Field sanitation such as removing or burning infested plant debris would be beneficial in reducing the inoculum potential of the pathogen in the soil.

Resistant Varieties

Two reports concerning resistance of peanut varieties to *Verticillium* wilt have been published. Smith (42) grew eighteen peanut accessions in soil naturally infested with *Verticillium* under field conditions. Ten promising accessions from the field test were further examined with artificial inoculation procedures under greenhouse conditions. Results from both field and greenhouse studies were conclusive enough to report Valencia and Spanish type peanuts were more susceptible than Virginia type. Based on the rating scale of 0 to 100, the New Mexico Valencia variety was highly susceptible with a disease severity index of 61% and 84% in greenhouse and field tests respectively. The line "Georgia Bunch 182-28" was reported highly resistant with a disease severity index of 24% and 12% for greenhouse and field tests respectively. Although varieties classified as resistant became infected, the vascular discoloration was restricted to roots and crowns.

The second reference was published by Frank and Krikun (15) and also reports resistance in peanuts to Verticillium wilt. They evaluated 28 peanut accessions under naturally infested field conditions. A local selection number 65-121 of the Schwarz-21 cultivar of Israel showed a combination of high degree of escape with low disease severity, resulting in an extremely low disease severity index.

Since resistance to Verticillium wilt in peanuts exists, a knowledge of the inheritance pattern of this resistance is needed to aid in planning its incorporation into adapted peanut cultivars. So far, no data are available about the mode of inheritance of Verticillium wilt resistance in peanuts, however, reports of the breeding behavior of resistance to Verticillium have not been consistent among host species in which it has been studied.

Schaible et al. (36) and Denby and Woolliams (10) concluded that resistance to Verticillium in tomato (Lycopersicon esculentum L.) is controlled by a single dominant gene, "Ve". They were able to transfer resistance by backcrossing. Lockwood and Markarian (22) reported that F_1 crosses between resistant and susceptible egg plants (Solanum melongena L.) were susceptible. They were able to obtain lines uniformly resistant to wilt in the F_3 generation, suggesting that inheritance was a simple recessive. Nelson (29) concluded from his studies that two pairs of complementary dominant genes, functioning additively and quantitatively equal, were responsible for controlling Verticillium resistance in peppermint (Mentha cruspa L.).

In potato (Solanum tuberosum L.) McLean (25) reported 40% of the F_1 progeny from susceptible x susceptible crosses were resistant. The largest proportion of resistant plants was obtained from resistant x susceptible and resistant x resistant crosses, ranging up to 100% in some resistant x resistant crosses. Conversely, Wilhelm (54) reported no resistant plants were obtained in F_1 progeny from susceptible x susceptible crosses in the cultivated strawberry (*Fragaria* spp.) and 53% was the highest proportion of resistant plants obtained from resistant x resistant crosses. The occurrence of susceptible plants among the progenies of resistant parents suggests that inheritance of Verticillium wilt resistance in strawberry is dominant over susceptibility, and the continuous gradation from susceptible to resistant suggests a quantitative type of inheritance.

Several experimental resistant F_1 hybrids of sunflowers, involving resistant or moderately resistant lines and segregating progenies of five crosses, were studied by Putt (33). Lack of dominance, dominance of resistance, dominance of susceptibility and heterosis for resistance to Verticillium wilt were found in single cross, three-way cross and top cross hybrids. In a susceptible x resistant cross, which lacked dominance, the disease distribution of the F_2 population fit a normal curve. In another cross of susceptible x resistant, 9% of the F_3 progeny plants were similar to the susceptible parent and 4% were similar to the resistant parent. The F_2 and F_3 of other crosses showed dominance and monofactorial inheritance of resistance. Thus, he concluded that the Verticillium wilt reaction in some lines was controlled by relatively few genes. In other lines resistance was controlled by a single dominant gene (V_1), whereas, in some lines, dominance of susceptibility was observed.

Inheritance of tolerance to Verticillium wilt in American upland cotton is not well understood, and diversified reports have been published. Cotton (9) and Fisher (12) noted tolerance could be transmitted to progenies only in certain crosses while in others, transgressive segregation occurred. Wilhelm *et al*. (56) reported dominance for

Verticillium wilt resistance among Upland x Sea Island crosses. Nearly true breeding lines were selected from Gossypium barbadense sources, but none were found from upland cottons. The F_1 progenies from Sea Island (resistant) x Upland (susceptible) crosses were mostly resistant, although a few were intermediate in susceptibility. Susceptible x susceptible upland types produced susceptible progenies.

Barrow (4) also noted a dominant type of inheritance for resistance when a mild strain of Verticillium albo-atrum was used to test tolerant x susceptible upland cotton crosses. Results from single crosses indicated some heterozygosity was present within the tolerant parent, since it was possible to group the F_1 progenies into tolerant, susceptible, or segregating (1:1) categories. Barnes and Staten (3) noted average effects among wide and narrow crosses with Acala parents under field conditions. Among 43 F_1 populations, 5 were more tolerant than either parent, 33 were intermediate and 5 were more susceptible to Verticillium wilt than either parent. Mildly tolerant x susceptible crosses generally produced susceptible F_1 plants, and tolerant x susceptible crosses produced F_1 plants with intermediate reactions.

Robert and Staten (34) investigated broad-sense heritability estimates for tolerance to Verticillium wilt and selection efficiencies of (tolerant x susceptible) Gossypium hirsutum L. populations under severe Verticillium wilt field conditions, using F_2 and F_3 generations. Heritability estimates varied from 0 to .833, depending upon exposure level, generation tested, and type of parental tolerance present. Selection efficiencies were 0 for the cross tolerant (8229) x (Lankart 57) susceptible, as none of the F_2 plants produced any tolerant F_3 progencies. Only 8 out of the 72 progenies were rated as intermediate in disease severity. Approximately 91% of the F_3 were susceptible for this cross. All F_2 plants that were completely defoliated were homozygous for susceptibility. Frequency distribution and means of parents and F_2 progenies indicated tolerance to be highly recessive for the reciprocal cross 8861 (T) x Lankart 57 (S). Both F_1 populations equalled the Lankart 57 (susceptible parent) in susceptibility, and the F_2's approached the susceptible parent. Maternal effects for wilt tolerance were not noted. The F_2's of this reciprocal cross of 8861 (T) x Lankart 57 (S) produced about 5% tolerant plants. The possibility then exists for double homozygous recessive genes conditioning Verticillium wilt tolerance under those conditions.

Verhalen et al. (47) reported from a quantitative genetic study of Verticillium wilt resistance among selected lines of upland cotton that Verticillium wilt resistance displayed partial dominance except in one environment where overdominance was found to occur. Direction of dominance was toward greater susceptibility. The frequency of negative versus positive alleles in the parents was likely unequal and biased towards greater susceptibility. Narrow-sense heritability estimates suggested that rapid genetic advances through selection could be made in most environments.

Environmental factors, particularly temperature, have marked effects on the expression of levels of resistance to Verticillium wilt. Bell and Presley (6) reported that both resistant and susceptible strains of cotton were susceptible at a greenhouse mean air temperature of 22°C and were resistant at 32°C. At 25-29°C susceptible varieties reacted as susceptible and resistant varieties as resistant; whereas, tolerant varieties were susceptible at 25°C, tolerant at 27°C and resistant at 29°C. Brinkerhoff (7) reported, from his studies using cotton seedlings grown in nutrient solutions, that the expression of resistance and tolerance was more pronounced when inoculated plants were first incubated under a high-day, low-night temperature regime, and then held at lower temperatures, than when plants were incubated at a continuous temperature of 27°C. The initial high-day, low-night temperature regime did not favor symptom expression, but apparently accentuates the expression of genetic resistance which in turn restricts the pathogen and disease when the plants are returned to favorable temperatures.

Talboys (45) showed that Verticillium wilt of strawberries incited by the microsclerotial form (MS) of Verticillium was more severe during a warm summer that produced water stress, and that, under these conditions, differences in virulence of isolates of the pathogen could be shown in a tolerant or resistant cultivar. During a cool moist season when symptoms were less pronounced, differences in virulence of the pathogen were obscured. Presumably the disease was less damaging in the cool season.

On the other hand, Garber and Presley (16) reported that two cotton strains grown in two seasons had different levels of field tolerance to Verticillium wilt. Both the susceptible as well as the tolerant strains were seriously affected by the disease in a warm summer. In a cool summer, the relative disease tolerances of the strains were readily separated. They concluded that high temperatures can mask or obscure the influence of the pathogen population and virulence in determining the disease severity.

Abdul-Raheem and Bird (1) studied the interrelationship of resistance and susceptibility of cotton to Verticillium albo-atrum (microsclerotial form) and Fusarium oxysporum as influenced by soil temperature. Incidence and severity were reduced for Verticillium wilt and increased for Fusarium wilt as soil temperatures increased from 20 to 32°C. The favorable temperature range for Verticillium wilt was 20-28°C and for Fusarium wilt 28-32°C. The incubation period was shorter for Verticillium than for Fusarium wilt. The cotton strains were separated into three distinct disease response levels at 24 and 28°C with Verticillium and at 28°C and 32°C with Fusarium wilt. The relationship of response of the three cotton strains to both wilts was highest at a soil temperature of 28°C where development and symptom expression were similar.

CHAPTER III

MATERIALS AND METHODS

Germ Plasm Evaluation

Field Studies

The peanut germ plasm used for the initial screening under field conditions included 152 accessions (Table I). Among these were 8 commercial varieties and 144 plant introductions and selections of Spanish, Virginia Bunch and Runner types. All were of the same species (Arachis hypogaea L.).

The peanuts in the field studies during 1971 and 1972 were grown in the same Verticillium infested area on a farm near the Caddo Peanut Research Station, Ft. Cobb, Oklahoma. In 1971 and 1972, respectively, 89 and 90 accessions were grown (Table II). In 1972, 21 of the more promising entries from 1971 plus 6 commercial Spanish varieties were planted along with 63 new entries. The soil type in the test area was sandy loam. The field was cultivated and sprinkler irrigated as needed.

Randomized block designs were used. In respect to peanut introductions and selections, four replications for the 1971 test and six replications in the 1972 test were used. The commercial varieties were replicated 2 times each year. Plantings were made on June 14, 1971 and on June 1 in 1972, at the rate of about 5 seeds per foot of row and approximately one and one-half to two inches deep. The rows were

LEGEND FOR TABLE I

Seed Catalog, Southern Regional Plant
Introduction Station, Georgia,
Regional Project S-9, 1964-65

Botanical Group:

S = Spanish

V = Virginia

VL = Valencia

R = Runner

IS = Improved Spanish

Growth Habit:

S = Spanish

B = Virginia Bunch

R = Virginia Runner

TABLE I

152 PEANUT ACCESSIONS SCREENED FOR RESISTANCE TO VERTICILLIUM WILT

Oklahoma P-No[1]	Seed Catalog 1964-65 Plant Introduction No. or Other Identity[2]	Botanical Group	Origin	Growth Habit
0002	Argentine	S	Oklahoma	S
0006	Starr	S	Texas	S
0014	162524	S	Argentina	S
0047	237509	S		S
0112	Spanhoma	S	Oklahoma	S
0118	121070-3	S	Paraguay	S
0144	234417	S	China	S
0151	Krinkleleaf	S	Texas	S
0161	Tenn. Red	VL		VL
0190	Valencia	VL		VL
0214	242100	S	China	S
0215	Early Runner	R		R
0289	VA 61	V	Virginia	R
0291	P-151-1	S	Oklahoma	S
0315	259772	S	Nyasaland	
0316	259650	S	Cuba	S
0317	259660	S	Cuba	S
0322	259805	S	Nyasaland	S
0330	152125	S	Brazil	S
0332	259800	S	Nyasaland	S
0333	264159	S	Australia	S
0335	268768	S	N. Rhodesia	S
0337	259637	R	Cuba	R
0338	259671	V	Cuba	V
0339	259678	V	Cuba	V
0351	268599	S	N. Rhodesia	S
0352	268601	S	N. Rhodesia	S
0360	268616	S	N. Rhodesia	S

TABLE I (Continued)

Oklahoma P-No	Seed Catalog 1964-65 Plant Introduction No. or Other Identity	Botanical Group	Origin	Growth Habit
0361	268616	S	N. Rhodesia	S
0362	268626	S	N. Rhodesia	S
0364	268633	S	N. Rhodesia	S
0365	268635	S	N. Rhodesia	S
0369	268637	S	N. Rhodesia	S
0373	268647	S	N. Rhodesia	S
0384	268680	S	N. Rhodesia	S
0386	268686	S	N. Rhodesia	S
0393	268692	S	N. Rhodesia	S
0400	268706	S	N. Rhodesia	S
0408	268711	S	N. Rhodesia	S
0410	268716	S	N. Rhodesia	S
0415	268737	S	N. Rhodesia	S
0416	268739	S	N. Rhodesia	S
0419	268740	S	N. Rhodesia	S
0420	268742	S	N. Rhodesia	S
0421	268748	S	N. Rhodesia	S
0422	268749	S	N. Rhodesia	S
0423	268752	S	N. Rhodesia	S
0424	268758	S	N. Rhodesia	S
0425	268759	S	N. Rhodesia	S
0426	268760	S	N. Rhodesia	S
0427	268767	S	N. Rhodesia	S
0431	268778	S	N. Rhodesia	S
0433	268789	S	N. Rhodesia	S
0436	268795	S	N. Rhodesia	S
0439	268808	S	N. Rhodesia	S
0440	268811	S	N. Rhodesia	S
0441	268812	S	N. Rhodesia	S
0442	268818	S	N. Rhodesia	S

TABLE I (Continued)

Oklahoma P-No	Seed Catalog 1964-65 Plant Introduction No. or Other Identity	Botanical Group	Origin	Growth Habit
0446	268825	S	N. Rhodesia	S
0450	268828	S	N. Rhodesia	S
0454	268830	S	N. Rhodesia	S
0455	268832	S	N. Rhodesia	S
0458	270784	S	N. Rhodesia	S
0460	270789	S	N. Rhodesia	S
0461	270804	S	N. Rhodesia	S
0466	271021	S	N. Rhodesia	S
0467	271022	S	N. Rhodesia	S
0468	274267	S	N. Rhodesia	S
0470	261989	S	Paraguay	S
0474	261997	S	Paraguay	S
0484	262022	S	Paraguay	S
0495	262046	S	Brazil	S
0511	261933	S	Paraguay	S
0528	261985	S	Paraguay	S
0552	248763	S	India	S
0553	248766	S	India	S
0554	248767	S	India	S
0555	248768	S	India	S
0557	247378	S	S. Africa	S
0559	240555	S		S
0562	240578	S		S
0570	268614	S	N. Rhodesia	S
0587	268737	S	N. Rhodesia	S
0594	268654	S	N. Rhodesia	S
0614	268686	S	N. Rhodesia	S
0621	268700	S	N. Rhodesia	S
0624	268703	S	N. Rhodesia	S
0626	268704	S	N. Rhodesia	S
0628	268707	S	N. Rhodesia	S

TABLE I (Continued)

Oklahoma P-No	Seed Catalog 1964-65 Plant Introduction No. or Other Identity	Botanical Group	Origin	Growth Habit
0632	268711	S	N. Rhodesia	S
0633	268712	S	N. Rhodesia	S
0635	268714	S	N. Rhodesia	S
0638	268717	S	N. Rhodesia	S
0639	268718	S	N. Rhodesia	S
0640	268719	S	N. Rhodesia	S
0653	268730	S	N. Rhodesia	S
0658	268736	S	N. Rhodesia	S
0659	268737	S	N. Rhodesia	S
0660	268738	S	N. Rhodesia	S
0661	268739	S	N. Rhodesia	S
0662	268739	S	N. Rhodesia	S
0664	268742	S	N. Rhodesia	S
0665	268743	S	N. Rhodesia	S
0666	268743	S	N. Rhodesia	S
0668	268745	S	N. Rhodesia	S
0670	268747	S	N. Rhodesia	S
0671	268747	S	N. Rhodesia	S
0675	268753	S	N. Rhodesia	S
0676	268754	S	N. Rhodesia	S
0678	268761	S	N. Rhodesia	S
0690	268773	S	N. Rhodesia	S
0693	268774	S	N. Rhodesia	S
0698	268782	S	N. Rhodesia	S
0700	268784	S	N. Rhodesia	S
0701	268785	S	N. Rhodesia	S
0705	268790	S	N. Rhodesia	S
0713	268796	S	N. Rhodesia	S
0714	268796	S	N. Rhodesia	S
0719	268801	S	N. Rhodesia	S

TABLE I (Continued)

Oklahoma P-No	Seed Catalog 1964-65 Plant Introduction No. or Other Identity	Botanical Group	Origin	Growth Habit
0725	268806	S	N. Rhodesia	S
0726	268807	S	N. Rhodesia	S
0730	268811	S	N. Rhodesia	S
0741	268823	S	N. Rhodesia	S
0743	268825	S	N. Rhodesia	S
0745	268827	S	N. Rhodesia	S
0750	268833	S	N. Rhodesia	S
0752	268835	S	N. Rhodesia	S
0754	270776	S	N. Rhodesia	S
0770	270846	S	N. Rhodesia	S
0773	268789	S	N. Rhodesia	S
0777	259701	S	Argentina	S
0782	259765	S	Nyasaland	S
0788	259821	S	Nyasaland	S
0790	259827	S	Nyasaland	S
0796	262055	S	Brazil	S
0797	262065	S	Brazil	S
0800	261921	S	Argentina	S
0860	268680	S	N. Rhodesia	S
0870	268706	S	N. Rhodesia	S
0871	268752	S	N. Rhodesia	S
0873	268756	S	N. Rhodesia	S
0874	268759	S	N. Rhodesia	S
0876	268780	S	N. Rhodesia	S
0891	259718	S	Peru	S
0900	259603	S	Australia	S
1258	Tifspan	S	Georgia	S
1259	Spancross	S	Georgia	S
1436	Dixie-Spanish	S	Georgia	S

TABLE I (Continued)

Oklahoma P-No	Seed Catalog 1964-65 Plant Introduction No. or Other Identity	Botanical Group	Origin	Growth Habit
1439	Spantex	S	Texas	S
1443	Comet	S	Oklahoma	S
2339	Florunner	V	Florida	R
2399	Georgia Bunch 182-28	V	Georgia	B

[1]Oklahoma Inventory Number of Peanut Accessions

[2]USDA Plant Introduction Inventory Number of Peanuts or Other Identity

TABLE II

PEANUT REACTION TO VERTICILLIUM WILT: PREVALENCE OF WILT INFECTION FOR ACCESSIONS IN FIELD EXPERIMENTS OF 1971 AND 1972

1971		1972	
Oklahoma P-No	Percent Wilt	Oklahoma P-No	Percent Wilt
0002	47.9	0002	40.0
0006	48.6	0006	42.8
0112	46.2	0014	38.1
0118	44.1	0047	30.3
0315	49.3	0112	58.6
0316	60.6	0144	48.5
0317	57.3	0151	36.6
0333	49.2	0161	51.0
0335	54.2	0190	63.2
0338	25.7	0214	34.8
0361	39.7	0215	46.0
0362	37.5	0289	37.5
0364	43.1	0291	40.8
0369	54.9	0322	35.4
0408	68.6	0330	33.3
0415	53.1	0332	42.8
0419	51.4	0337	58.5
0421	40.3	0338	50.7
0423	55.8	0339	53.9
0424	41.1	0351	36.9
0425	36.4	0352	48.7

TABLE II (Continued)

1971		1972	
Oklahoma P-No	Percent Wilt	Oklahoma P-No	Percent Wilt
0426	46.9	0360	30.7
0427	57.2	0361	35.7
0431	31.0	0362	45.3
0440	45.6	0365	34.4
0441	64.9	0373	33.6
0442	12.3	0384	47.5
0446	39.5	0386	60.1
0450	42.8	0393	47.8
0454	62.1	0400	42.1
0455	49.0	0410	44.9
0466	54.7	0416	40.2
0552	33.6	0420	33.3
0553	42.1	0422	45.4
0554	51.8	0425	42.5
0555	40.0	0431	32.6
0559	39.3	0433	42.3
0562	50.6	0436	53.5
0570	42.6	0439	41.3
0587	61.2	0442	45.7
0614	40.1	0446	40.8
0621	46.2	0458	46.0
0624	32.5	0460	53.9
0628	38.1	0461	33.5

TABLE II (Continued)

1971		1972	
Oklahoma P-No	Percent Wilt	Oklahoma P-No	Percent Wilt
0633	41.9	0467	49.0
0635	53.7	0468	44.2
0638	47.4	0470	63.1
0639	44.5	0474	43.0
0640	52.5	0484	89.3
0658	47.2	0495	65.8
0661	60.5	0511	63.7
0662	42.7	0528	91.9
0664	31.7	0552	41.9
0665	69.6	0555	53.7
0668	55.8	0557	26.3
0670	44.2	0559	44.4
0675	63.4	0594	47.8
0678	45.5	0614	38.6
0693	43.9	0624	36.5
0698	61.3	0626	42.1
0700	67.5	0628	27.0
0701	38.8	0632	35.5
0705	44.3	0653	53.9
0713	54.4	0659	51.0
0719	30.5	0660	41.3
0725	51.1	0664	33.9
0726	49.1	0666	40.0

TABLE II (Continued)

1971		1972	
Oklahoma P-No	Percent Wilt	Oklahoma P-No	Percent Wilt
0730	37.4	0671	49.6
0741	43.3	0676	34.4
0743	45.1	0690	48.7
0745	70.1	0701	49.8
0750	54.3	0714	39.8
0752	46.2	0719	64.6
0754	57.9	0730	47.3
0770	54.5	0777	51.3
0773	47.4	0788	40.1
0782	61.6	0800	43.8
0790	44.3	0860	32.0
0796	55.0	0870	60.9
0797	52.6	0871	30.9
0860	37.9	0874	36.9
0870	40.0	0876	48.4
0873	66.0	0900	35.4
0891	42.6	1258	58.9
1258	48.9	1259	41.3
1259	63.4	1436	52.2
1436	64.0	1439	51.1
1439	62.5	1443	50.3
1443	53.2	2339	47.3
		2399	37.3

spaced 36 inches apart. Each introduction and selection was planted in ranges in which single rows 24 feet long in 1971 and 16 feet long in 1972 were used. A narrow alley separated the ranges. However, the commercial varieties were planted in double rows with the same lengths as previously mentioned with two replicates. Each replicate consisted of 8 and 12 ranges in tests of 1971 and 1972 respectively. Verticillium wilt symptoms were observed about two months after planting, near the middle of August and maturity time. Entries were rated for disease prevalence before harvest time. Data were obtained on the percentage of diseased plants from 8 feet of row in 1971 by counting the diseased and healthy plants. The *Verticillium* infected plants were diagnosed by vascular discoloration of roots, crown and stem. In 1972, entries were scored for disease development by measuring the length of the Verticillium wilt infected portion of the row and converting to a percentage of the total row. The data were analyzed statistically. Isolation of the *Verticillium* organism was made from diseased plants each year at the end of the test.

Greenhouse Studies

Greenhouse Test 1. Before screening the germ plasm by artificial inoculation, it was important to determine which isolates of *Verticillium* were pathogenic.

Seven old and 3 new isolates of *Verticillium* obtained from peanut debris were tested for pathogenicity and compared with new isolates from cotton plants (Table III). The inocula were increased in a potato-dextrose-broth shake culture for 6 days. The cultures were filtered through cheese cloth to remove mycelial fragments.

TABLE III

PATHOGENICITY OF *VERTICILLIUM* ISOLATES OBTAINED FROM COTTON AND PEANUTS ON PEANUT SEEDLINGS[1]

Oklahoma P-No	Peanut Isolates: Old Isolates P_1	P_2	P_3	P_4	P_5	P_6	P_7	Peanut Isolates: New Isolates P_8	P_9	P_{10}	Cotton Isolates: New Isolates C_1	C_2
P-0006	1	1	2	1	1	1	1	4	4	4	4	4
P-0112	4	1	4	2	1	1	4	4	4	4	4	4
P-1258	1	4	2	2	2	2	3	4	4	4	4	4
P-1259	1	1	1	2	1	1	1	4	4	3	4	4
P-1436	1	1	2	2	1	1	2	4	4	4	4	4
P-1439	2	1	1	2	1	1	2	4	4	4	4	4
P-1443	1	2	2	1	1	1	1	4	4	4	4	4

[1]Key for rating wilt severity:

1 = No disease

2 = Vascular discoloration of root and crown

3 = Vascular discoloration up 3/4 of stem

4 = Severe leaf chlorosis with brown vascular discoloration of stem up to top leaves

Concentrations of inocula were not determined but the stock suspensions were diluted 1 to 10 and were immediately used for inoculating 10-day old peanut seedlings. Seedlings were inoculated by a root-dip method in which seedlings were uprooted, roots were washed with tap water, then dipped in inoculum for 1 minute, and transplanted into 4 inch pots filled with vermiculite.

The plants were incubated in a greenhouse at a temperature regime of $85\pm2^{\circ}F$ day and $70\pm2^{\circ}F$ night temperatures. Plants were rated for disease severity 21 days after inoculation by means of the following rating scale:

1 = no disease

2 = vascular discoloration of root and crown

3 = vascular discoloration up 3/4 of stem

4 = severe leaf chlorosis with brown vascular discoloration of stem up to top leaves

Greenhouse Test 2. A test was designed to determine satisfactory inoculum levels for the most virulent peanut and cotton isolates of *Verticillium* (Table IV).

This test was conducted on the Spanhoma variety in the greenhouse. All the procedures and methods of inoculation and rating for disease severity were the same used for greenhouse test 1, with the exception that concentrations of conidial suspensions were determined with a hemocytometer and adjusted to $3x10^6$, $4.5x10^6$ and $6x10^6$ conidia/ml.

Greenhouse Test 3. Since the results of field studies in 1971, shown in Table II, indicated that there were large differences in levels of resistance or susceptibility to Verticillium wilt among the 89 peanut accessions tested, it was desirable to further examine

TABLE IV

COMPARATIVE PATHOGENICITY OF VERTICILLIUM ISOLATES AT VARIOUS INOCULUM LEVELS ON PEANUT SEEDLINGS

Inoculum Concentration	Disease Severity Ratings[1]	
	Peanut Isolate	Cotton Isolate
3×10^6 spores/ml	3.08	2.75
4.5×10^6 spores/ml	3.25	2.93
6×10^6 spores/ml	3.63	2.95

[1]Rating scale for wilt severity:

1 = No disease

2 = Vascular discoloration of root and crown

3 = Vascular discoloration up 3/4 of stem

4 = Severe leaf chlorosis with brown vascular discoloration of stem up to top leaves

promising entries under controlled environments of a greenhouse and a growth chamber.

Entries (Table V) showing less than 40% disease prevalence in the field study in 1971, two adapted commercial varieties (Argentine and Dixie Spanish) and a line "Georgia Bunch 182-28", previously reported to be highly resistant to Verticillium wilt (42), were studied under greenhouse conditions.

Sixteen seeds of each entry were Arasan treated and planted on Nov. 10, 1971 in a flat filled with vermiculite. Four flats (replicates) were planted for each entry, and arranged in a completely randomized design. The temperature regime of $85 \pm 2^{\circ}F$ day and $70 \pm 2^{\circ}F$ night was maintained with relative humidity at 80%.

Inoculation Procedures. The most pathogenic isolate of *Verticillium* obtained from peanuts in greenhouse test 1 of this study (Table III) was increased on potato-dextrose-broth for 6 days at room temperature. An inoculum level of 4.5×10^{6} conidia/ml was found satisfactory for pathogenicity in the test comparing inoculum levels (Table IV). Therefore, this dosage was used for all subsequent artificial inoculation tests in this study. The inoculation techniques and procedures were similar to those employed for greenhouse tests 1 and 2 of this study.

The Disease Rating Scale and Procedures. Verticillium wilt severity of individual plants was evaluated by rating them 3 times after inoculation: 1st rating after 20 days, 2nd rating after 40 days and final rating 60 days after inoculation. A thirteen-point rating scale (Figure 5-a,b,c) was used, where "1" was no disease and "13" indicated dead plants. Figures 5-a through 5-c show peanut seedlings which

TABLE V

PEANUT ACCESSIONS SCREENED FOR RESISTANCE TO VERTICILLIUM WILT IN GREENHOUSE AND GROWTH CHAMBER ENVIRONMENTS

Oklahoma P-No	P.I. Number or Other Identity
0002	Argentine
0338	259671
0361	268616
0362	268626
0422	268749
0425	268759
0431	268778
0436	268795
0442	268818
0446	268825
0552	248763
0555	248768
0559	240555
0614	268686
0624	268703
0628	268707
0664	268742
0701	268785
0719	268801
0730	268811
0860	268680
0870	268706
1436	Dixie Spanish
2399	Georgia Bunch 182-28

Figure 5. Verticillium Wilt Severity Rating Scale
(a) Rating Categories 1 Through 4
* (Rating Scale, p. 90)

(b) Rating Categories 5 Through 7

Figure 5 (Continued)

(c) Rating Categories 8 Through 13

illustrate each category of the rating scale.

Reisolations of the Verticillium fungus from diseased plants were made before termination of the test.

The data for disease severity were analyzed for F and t values as presented by Snedecor (43). The disease rating means for the significant treatments (P-nos) were analyzed according to Duncan's New Multiple Range test (43).

Growth Chamber Studies

The same 24 accessions which were used in greenhouse test 3 (Table V) were further examined in growth chamber environments in two tests which were run at different times. In both tests 10 seedlings of each entry were grown in flats of vermiculite. Each entry was repeated twice and arranged in a completely randomized design. A temperature regime of $80\pm2^{o}F$ day temperature and $66\pm2^{o}F$ night temperature was maintained. Day-light fluorescent bulbs provided about 4000 foot candles of light in a 12-hour photoperiod.

Inoculation and Disease Rating Procedures. Ten-day old seedlings were inoculated and rated for disease reaction by methods previously described. Comparative disease severity reactions of certain accessions are shown in Figures 6 through 9. Reisolations of the Verticillium fungus were made from diseased plants at the end of the tests.

The data were analyzed and means for disease severity were grouped according to Duncan's New Multiple Range Test.

Figure 6. Verticillium Wilt Reaction From a Growth Chamber Test
A = P-0860 (Susceptible)
B = Argentine (Tolerant)

Figure 7. Verticillium Wilt Reaction From a Growth Chamber Test
A = Georgia Bunch 182-28 (Intermediate)
B = P-0614 (Intermediate)

Figure 8. Verticillium Wilt Reaction From a Growth Chamber Test
A = P-0425 (Tolerant)
B = P-0362 (Susceptible)

Figure 9. Verticillium Wilt Reaction From a Growth Chamber Test
A = P-0559 (Tolerant)
B = P-0446 (Tolerant

Mode of Inheritance Studies

Diallel hand crosses were made by conventional methods among four selected parents (P-446, P-431, P-362 and P-870) grown in a plant growth chamber. The growth chamber was illuminated with fluorescent light bulbs providing 4000 foot candles of light during a 12-hour photoperiod from 7 p.m. to 7 a.m. The temperature regime was $85\pm2^{\circ}$F day temperature (while the lights were on) and $70\pm2^{\circ}$F at night.

Plants were emasculated and pollinated during the hours of 9:00 to 10:00 a.m. using pollen from plants grown in a greenhouse under a normal day-night regime.

Two accessions tolerant to Verticillium wilt, P-431 and P-446, and two susceptible accessions, P-362 and P-870, were used as parents in the crosses (Table IX). The diallel cross families were designed as shown in Figure 10.

Part of the F_1 seeds from successful diallel cross families were planted in pots of sterilized soil and grown under greenhouse conditions. F_2 seeds were obtained from these F_1 plants.

The F_1 seeds from certain crosses were insufficient to run complete diallel cross F_1 families. Therefore attempts were made to establish vegetative cuttings from F_1 plants to obtain more adequate populations for each F_1 progeny. Unfortunately, the cuttings were not successful and thus the diallel studies were discontinued. However, remnant seed of the following populations of F_1 and F_2 progenies and their reciprocals were used to evaluate resistance to Verticillium wilt:

1) P-431 Verticillium wilt - tolerant parent (T)
2) P-446 Verticillium wilt - tolerant parent (T)

PARENTS ♀ ⟷ ♂	P-no. 362	P-no. 431	P-no. 446	P-no. 870
P-no. 362 s	⊗	×	×	×
P-no. 431 t	×	⊗	×	×
P-no. 446 t	×	×	⊗	×
P-no. 870 s	×	×	×	⊗

⊗: *selfed* t: *Tolerant*

×: *crossed* s: *Susceptible*

Figure 10. 4-Parent Diallel Cross Families of Peanut Accessions

3) P-362 Verticillium wilt - susceptible parent (S)

4) P-870 Verticillium wilt - susceptible parent (S)

5) 431x362 P-362 as male parent (F_1,F_2)

6) 362x431 P-431 as male parent (F_1,F_2)

7) 446x362 P-362 as male parent (F_1,F_2)

8) 431x870 P-870 as male parent (F_1,F_2)

9) 870x431 P-431 as male parent (F_1,F_2)

10) 446x870 P-870 as male parent (F_1,F_2)

11) 870x446 P-446 as male parent (F_1,F_2)

12) 446x431 P-431 as male parent (F_1,F_2)

13) 431x446 P-446 as male parent (F_2)

14) 870x362 P-362 as male parent (F_2)

Because several F_1 plants died in the growth chamber while establishing the vegetative cuttings, it was necessary to plant all available seeds from every population listed above. Seeds and seedlings were handled as previously described. The test was run in a completely randomized design with unequal number of plants per various populations.

The 12-day old seedlings were inoculated by the root-dip method at an inoculum level of $4.5x10^6$ conidia/ml. Check plants were uprooted and replanted in the same pot but were not inoculated. The temperature for the first 20 days after inoculation in the growth chamber was maintained at 80 ± 2^oF in the day time and 66 ± 2^oF at night. Illumination was as previously described. After 20 days, the temperature was lowered to 75 ± 2^oF in the day time and 65 ± 2^oF at night.

Based on the rating scale shown in (Fig. 5-a,b,c), all plants were rated for disease severity 19 days after inoculation and rated a second time 15 days later.

CHAPTER IV

RESULTS AND DISCUSSION

Germ Plasm Evaluation

Field Studies

Field Test 1. Spanhoma and Argentine had the least mean wilt prevalence among the varieties of 46.2 and 47.9 percent, respectively, whereas the Dixie Spanish variety had the highest mean wilt prevalence of 64.0 percent (Table II). Mean squares for percent wilt reaction for the commercial varieties are given in Table XIV.

The F ratio indicated no significant differences among the commercial varieties for Verticillium wilt reaction and the wilt prevalence percentage range was also narrow. Therefore these varieties could not be specifically categorized for Verticillium wilt tolerance or resistance from the field test data of 1971.

The mean squares for percent Verticillium wilt prevalence among the accessions (introductions and selection material) and between replicates are presented in Table XV. Although the F ratio does not suggest significant differences among the accessions, there are actually large differences among mean wilt prevalence percentages, ranging from 12.3 (P-442) to 70.1 (P-745) as shown in Table II. However, the F ratio for replicates was higher than expected, thus indicating highly significant differences for disease prevalence in the various replicates.

This can be attributed to soil heterogeneity for inoculum potential of the fungus and other environmental factors.

The highly significant differences among replicates most probably had major influence on the averages of wilt prevalence percentages of the accessions from all the replicates, indicating them (accessions) non-significantly different statistically. Even then some of the accessions had very low average wilt prevalence percentage (Table II) of 12.3 (P-442), which suggests that there are some potentialities among certain accessions for different levels of resistance or tolerance to Verticillium wilt.

It was thus desirable to examine such accessions of low disease prevalence percentages further under the controlled environments of a greenhouse and a growth chamber. Entries showing less than 40% prevalence of Verticillium wilt were selected from the introductions and selection material while among the commercial varieties Argentine and Dixie Spanish were used for further critical studies in the greenhouse and growth chamber.

<u>Field Test 2</u>. Mean squares for percent wilt prevalence for the commercial varieties are given in Table XVI. Again, as in the 1971 test, Spanhoma and Argentine had the lowest mean wilt prevalence percentages of 38.1 and 40.0, respectively, whereas Dixie Spanish had the highest mean wilt prevalence of 52.2% (Table II). This reaction trend among varieties is consistent with that noticed in the 1971 test. In general the disease reaction or mean percent wilt prevalence of varieties exhibited the same range in both years. The 1972 means ranged from 38.1 (Spanhoma) to 52.2 (Dixie Spanish) while the 1971 means ranged from 46.2 (Spanhoma) to 64.0 (Dixie Spanish).

For the plant introductions and selections, the mean wilt prevalence percentages are presented in Table II and the mean squares for them are given in Table XVII. The F ratio for accessions was significant at the .01 level of probability, suggesting the presence of highly significant differences among the accessions. The means for percent wilt prevalence ranged from 26.3 (P-557) to 91.9 (P-528). Besides the significant F ratio for accessions, the F ratio for replicates was also significant at the .01 level of probability, showing differential disease prevalence in the various replicates.

Seven of the 23 selected accessions which were repeated from the 1971 test showed consistency in their performance and had the lowest percent disease prevalence values. These accessions were: P-361, P-431, P-446, P-624, P-628, P-664, and P-860. However, among the accessions tested for the first time in 1972, twenty-one had mean disease prevalence percentages of 40 or less (Table II). These included the Georgia Bunch 182-28 line which had been previously reported to have resistance to Verticillium wilt.

Greenhouse Studies

Greenhouse Test 1. A pathogenicity test was run for old as well as new _Verticillium_ isolates on peanuts. These isolates were obtained from diseased cotton and peanut plants. The disease reaction, expressed as wilt severity, is presented in Table III. Every new isolate, either from cotton or peanut, was more pathogenic on peanuts than the old isolates which had apparently lost their virulence to a great extent through long culturing on artificial medium.

Since the new isolates showed higher pathogenicity, they were used in subsequent inoculation tests for screening the germ plasm.

Greenhouse Test 2. After confirming the virulence of the isolates of *Verticillium*, a satisfactory inoculum potential and an adequate inoculum technique were determined. Three general inoculation methods have been reported (8). These are root-dips in conidial suspensions, spraying spores on the root balls and injection of conidial suspensions directly into the vascular system by stem puncture. The stem puncture technique was the most rapid and satisfactory (8). Simply for convenience the root-dip method was used during this study.

Inoculum potential has a great bearing on Verticillium wilt development, severity of symptoms and time of plant death (46). Therefore a test was designed to determine an adequate inoculum level with the most pathogenic isolate of *Verticillium*. The disease severity ratings for different inoculum levels are presented in Table IV.

Increased inoculum dosages generally resulted in higher ratings of disease severity with isolates from either cotton or peanuts. Isolates from peanuts showed more pathogenicity on peanuts at corresponding doses than did the cotton isolates. The inoculum level of 4.5×10^6 conidia/ml showed intermediate severity ratings and thus was considered adequately effective for causing the disease. The inoculum potential of 4.5×10^6 spores/ml was used as a standard in all subsequent inoculation tests of this study.

Greenhouse Test 3. Disease reactions of the 24 selected peanut accessions screened in the greenhouse are presented in Table VI. Since the plants were rated three times for disease severity the mean squares were computed (Table XVIII) both for variable ratings (Dates) and P-No (Accessions).

TABLE VI

MEAN VERTICILLIUM WILT SEVERITY RATINGS OF 24 PEANUT ACCESSIONS IN GREENHOUSE TEST 3[1]

Oklahoma P-No	1st Rating[2]	2nd Rating[2]	3rd Rating[2]	Overall Mean
0002	5.8	8.5	10.8	8.4
0338	3.8	6.9	10.6	7.1
0361	7.1	10.8	13.0	10.3
0362	6.7	11.3	13.0	10.3
0422	5.7	8.8	12.5	8.9
0425	3.7	7.6	10.9	7.4
0431	3.3	7.7	9.8	6.9
0436	4.3	9.2	11.7	8.4
0442	5.8	8.5	10.8	8.3
0446	3.7	7.1	9.7	6.8
0552	5.3	8.2	11.6	8.4
0555	5.5	8.0	10.1	7.9
0559	4.7	7.9	9.7	7.4
0614	6.9	8.4	11.2	8.9
0624	5.4	8.5	11.3	8.4
0628	5.7	8.1	9.9	7.9
0664	5.9	8.5	11.4	8.6
0701	6.1	8.5	9.5	8.1
0719	6.1	8.4	9.8	8.1
0730	6.1	8.5	11.4	8.8
0860	6.5	9.7	12.1	9.5
0870	6.3	9.7	13.0	9.7
1436	6.1	8.9	11.9	8.9
2399	3.9	10.6	12.2	8.9
Mean	5.4	8.7	11.2	8.4

[1]Rating scale of 1 to 13; "1" indicating no disease, "13" indicating completely defoliated, dead plants

[2]Rating intervals of 20 days starting 20 days after inoculation

Highly significant differences were present among P-Nos in individual ratings as well as on the basis of overall averages of the three ratings. The mean disease severity ratings are given in Table VI. In the first rating, there were small differences among P-Nos as compared to later rating dates. At the second rating date there were enhanced disease reactions of P-Nos and the differences among P-Nos were highly significant. Although the disease severity ratings were higher in the third rating than those of earlier ratings, the differences among P-Nos were small. In fact, the susceptible accessions had collapsed completely by the third rating (60 days after inoculation). For visual comparison, Figures 6 through 9 give adequate evidence for the reactions of certain accessions.

Averaging the rating values for disease severity of the three rating dates, mean wilt severity ratings were obtained (Table VI) which ranged from 6.8 (P-446) to 10.3 (P-362). The corresponding rating values for these P-Nos at the first rating were 3.7 (P-446) and 6.7 (P-362), and at the second rating 7.1 (P-446) and 11.3 (P-362). In general there were consistent increases in severity of disease from the first rating to subsequent ratings for every accession, but plants of susceptible accessions (P-Nos. 361, 362, 860 and 870) were almost completely defoliated and dead by the third rating date. Figure 11 reveals the mean reactions of the 24 peanut accessions under greenhouse conditions.

Since the Georgia Bunch 182-28 line (P-2399) had been reported (42) highly resistant to Verticillium wilt, it was considered as a standard entry in these studies. The P-Nos. 338, 425, 431, 446, and 559 had significantly lower disease severity ratings than Georgia

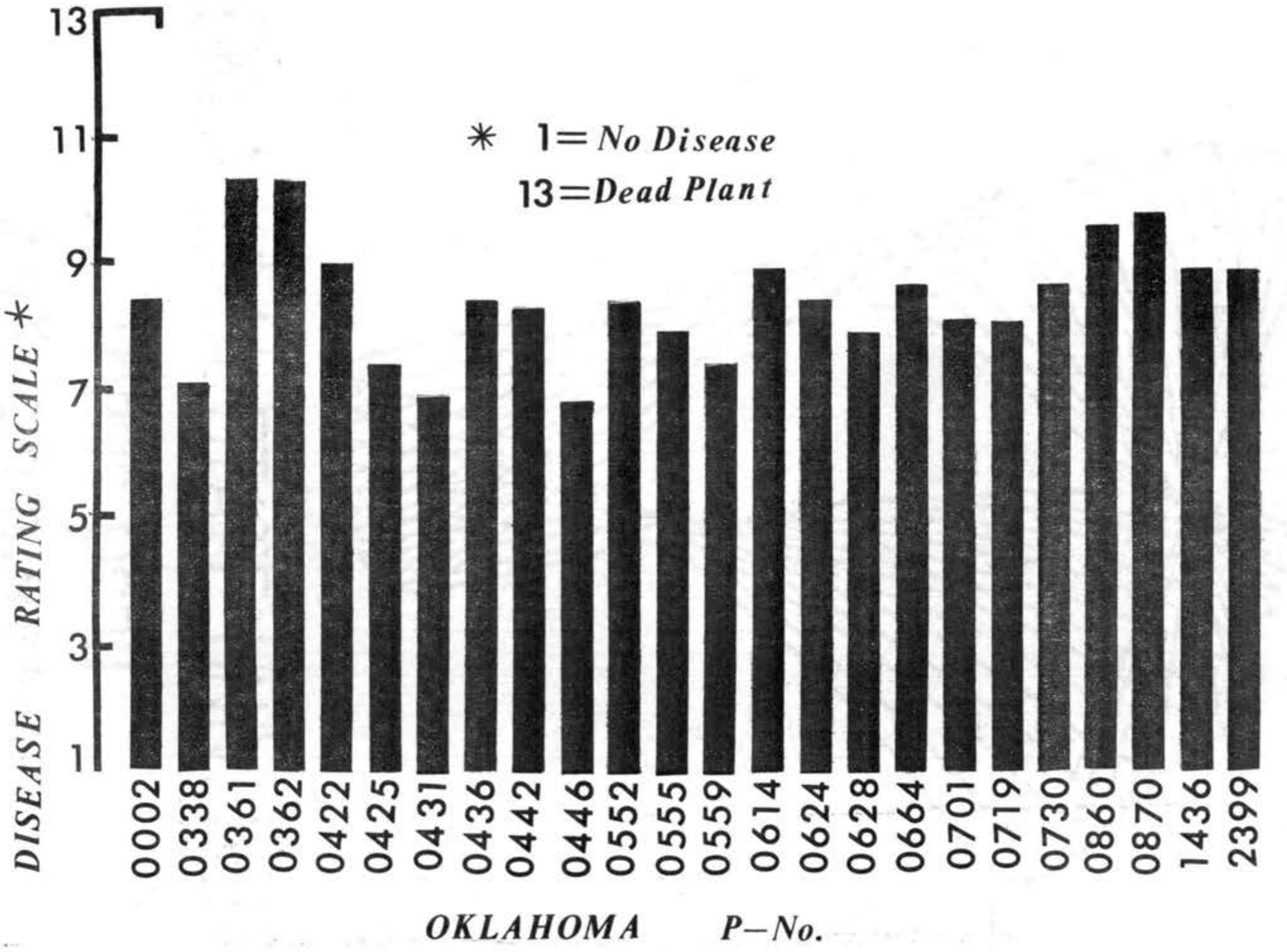

Figure 11. Mean Verticillium Wilt Severity Ratings of 24 Peanut Accessions in Greenhouse Test 3

Bunch 182-28 in second and third ratings as well as in the overall mean rating values. P-Nos. 361, 362, 860 and 870 had significantly higher disease severity ratings than Georgia Bunch 182-28, especially from the first, third and overall rating values. The Argentine variety and P-Nos. 422, 436, 552, 614, 664, 730 and 1436 did not differ significantly from Georgia Bunch 182-28 (P-2399).

Growth Chamber Studies

The same 24 accessions which were screened in the greenhouse were further examined under growth chamber conditions. The mean disease severity ratings are presented in Table VII. From the analysis of variance (Table XIX) the F ratio signifies the presence of significant differences among different ratings (Dates) and P-Nos (Accessions).

Reactions of the accessions in the growth chamber were consistent with those in the greenhouse. A significant correlation coefficient was obtained between the results of the greenhouse and growth chamber. The magnitudes of disease severity of the individual accessions were lower in the growth chamber than in the greenhouse. This was probably due to the different temperature regimes of the growth chamber (66 to 80°F) and the greenhouse (70 to 85°F). The higher temperature regime in the greenhouse favored Verticillium wilt development and obscured the expression of resistance of the accessions. Such findings have been reported (6, 7, 45) with other crops like cotton and strawberry.

The overall means of the three ratings are presented graphically in Figure 12. Again, Georgia Bunch 182-28 (P-2399) was considered as a standard for the test. The same P-Nos as previously reported from

TABLE VII

MEAN VERTICILLIUM WILT SEVERITY RATINGS OF 24 PEANUT ACCESSIONS IN THE GROWTH CHAMBER TEST[1]

Oklahoma P-No	1st Rating[2]	2nd Rating[2]	3rd Rating[2]	Overall Mean
0002	4.0	7.9	10.2	7.4
0338	3.7	7.4	9.7	6.9
0361	4.9	10.8	12.2	9.3
0362	5.9	10.4	12.5	9.6
0422	2.8	8.7	11.2	7.5
0425	3.8	6.7	9.9	6.7
0431	2.6	7.4	10.1	6.7
0436	2.9	8.4	10.9	7.4
0442	2.6	7.8	10.4	6.9
0446	3.9	6.9	10.2	6.9
0552	4.1	8.7	11.2	7.9
0555	4.3	7.6	10.3	7.4
0559	3.4	7.7	10.2	7.1
0614	5.2	8.5	11.1	8.3
0624	4.4	7.8	10.5	7.6
0628	4.7	6.9	10.4	7.3
0664	4.0	7.9	10.6	7.5
0701	4.9	10.2	11.9	8.9
0719	4.9	8.5	11.0	8.2
0730	5.4	8.9	11.2	8.5
0860	7.1	11.9	12.7	10.6
0870	5.9	10.5	13.0	9.8
1436	4.1	8.0	10.4	7.5
2399	2.9	10.6	12.4	8.6
Mean	4.3	8.6	11.0	8.0

[1]Rating scale of 1 to 13; "1" indicating no disease, "13" indicating completely defoliated, dead plants.

[2]Rating intervals of 20 days starting 20 days after inoculation.

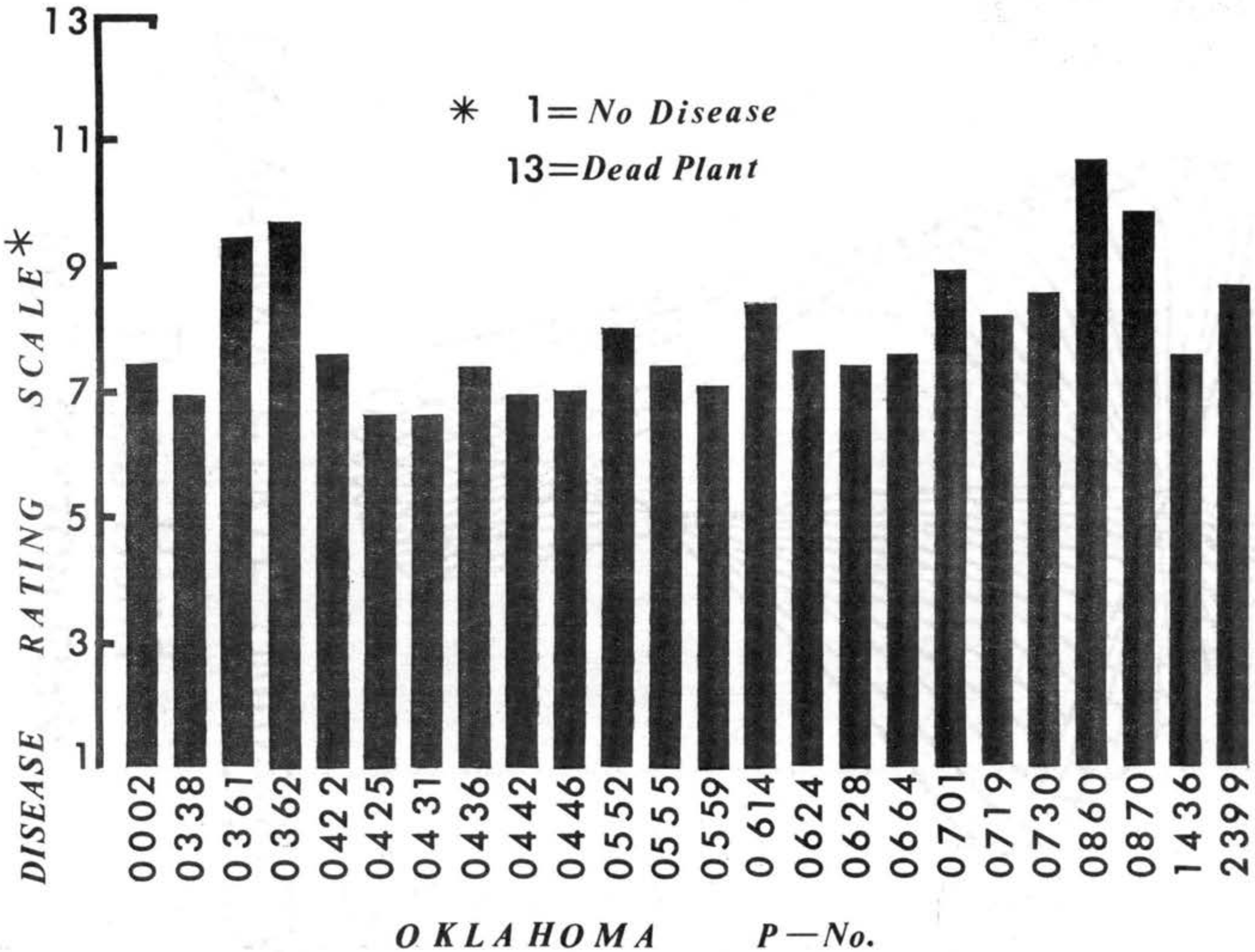

Figure 12. Mean Verticillium Wilt Severity Ratings of 24 Peanut Accessions in Growth Chamber Test

the greenhouse test 3 plus P-442 had lower disease values than Georgia Bunch 182-28. Accession numbers P-361, P-362, P-860 and P-870 were again more susceptible than Georgia Bunch 182-28. The remaining accessions did not differ significantly from Georgia Bunch 182-28.

For practical interpretation it was important to put together the data of the greenhouse and growth chamber; and to draw some conclusions, if possible. Hence combined mean disease severity data for all the accessions are presented in Table XIII, and also shown graphically in Figure 13. With the help of Duncan's New Multiple Range Test, the accessions were categorized into three groups: tolerant, intermediate and susceptible (Table IX).

The Spanish peanut variety, Argentine, and 9 of the introductions, P-338, P-425, P-431, P-436, P-442, P-446, P-555, P-559 and P-628, were placed in the tolerant group. In contrast, the line Georgia Bunch 182-28 (P-2399), previously reported (42) to be highly resistant, ranked in the intermediate group. However, one important feature of this line was noticed that at early rating date (20 days after inoculation) it was quite competent to withstand the disease reaction as did the other tolerant lines to give very low disease severity rating values at early disease development stage (Table VI, VII). Nevertheless, it could not tolerate disease severity at advanced stage of disease and collapsed at 2nd rating dates (40 days after inoculation), whereas the other promising accessions of the tolerant group (Table IX) were strong enough, even after 60 days of inoculation (Fig. 7, 8, 9). Accessions P-361, P-362, P-860 and P-870 were placed in the susceptible group. These four accessions had maximum disease severity ratings in both the greenhouse and growth chamber tests (Table VI, VII).

TABLE VIII

MEAN VERTICILLIUM WILT SEVERITY RATINGS OF 24 PEANUT ACCESSIONS IN GREENHOUSE AND GROWTH CHAMBER TESTS[1]

Greenhouse Test 3		Rank	Growth Chamber Test	
Okla. P-No	Mean		Okla. P-No	Mean
0446	6.8	1	0431	6.7
0431	6.9	2	0425	6.7
0338	7.1	3	0442	6.9
0425	7.4	4	0338	6.9
0559	7.4	5	0446	6.9
0555	7.9	6	0559	7.1
0628	7.9	7	0628	7.3
0701	8.1	8	0002	7.4
0719	8.1	9	0555	7.4
0442	8.3	10	0436	7.4
0002	8.4	11	0664	7.5
0436	8.4	12	1436	7.5
0552	8.4	13	0422	7.5
0624	8.4	14	0624	7.6
0664	8.6	15	0552	7.9
0730	8.8	16	0719	8.2
0614	8.9	17	0614	8.3
2399	8.9	18	0730	8.5
1436	8.9	19	2399	8.6
0422	8.9	20	0701	8.9
0860	9.5	21	0361	9.3
0870	9.7	22	0362	9.6
0361	10.3	23	0870	9.8
0362	10.3	24	0860	10.6

[1]Means enclosed by the same line are not significantly different at the 5% level of probability, according to Duncan's New Multiple Range Test.

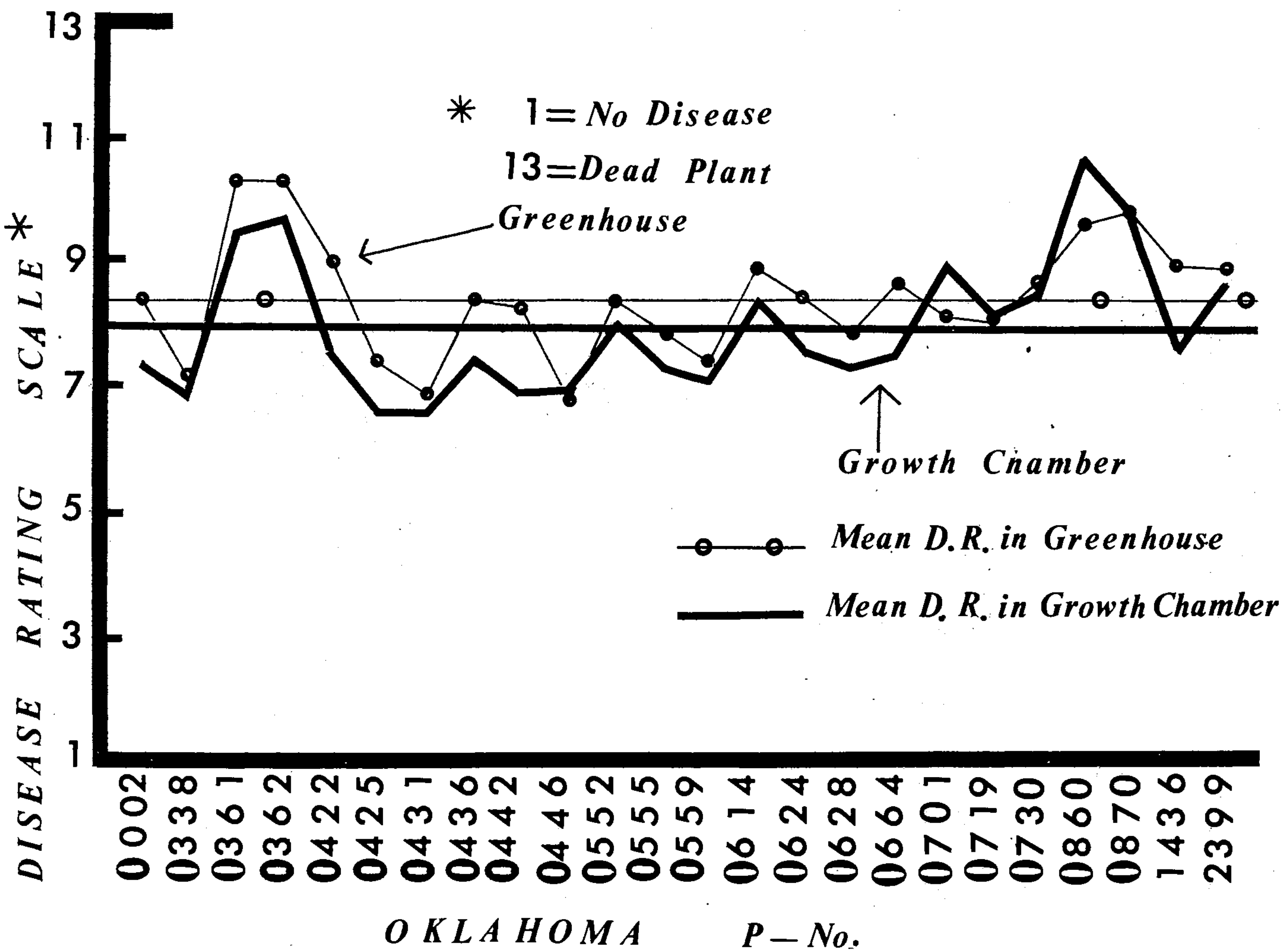

Figure 13. Mean Verticillium Wilt Severity Ratings of 24 Peanut Accessions in Greenhouse with Their Respective Ratings in Growth Chamber

TABLE IX

VERTICILLIUM WILT MEAN SEVERITY RATINGS OF 24 PEANUT ACCESSIONS IN GREENHOUSE AND GROWTH CHAMBER TESTS[1]

Okla. P-No.	Variety or P.I. No.	Greenhouse[2]	Growth Chamber[2]	Average Severity
Tolerant Group				
0431	268778	6.9	6.7	6.8
0446	268825	6.8	6.9	6.8
0338	259671	7.1	6.9	7.0
0425	268759	7.4	6.7	7.1
0559	240555	7.4	7.1	7.2
0442	268818	8.3	6.9	7.6
0628	268707	7.9	7.3	7.6
0555	248768	7.9	7.4	7.6
0002	Argentine	8.4	7.4	7.9
0436	268795	8.4	7.4	7.9
Intermediate Group				
0624	268703	8.4	7.6	8.0
0664	268742	8.6	7.5	8.0
0719	268801	8.1	8.2	8.1
0552	248763	8.4	7.9	8.1
1436	Dixie Spanish	8.9	7.5	8.2
0422	268749	8.9	7.5	8.2
0701	268785	8.1	8.9	8.5
0730	268811	8.8	8.5	8.7
0614	268686	8.9	8.3	8.6
2399	Georgia Bunch 182-28	8.9	8.6	8.7
Susceptible Group				
0870	268706	9.7	9.8	9.7
0361	268616	10.3	9.3	9.8
0362	268626	10.3	9.6	9.9
0860	268680	9.5	10.6	10.0

[1]Rating scale: 1 = no disease and 13 = completely defoliated, dead plant

[2]Average of 3 ratings

Since the inoculum potential for inoculating the accessions was presumably much higher than that under field conditions, the accessions in the tolerant group might be considered resistant. It is hoped that resistance levels of these tolerant accessions could be intensified, if critical plant selection and hybridization work are carried out among the best selected material.

To achieve the objectives of intensifying the levels of resistance in certain accessions and to incorporate resistance into adapted peanut varieties, it is important to study the mode of inheritance of resistance to Verticillium wilt in peanuts. Thus, crosses among four selected accessions representing both tolerant and susceptible groups were initiated. The details of this work are described under a separate heading in the latter part of this thesis.

Inheritance Study

Peanut plants in the genetic study were rated twice for disease reaction, however, the data from the first rating did not show pronounced disease reaction. Therefore, only data for the second rating were analyzed for inheritance pattern.

Even though the disease reaction was placed in one of the thirteen categories, for practical purposes it was desirable to classify the plants as either resistant or susceptible. Since no plants were immune or had a really high level of resistance, the more resistant plants are referred to as tolerant rather than resistant. After studying the frequency distribution of plants in the thirteen disease categories (Fig. 14) and reviewing the criteria of the various categories (Fig. 5a,b,c) it appeared that a natural and logical break occurred

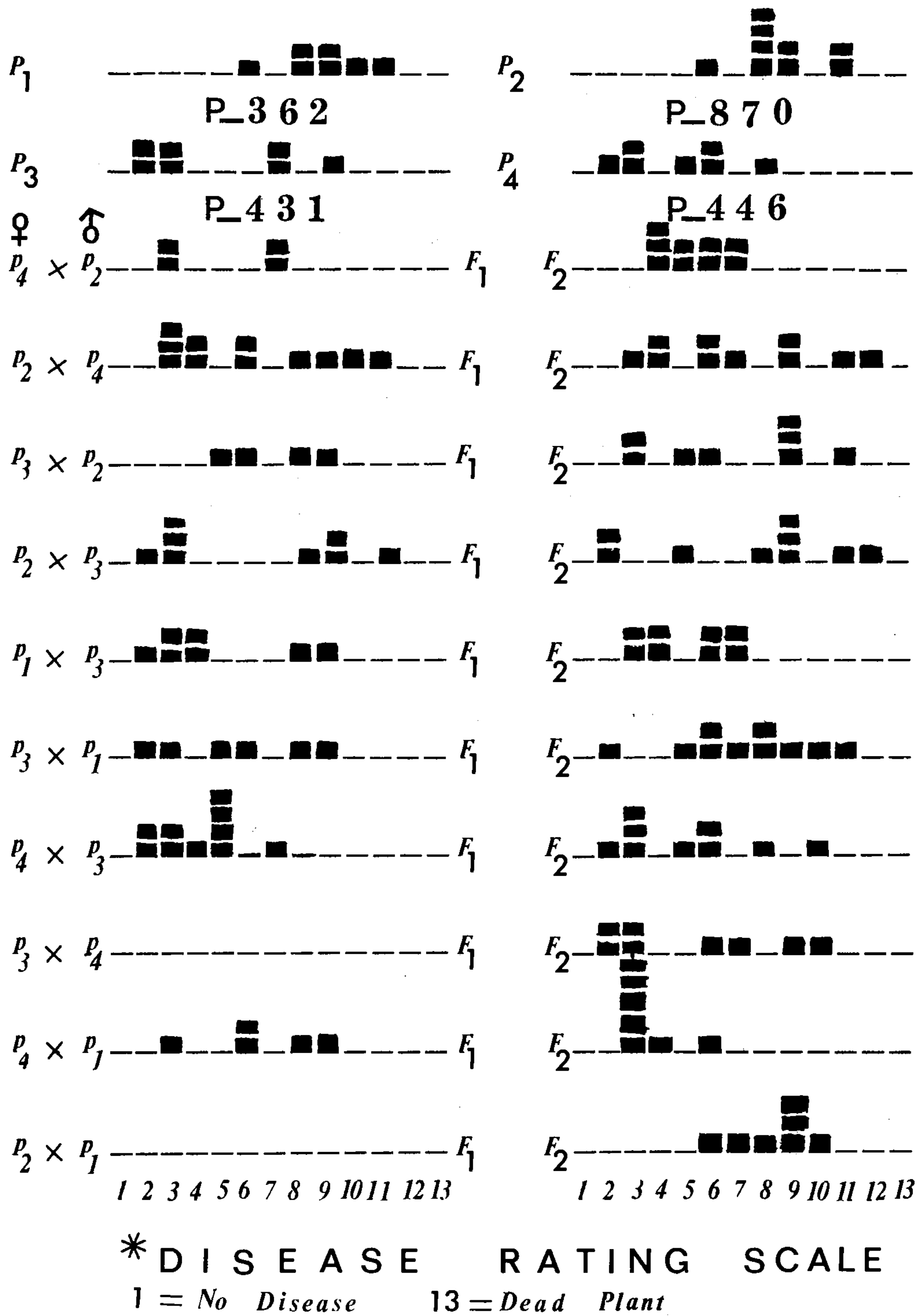

Figure 14. Frequency Distribution of Plants Rated* for Verticillium Wilt Severity in F_1 and F_2 Generations with Their Reciprocals and Parents;

P_1 = P-362 (Susceptible)
P_2 = P-870 (Susceptible)
P_3 = P-431 (Tolerant)
P_4 = P-446 (Tolerant

*Each block in the histogram represents one plant in the rating scale category between categories 4 and 5. Therefore, plants rated 1 to 4 were classified as tolerant or resistant and those rated 5 to 13 were classified as susceptible.

F_1's Derived from Crosses and Their Reciprocals

The mean disease severity ratings of F_1 hybrids and the percentage of plants in each F_1 population rated into the thirteen disease categories are presented in Table X. The mean rating values of the parents involved in these crosses are also given. Based on the two classes of tolerant and susceptible, all plants in the two parental populations of P-362 and P-870 were classified as susceptible. Since these two accessions were selected as two of the most susceptible lines in the preliminary tests, this result was as expected.

Plants in the two parental populations of P-431 and P-446 were placed in approximately equal numbers in the tolerant and susceptible categories, however, none of the plants in these two populations received as severe a susceptibility rating as plants in the previous two populations.

Because population sizes for individual crosses were small and distinct maternal differences were not evident, reciprocal crosses were combined for analysis.

The mean disease severity rating of F_1 hybrids was either similar to, or slightly lower than, the mean of the parents which constituted that particular cross (Table X). The mean rating of the tolerant (P-446) x tolerant (P-431) cross was lower than either parent's mean rating value suggesting a heterotic or intensifying effect for wilt tolerance or resistance. Similar findings have been reported in

TABLE X

FREQUENCY DISTRIBUTION (%) OF PLANTS RATED FOR VERTICILLIUM WILT SEVERITY IN F_1 HYBRIDS AND PARENTS

Population or Pedigree	Verticillium Wilt Severity Rating Categories[1] Tolerant or Resistant					Susceptible										Mean Wilt Severity Rating	
	1	2	3	4	Total of Col 1-4	5	6	7	8	9	10	11	12	13	Total of Col 5-13	Mean of F_1	Mean of Parents
P-362(S)Parent	--	--	--	--	--	--	14.3	--	28.6	28.6	14.3	14.3	--	--	100.00	--	8.7
P-870(S)Parent	--	--	--	--	--	--	11.1	--	44.4	22.1	--	22.1	--	--	100.00	--	8.7
P-431(T)Parent	--	28.6	28.6	--	57.1	--	--	28.6	--	14.3	--	--	--	--	42.9	--	4.7
P-446(T)Parent	--	14.3	28.6	--	42.9	14.3	28.6	--	14.3	--	--	--	--	--	57.1	--	4.7
P-870 x P-431* + reciprocal	--	8.3	24.9	--	33.2	8.3	8.3	--	17.0	24.9	--	8.3	--	--	66.8	6.5	6.7
P-870 x P-446 + reciprocal	--	--	33.5	13.4	46.9	--	13.1	13.1	6.7	6.7	6.7	6.7	--	--	53.1	5.5	6.7
P-431 x P-362 + reciprocal	--	15.4	23.1	15.4	53.9	7.7	7.7	--	15.4	15.4	--	--	--	--	46.2	5.1	6.7
P-446 x P-362 No reciprocal	--	--	20.0	--	20.0	--	40.0	--	20.0	20.0	--	--	--	--	80.0	6.4	6.7
P-446 x P-431 No reciprocal	--	20.0	20.0	10.0	50.0	40.0	--	10.0	--	--	--	--	--	--	50.0	4.1	4.7

[1] 1=no disease, 13=dead plant, Tolerant or Resistant = rating categories 1 to 4, Susceptible = rating categories 5 to 13

*The first parent listed in pedigree is female and second is male. This procedure is used throughout the thesis strawberries where the highest proportion of resistant plants (53%) was obtained from resistant x resistant crosses (54).

Mean disease rating values of most of the hybrids of susceptible (P-870, P-362) x tolerant (P-446, P-431) crosses approached the mean rating value of the susceptible parent involved, suggesting the dominance of susceptibility. Similar trends of Verticillium wilt reaction have been reported from F_1 progenies of crosses between resistant and susceptible plants of eggplant and sunflowers (22, 33). It is further revealed from this study that results from certain crosses between tolerant x susceptible indicated that some heterozygosity was present within the tolerant parents, because their F_1 progenies showed a 1:1 ratio of tolerant to susceptible plants.

F_2's Derived from Crosses and Their Reciprocals

Small population sizes and lack of obvious maternal differences again prompted the combining of reciprocal populations for analysis.

The frequency distributions and mean wilt severity ratings of parents and F_2 progenies are presented in Table XI. For the crosses P-870 x P-431, P-870 x P-446 and P-362 x P-431, the distribution of the F_2 plants was skewed toward susceptibility. In the cross of P-446(T) x P-362(S) the situation was quite opposite and the distribution of the F_2 plants was heavily skewed toward tolerance with 88% of the plants in disease categories 2 or 3 and 12% in category 6. According to the two attribute classes, the proportion of tolerant to susceptible plants in this particular cross fit a 3:1 ratio. The chi-square test was consistent with the hypothesis of a 3:1 ratio of tolerant to susceptible and the P value ranged from .25-.5 (Table XII). Thus

TABLE XI

FREQUENCY DISTRIBUTION (%) OF PLANTS RATED FOR VERTICILLIUM WILT SEVERITY IN F_2 GENERATIONS AND PARENTS

Population or Pedigree	Verticillium Wilt Severity Rating Categories[1]: Tolerant or Resistant					Susceptible										Mean Wilt Severity Rating	
	1	2	3	4	Total of Col 1-4	5	6	7	8	9	10	11	12	13	Total of Col 5-13	Mean of F_2	Mean of Parents
P-362(S)Parent	--	--	--	--	--	--	14.3	--	28.6	28.6	14.3	14.3	--	--	100.0	--	8.7
P-870(S)Parent	--	--	--	--	--	--	11.1	--	44.4	22.1	--	22.1	--	--	100.0	--	8.7
P-431(T)Parent	--	28.6	28.6	--	57.1	--	--	28.6	--	14.3	--	--	--	--	42.9	--	4.7
P-446(T)Parent	--	14.3	28.6	--	42.9	14.3	28.6	--	14.3	--	--	--	--	--	57.1	--	4.7
P-870 x P-431 + reciprocal	--	11.7	11.7	--	23.4	11.6	5.9	--	5.9	35.4	--	11.6	5.9	--	76.6	7.1	6.7
P-870 x P-446 + reciprocal	--	--	5.3	26.5	31.8	10.6	21.2	15.9	--	10.6	5.3	5.3	--	--	68.2	6.2	6.7
P-431 x P-362 + reciprocal	--	5.6	11.2	11.2	28.0	5.6	22.0	16.4	11.2	5.6	5.6	5.6	--	--	72.0	6.1	6.7
P-446 x P-362 No reciprocal	--	77.8	11.1	--	88.9	--	11.1	--	--	--	--	--	--	--	11.1	3.4	6.7
P-870 x P-362 No reciprocal	--	--	--	--	--	--	14.3	14.3	14.3	42.0	14.3	--	--	--	100.0	8.3	8.7
P-446 x P-431 + reciprocal	--	17.6	29.5	--	47.1	5.9	17.6	5.9	5.9	5.9	11.7	--	--	--	52.9	5.2	4.7

[1] 1 = no disease; 13 = dead plant; Tolerant = categories 1 to 4; Susceptible = categories 5 to 13

TABLE XII

CHI-SQUARE AND P VALUES FOR F_2 GENERATIONS OF FOUR PEANUT CROSSES

Pedigree of F_2 Progenies	Phenotypes	Number of Plants Observed	Number of Plants Expected[1]	Difference	X^2	P-Value
P-870(S) x P-431(T)	Susceptible	13	12.75	0.25	0.0049	
+ reciprocal	Tolerant	4	4.25	-0.25	0.0147	
					0.0196	.7 to .9
P-870(S) x P-446(T)	Susceptible	13	14.25	-1.25	0.1096	
+ reciprocal	Tolerant	6	4.75	1.25	0.3289	
					0.4385	.5 to .7
P-431(T) x P-362(S)	Susceptible	13	13.50	-0.50	0.0182	
+ reciprocal	Tolerant	5	4.50	-.50	0.0555	
					0.0740	.7 to .9
P-446(T) x P-362(S)	Susceptible	1	2.25	-1.25	0.6944	
No reciprocal	Tolerant	8	6.75	1.25	0.2315	
					0.9259	.5 to .25

[1]Expected values based on the hypothesis of 3:1 ratio of Susceptible to Tolerant plants except for P-446 x P-362 population where the hypothesis was 3 Tolerant to 1 Susceptible.

resistance or tolerance in line P-446 should be controlled by a single dominant gene. The monogenic dominance inheritance for resistance to Verticillium wilt has also been reported in tomatoes (10, 36) and sunflowers (33). However, it is important to note that this 8:1 population fit the 3:1 ratio only because of the extremely small population size in all likelihood. Since this was the only population studied that direction of dominance was toward tolerance, no reciprocal population was available, the ratio fit because of small numbers, and the tolerant parent was used as the female in the attempted cross, the most probable explanation would be to assume that the attempted cross was unsuccessful and that the population involved was actually the selfed progeny of the tolerant parent P-446.

The tolerant parent (P-446) crossed with a different susceptible (P-870) had a mean disease severity rating close to the mid-parent value in the F_1 (Table X). In the F_2 generation the mean disease severity rating value of this cross was also closer to the mean rating of the parents and the frequency distribution of the F_2 population was skewed toward susceptibility (Table XI). Thus, the preponderance of susceptible material in the cross (P-446 x P-870) compared with the cross (P-446 x P-362) indicates that the genotypes governing susceptibility in the lines P-870 and P-362 should be substantially different or that P-446 may be homozygous but not homogeneous for the loci affecting Verticillium wilt reaction, or that the P-446 x P-362 population was indeed a self of P-446 rather than the actual cross.

In the crosses P-870 x P-431, P-870 x P-446 and P-431 x P-362, qualitative inheritance of Verticillium reaction could be determined when the two broad reaction classes were considered. The data in

Table XI indicates that tolerance was recessive in these crosses. The proportion of susceptible to tolerant plants in each of the three segregating populations fit the 3:1 ratio excellently (Table XII).

Recessiveness for resistance to Verticillium wilt has been shown in eggplant (Solanum melongena), sunflowers (Helianthus annuus L.) and American Upland cotton (Gossypium hirsutum L.) (22, 33, 34).

No clear-cut segregation occurred in the F_2 progeny of the cross of P-446(T) x P-431(T) (Table XI). Only two plants with a rating of 10 and one plant each in rating categories 7, 8, and 9 did appear in the F_2 progeny. However, one plant with a rating of 9 occurred in the parental line P-431 and most of the plants had ratings below 6 in both parental populations. Thus the genes with major bearing on the resistance or tolerance of these two lines should be the same.

Although the F_1 progeny of the susceptible x susceptible cross (P-870 x P-362) was missing, the F_2 progeny was evaluated. As mentioned previously, all plants in the F_2 were susceptible (Table XI). The mean wilt severity rating was similar to the average rating of the parents. Most of the plants were rated in categories 8 to 11 of the rating scale. As far as can be determined from this particular cross, the two susceptible parents (P-870 and P-362) may have identical or at least very similar genotypes for Verticillium wilt reaction.

Heritability Estimates

A knowledge of the heritability of any character is desirable to enable breeders to make effective and efficient manipulation of genotypes in advanced generations through selection from a variable source population.

Heritability in the broad-sense is generally defined as the ratio of the total amount of genetic variation to the total phenotypic variation, usually expressed as $(\frac{\sigma^2 G}{\sigma^2 P})$.

Broad-sense heritability can be estimated by the formula:

$$H = \frac{VF_2 - \frac{VP_1 + VP_2 + VF_1}{3}}{VF_2}$$

where:

H = Broad-sense heritability

VF_2 = Phenotypic variance of the F_2, which estimates the total variance

$\frac{(VP_1 + VP_2 + VF_1)}{3}$ = Mean phenotypic variance of non-segregating populations, which estimates the total environmental variance

The broad-sense heritability estimates and the variances of the populations are given in Table XIII. Reciprocal crosses were combined where available to increase the population size. The heritability range was from -3.84 to 0.44. Since heritabilities theoretically cannot be negative, the most reasonable value for such an estimate is zero.

These crosses can be compared two at a time with either the susceptible parent or the tolerant parent in common. It can be assumed that any differences noted are due to genetic differences between the tolerant (P-446, P-431) parental lines or between the susceptible lines (P-362, P-870). The P-431 line retained its leaves even at advanced stages of disease development. In contrast, P-446 increased its tolerance value by strengthening its main stem through new regrowth near the crown of the plant.

The four lines used in this study are plant introductions. The degree of genetic homozygosity and homogeneity in each of these lines

TABLE XIII

SUMMARY OF BROAD-SENSE HERITABILITY ESTIMATES FROM F_2 GENERATIONS ASSOCIATED WITH VERTICILLIUM WILT TOLERANCE FROM PEANUT CROSSES AMONG TOLERANT AND SUSCEPTIBLE LINES

Population or Pedigree	Variances (σ^2) of Populations Involved[1]				Broad-Sense Heritability: $H = \frac{\sigma^2 F_2 - \frac{\sigma^2 P_1 + \sigma^2 P_2 + \sigma^2 F_1}{3}}{\sigma^2 F_2}$
	P_1	P_2	F_1	F_2	
P-870(S) x P-431(T)	2.50	8.40	8.17	11.28	.436
P-870(S) x P-446(T)	2.50	4.57	7.74	5.14	.0389
P-362(S) x P-431(T)	3.80	8.24	6.91	6.06	0.00*
P-446(T) x P-362(S) No reciprocal	4.57	4.95	5.30	1.02	0.00*

[1] P_1 (Population 1) represents the progeny of the first parent listed in the pedigree and P_2 represents the progeny of the second parent of the pedigree.
F_1,F_2 populations represent the respective progenies including their reciprocals unless otherwise indicated.
*Negative estimate for which the most reasonable value is zero.

is unknown for any character and certainly for reaction to Verticillium wilt. Naturally, any genetic variation within the parental lines could result in genetic variation in the F_1 generation. Genetic variation in the three supposedly "non-segregating" populations would inflate the estimate of environmental variance and reduce the estimate of heritability.

In general, estimates of heritability apply to the specific populations and the particular set of environmental conditions from which the estimates are obtained. Therefore heritability estimates reported in this study may not be applicable under different conditions.

CHAPTER V

SUMMARY AND CONCLUSIONS

Germ Plasm Evaluation

The objectives of this study were to evaluate peanut germ plasm for possible sources of resistance to Verticillium wilt and to study the mode of inheritance of Verticillium wilt resistance in peanuts.

A preliminary screening of 152 peanut accessions, comprised of 8 commercial varieties and 144 plant introductions and selections, was made in a field naturally infested with Verticillium in 1971 and 1972. In the 1971 test, the disease prevalence data (for percent of Verticillium infected plants) were recorded 90 days after planting. Accessions which showed less than 40% disease incidence were selected for further evaluation under the controlled environments of a greenhouse and a growth chamber.

Twenty-one selected accessions from the field study of 1971, two commercial varieties (Argentine and Dixie Spanish) and a line (Georgia Bunch 182-28) previously reported resistant to Verticillium wilt, were artificially inoculated and examined under greenhouse and subsequently under growth chamber conditions. The procedures and techniques were the same in both environments except in the growth chamber the temperature regime was maintained at 65 to 80^{o}F while the greenhouse was 5^{o} higher. The root-dip method of inoculation with

4.5×10^6 spores/ml was used for inoculating the 10-day old seedlings.

Individual plants were rated 3 times after inoculation at 20 day intervals for disease severity based on an arbitrary rating scale of 1 to 13, where 1 indicated no disease and 13 indicated a dead plant. The mean severity rating values from both locations were analyzed separately as well as combined. Significant correlation coefficients were obtained between the results of the greenhouse and growth chamber, indicating comparable results in both studies. All inoculated plants became infected but the degree of disease severity was quite variable in different accessions. Thus, with the aid of Duncan's New Multiple Range Test, the accessions were divided into tolerant, intermediate and susceptible groups.

The Spanish peanut variety, Argentine, and 9 of the introductions (P-338, P-425, P-431, P-436, P-442, P-446, P-555, P-559, and P-628) were placed in the tolerant group. In contrast, Georgia Bunch 182-28, previously reported to be highly resistant, and 9 other accessions ranked in the intermediate group. Accessions in the susceptible group included P-361, P-362, P-860 and P-870. Under less inoculum pressure such as field conditions, the accessions in the tolerant group might prove to be resistant.

Inheritance Study

Since prior study indicated that tolerance or resistance to Verticillium wilt was present in peanuts, it was desirable to determine the inheritance pattern of resistance which might facilitate its incorporation into commercial varieties.

A four-parent diallel cross was attempted by conventional methods using two tolerant (P-431, P-446) and two susceptible (P-362, P-870) peanut accessions. Some plants died in the growth chamber while making the crosses due to a root rot fungus. F_1 seeds from the successful crosses were harvested. Part of the F_1 seeds was planted to obtain F_2 seeds. Progenies of F_1 and F_2 generations were evaluated along with the parents under growth chamber conditions. The inoculation and data collection procedures were the same as used for germ plasm screening tests.

Susceptibility appeared to be controlled by a single dominant gene. However, tolerance was somewhat intensified in a hybrid of the two tolerant parents. Broad-sense heritability estimates for tolerance to Verticillium wilt varied from 0 to .44 for F_2 generations of tolerant x susceptible crosses.

Conclusion

Breeding for the development of peanut varieties resistant to Verticillium wilt appears to be encouraging even though all of the questions have not been answered. A reasonable level of tolerance appears to be present in the germ plasm. Although tolerance appears to be primarily governed by a single recessive gene, more critical studies should be undertaken to determine if genes are present that can increase tolerance or resistance to Verticillium wilt in peanuts.

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APPENDIX

TABLE XIV

ANALYSIS OF VARIANCE FOR PERCENT WILT PREVALENCE OF PEANUT VARIETIES IN 1971 FIELD TEST

Source of Variation	DF	Sum of Squares	Mean Squares	F Ratio
Replicates	1	15885.081	15885.081	22.95**
P-No (Varieties)	5	4387.111	877.4223	1.2
P-No x Rep (Error)	5	3460.595	692.1190	
Plot (Rep x P-No)	84	73434.760	874.2233	
Row (Rep x P-No x Plot)	96	14449.270	150.5132	
Corrected Total	191	111616.817	584.3812	

**Significant at the .01 level of probability

TABLE XV

ANALYSIS OF VARIANCE FOR PERCENT WILT PREVALENCE OF PEANUT ACCESSIONS (INTRODUCTIONS AND SELECTIONS) IN 1971 FIELD TEST

Source of Variation	DF	Sum of Squares	Mean Squares	F Ratio
Replicates	3	58779.855	19593.2849	29.2**
P-No (Accessions)	80	59506.572	743.8322	1.1
Rep x P-No (Error)	240	161016.766	670.9032	
Corrected Total	323	279303.193	864.7158	

**Significant at the .01 level of probability

TABLE XVI

ANALYSIS OF VARIANCE FOR PERCENT WILT PREVALENCE OF PEANUT VARIETIES IN 1972 FIELD TEST

Source of Variation	DF	Sum of Squares	Mean Squares	F Ratio
Replicates	1	4056.22	4056.22	4.13
Varieties	5	9589.94	1917.98	1.95
Rep x Varieties (Error)	5	4906.49	981.29	
Plot x Rep x Var	132	138663.81	1050.49	
Row x Rep x Plot x Var	144	26119.59	181.39	
Corrected Total	287	183336.10	638.81	

TABLE XVII

ANALYSIS OF VARIANCE FOR PERCENT WILT PREVALENCE OF PEANUT ACCESSIONS (INTRODUCTIONS AND SELECTIONS) IN 1972 FIELD TEST

Source of Variation	DF	Sum of Squares	Mean Squares	F Ratio
Replicates	5	12354.26	2470.85	6.10**
P-No (Accessions)	81	72470.37	894.69	2.19**
Rep x P-No (Error)	405	165137.25	407.75	
Corrected Total	491	249961.9	509.10	

**Significant at the .01 level of probability

TABLE XVIII

ANALYSIS OF VARIANCE FOR DISEASE SEVERITY
RATINGS OF 24 PEANUT ACCESSIONS
IN THE GREENHOUSE TEST 3

Source of Variation	DF	Sum of Squares	Mean Squares	F Ratio
P-No (Accessions)	23	241.733	10.510	28.413**
Flat (P-No) Error (a)	72	26.638	0.369	
Date (Rating)	2	1582.912	791.456	2211.759**
P-No x Date	46	97.247	2.114	5.907**
Flat x Date (P-No) Error (b)	144	51.528	0.357	
Corrected Total	287	2000.059	6.968	

**Significant at the .01 level of probability

TABLE XIX

ANALYSIS OF VARIANCE FOR DISEASE SEVERITY RATINGS OF 24 PEANUT ACCESSIONS IN THE GROWTH CHAMBER TEST

Source of Variation	DF	Sum of Squares	Mean Squares	F Ratio
Trial	1	363.1957	363.1957	178.05**
P-No	23	305.4469	13.2803	6.51**
Trial x P-No (Error a)	23	46.9177	2.0399	
Plot (Trial x P-No)	48	23.9268	0.4984	
Period (Rating)	2	2250.6132	1125.3066	20.50*
Trial x Period (Error b)	2	109.7761	54.8880	
P-No x Period	46	78.3521	1.7033	1.42^NS
Trial x P-No x Period (Error c)	46	51.6350	1.1225	
Plot x Period (Trial x P-No)	96	26.2266	00.2731	
Corrected Total	287	3256.0906	11.3452	

**Significant at the .01 level of probability
*Significant at the .05 level of probability
NS Not significant

Disease Rating Scale

Category Number	Description
1	No disease
2	Severe chlorosis of leaves
3	Severe chlorosis of leaves + 25% defoliation + epical growth
4	Severe chlorosis of leaves + 25% defoliation + epical growth with basal growth
5	Severe chlorosis of leaves + 25% defoliation + no epical growth but basal growth
6	Severe chlorosis of leaves + 50% defoliation + epical growth
7	Severe chlorosis of leaves + 50% defoliation + no epical but basal growth
8	Severe chlorosis of leaves + 75% defoliation + epical growth
9	Severe chlorosis of leaves + 75% defoliation + no epical growth but basal growth
10	100% defoliation + epical growth
11	100% defoliation + no epical but basal growth
12	Dying plant
13	Completely defoliated, dead plant

VITA

Baz Mohammad Khan

Candidate for the Degree of

Doctor of Philosophy

Thesis: BREEDING PEANUTS (ARACHIS HYPOGAEA L.) FOR RESISTANCE TO VERTICILLIUM WILT

Major Field: Crop Science

Biographical:

Personal Data: Born in Gul Imam, D. I. Khan, Pakistan, January 4, 1939, the son of Mr. and Mrs. Khan Mohammad Khan Kundi.

Education: Graduated from High School Tank, D. I. Khan in May, 1955; received Bachelor of Science (Honour) degree in Agriculture from University of Peshawar, Pakistan in May, 1960, with a major in Entomology; received Master of Science degree from the American University of Beirut, Lebanon in July, 1964, with major in Plant Pathology; completed requirements for the Doctor of Philosophy degree in May, 1973, with major in Crop Science at Oklahoma State University.

Professional Experience: Research Assistant in Entomology and Plant Pathology Departments, at Agricultural Research Institute, Tarnab, Peshawar, Pakistan, 1960-1968; Assistant Plant Pathologist, at the same Institute, 1968-73.