

SURVIVAL OF CERCOSPORA ARACHIDICOLA, CERCOSPORIDIUM  
PERSONATUM, AND PRIMARY INFECTION OF  
PEANUTS IN OKLAHOMA

By

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## CHAPTER I

### INTRODUCTION

The peanut or groundnut (Arachis hypogaea L.) is a major agricultural crop in many parts of the world. In the southern United States, peanuts are grown extensively for cash return and for hay. In 1976, it was estimated that 98% of the total peanut production of 1,680,728 tons grown on 600,000 ha were produced by seven southern states for a farm value of about \$740 million, thus ranking peanuts among the ten most important crops in the country (19). In Oklahoma, peanut production is economically important. According to 1979 statistics, in spite of an estimated loss of \$20,106,555 due to disease, Oklahoma growers produced over 132,100 tons of peanuts for a cash return of approximately \$55,482,000. Losses due to Cercospora and Cercosporidium leafspots in the State of Oklahoma for 1979 were estimated to be 3.75% (68).

The major foliar diseases affecting peanuts worldwide continue to be those of leafspots caused by Cercospora arachidicola Hori, and Cercosporidium personatum (Berk. & Curt.) Deighton. The diseases, if not appropriately controlled, often result in heavy economic losses for peanut growers. Under environmental conditions conducive to development of epiphytotics, 60 to 70% defoliation of the crop had been observed in some fields (67). Losses from these leafspots of peanuts are mainly due to extensive defoliation late in the season leading to reduction in yield of kernels and decrease in quality of hay. With no

control measures being applied, losses in yield of 20-40% have been reported (22, 39, 45, 67, 68, 69, 72, 73). In Oklahoma, all commonly grown varieties of peanuts are attacked, and these leafspot diseases occur regularly on irrigated and on dryland peanuts grown in more humid areas of the state (72). It has been estimated that in Oklahoma, a loss in yield of nuts of 20 to 30% may occur when a favorable environment for epiphytotic prevails.

Among measures for leafspot control, varietal resistance would seem to be the most economical and effective. However, sources of resistance found in wild peanuts are often limited to only one of the two pathogens (24). Jackson and Bell (25) reported that Higgins found that resistance had also been associated with such undesirable agronomic characters as lack of fruit-set, making highly resistant selections unacceptable.

The standard control procedure of leafspots on peanuts in the U.S. continues to be through application of fungicides. In the early years, sulfur dust alone or in combination with copper were perhaps the most commonly used chemicals (12, 44). With the advent of organic fungicides, carbamates such as maneb, zineb, and later systemic benzimidazole compounds were adopted in spray programs in different peanut production areas (4, 20, 60). Extensive use of fungicides to combat *Cercospora* and *Cercosporidium* leafspots of peanuts, while achieving dramatic success, led to development of some alarming problems. In areas where benomyl was regularly used, reports of isolates of *Cercospora* spp. and *Cercosporidium personatum* that were no longer sensitive to benomyl or other related compounds and, therefore, could tolerate large amounts of the fungitoxicant are well documented (5, 11, 18, 35,

63). Other recommended control practices aimed at reducing primary inoculum available for early season infection by Cercospora include crop rotation, and removal or burial of peanut debris or volunteer plants (21, 58). The extent to which these cultural practices are being adopted, could not be determined, but presumably their adoption is only on a small scale because of our lack of information about their efficiency. Gaps in our knowledge regarding some basic aspects of the life cycle of peanut leafspot fungi could well be illustrated by the following statement, "The source of inoculum is presumably from conidia or ascospores produced in or on peanut debris in the field" (25). For a disease control program to be successful, it is essential to have a thorough understanding of the life cycle of the causal organism or organisms. Overwintering represents the survival stage where the causal organism(s) has to carry over from one season to the other in the absence of the primary host.

In Oklahoma, peanuts are planted from mid-May until mid-June, and harvested by late September or early October, so there is almost a seven-month period during which the land is planted to a non-leguminous cover crop.

The objectives of this study were: (1) to find out the mode of survival of the leafspot fungi infecting peanuts under conditions prevailing in Oklahoma, (2) to look for the presence or absence of the perfect stage (Mycosphaerella spp.), and determine its role (if any) in the infection of the crop, and (3) to establish when, where, and how the initial infection of peanuts occurs.

## CHAPTER II

### LITERATURE REVIEW

#### Historical

According to Woodroof (75), a peanut leafspot that was attributed to Cladosporium personatum (B. & C.) was first reported by Berkeley in 1875 from material collected by Ravenel in South Carolina. The fungus was later transferred to the genus Cercospora and renamed Cercospora personata by Ellis and Everhart (16). Another species, Cercospora arachidicola was described by Hori from Japan. It was suggested by Woodroof (75) that the description given for C. arachidicola was the same as that of C. personata reported earlier from Southeastern United States.

The etiology of peanut leafspot was not clearly established until 1933 when Woodroof concluded that of the species of fungi reported to cause leafspot of peanuts, only C. arachidicola and C. personata were valid species (75). More recently, Deighton (14) proposed the new combination Cercosporidium personatum (B. & C.) Deighton as a synonym for C. personata. This new combination apparently is gaining wide acceptance among workers on Cercospora leafspot of peanuts (2, 3, 11, 63). Reported incidences of leafspot on peanuts indicate that both species occur worldwide wherever peanuts are grown (12, 17, 58, 75, 76). However, the frequency of either species is variable from one area to

another. Cercosporidium personatum is more common and is the major cause of loss in East Africa (22) and many parts of India (25), while C. arachidicola is the predominant species in Argentina (17), and the southern United States where it is usually found early in the season (26, 45, 75). Since 1976, a shift in prevalence of species where C. personatum has become more predominant have been reported from parts of the United States and Australia (37).

Jenkins (26), in 1938 after studying the morphology and life history of C. arachidicola and C. personata (C. personatum) over a period of three seasons, reported that both fungi produce spermogonia and perithecia in addition to conidia. He proposed the name Mycosphaerella arachidicola sp. nov. for the perithecial stage of C. arachidicola. Deighton (15) in 1967 proposed the name, Mycosphaerella arachidis Deighton, for the perfect stage of C. arachidicola because the name, M. arachidicola Chochrjokov, was previously used to describe the perfect stage of Ascochyta adzamehi, a fungus of apparently limited distribution in the Caucasus region, U.S.S.R., where it causes a different leaf-spot on peanuts.

#### Survival

For a plant pathogen to be established in an area it is assumed to have the ability to survive not only during its vital association with its host or hosts, but also during those seasons in which the hosts are not growing (the non-cropping period) (54). The peanut leaf-spot organisms are no exception and, in Oklahoma, the non-cropping period extends for almost seven months. There is general agreement that under situations where peanuts follow peanuts, Cercospora and

*Cercosporidium* leafspots occur early and are more serious (21, 25, 44). The source of inoculum for those early infections is "presumably" from ascospores or conidia produced in or on peanut debris in the field (21, 25, 26, 55). Conditions under which *C. arachidicola* and *C. personatum* survive the winter and mode of this survival are not clearly defined.

Much of the work on survival of *Cercospora* spp. has been conducted on species other than those which attack peanuts (8, 28, 46, 49, 64, 71). *Cercospora kikuchii* (Matsu and Tomoyasu) Chupp, the causal agent of leaf blight and purple seed stain of soybean was reported by Kilpatrick (31) to survive on infected soybean stems for 42 months when a bundle of infected stems was hung outside a laboratory window. Jones (28) observed abundant sporulation on overwintered soybean stems collected from different locations of different soil texture. He observed that on partially buried soybean stem samples, more sporulation occurred on above-ground than on underground portions indicating that burying the stems apparently reduces survival of the fungus greatly. Decomposition of the buried portions was not a factor in reduction of sporulation because very little decomposition was observed, especially in clay loam soil. Pool and McKay (49) stated that conidia of *Cercospora beticola* Sacc., the causal agent of leafspot on sugarbeets and several other crops, have a short longevity and, therefore, cannot survive normal field conditions of the winter season in Colorado or Wisconsin. While conidia of *C. beticola* survived for eight months if kept dry, as in the case of herbarium specimens, conidia on infected areas of leaves failed to germinate after 1-4 months exposure to field conditions. The authors concluded that, under ordinary conditions in the field, conidia play no important part in overwintering of the fungus. According to

Pool and McKay (49) the sclerotia-like bodies (stromata) of C. beticola embedded in the host tissue survived the winter when slightly protected in a pile of beet tops or when buried 2.5-12.5 cm in the soil. They also suggested that the organism under the above conditions serves as a source of primary infection the following year. Nagel (46) showed that C. beticola can live in the soil for a considerable time. In sterile soil culture, the fungus retained its viability and pathogenicity for a maximum of 27 months, while in naturally-infested soil, the pathogen survived for up to 20 months, but there was a marked decline in the Cercospora population in the soil. Cercospora beticola, as conidia and mycelium carried on seeds and in infected leaves on the ground, had been reported to survive unfavorable environmental conditions for 12-18 months even if buried 30-50 cm deep (8). Verma et al. (71) recovered C. beticola in periodical isolations up to six months after infected spinach-beet leaves were subjected to burial in sterilized or unsterilized soils (2.5-12.5 cm deep), storage in refrigerator at 0-6 C, or in the laboratory at -5 to 38 C. This led them to suggest that the pathogen can survive in decomposed or partially decomposed host debris on or in the soil. Solel (64) studied factors affecting survival of C. beticola under relatively hot and dry summer conditions in Israel. He found that longevity of the spores on infected sugarbeet debris left on the soil surface was limited to a maximum of three weeks, whereas that of mycelium lasted over three years. In debris buried at different depths in the soil, loss of viability of the mycelium increased with depth in contrast to a previous report by Canova (8) who claimed that survival of the fungus in Northern Italy was longer with increasing depth.



Jenkins (26) reported that the Mycosphaerella stage is initiated on fallen foliage in the field in Georgia during early fall by the formation of spermogonia and perithecia within old conidial stromata or within separate stromata that develop after the death of leaflets. Perithecial formation in nature is influenced by rainfall and, unless leaflets are kept wet during the time the spermatia are released, no perithecia will be formed. Mature perithecia and ascospores were never found in nature earlier than May 31, while on overwintering leaves collected in February and March and placed in moist chambers, mature perithecia and ascospores were obtained in 2-3 weeks. Jenkins further suggested that ascospores released early in the season constitute the source of primary inoculum initiating the early infection on peanuts in the field. However, apart from this report by Jenkins, the perfect stages [M. arachidicola (M. arachidis) and M. berkeleyii] had only been reported from Argentina by Frezzi (17). The importance of ascospores as a source of primary inoculum for leafspot infection is questionable (62).

Cercospora arachidicola and/or C. personatum have been reported to persist in the stromata (sclerotia-like bodies) in the dead refuse of diseased peanut tissue (55), as dormant mycelia in peanut debris (21, 56), and as conidia in the soil (21, 73). Wolf (73) showed that infective material (decaying leaves and stems) persist in the soil from one season to the next, and the early infections reaching the lower leaves from the soil presumably come from conidia produced in the lesions when favorable moist conditions prevail. Roldan and Querijero (55) concluded that C. personata (C. personatum) can persist in the form of stromata in the debris of infected peanut tissue. Shanta

(56), unable to find the perfect stage of C. personatum in India, assumed that the fungus survives as dormant mycelia on the infected debris of the previous crop. In a study of overwintering under conditions of constant temperature (-3 C), burial 30 cm deep in the soil of an open field where the ground was frozen to a depth of 30 cm for at least 3.5 months in the winter, and on a roof on the south side of a building, Miller (45) reported that after five months exposure of cultural material (C. arachidicola and C. personata grown on potato dextrose agar) and infected peanut tissue, only the material stored in sealed jars without moistened soil in a constant temperature chamber yielded C. personata (C. personatum), while infected material of C. arachidicola stored under all stated conditions was viable. He also found that an inoculum of C. arachidicola can persist on peanut hulls for at least 14 months under dry conditions. Infested soil from Virginia could also retain a viable inoculum of C. arachidicola for 12 months when stored dry at room temperature. Cultural material of C. arachidicola and C. personata (C. personatum) stored at room temperature was still viable four years later.

The ability of Cercospora mycelium to persist in peanut debris in a dormant state for at least six months was reported by Hemingway (21). He further stated that a viable inoculum presumably in the form of conidia, may persist in the soil. The idea that conidia have sufficient longevity to carry over from one crop to another was also shown by Frezzi (17). Miller (45) could not successfully determine whether conidia per se were capable of overwintering, although he stated that chlamydospores in the agar substratum did overwinter successfully. In Georgia, even though the cultural practice of deep plowing to bury the

surface debris was adopted, a second crop of peanuts was usually infected earlier and more extensively than if peanuts follow a different crop. The source of inoculum in this case is most probably in the soil (25). Boyle (7), suspecting that the primary inoculum may come from the soil, carefully buried peanut debris from previous seasons at a depth of 12.5-17.5 cm below the soil surface, but no reduction in the severity of disease resulted. Soil applications of a fungicide did not consistently affect infection and defoliation, but greater yields were obtained whenever supplementary application of fungicides to the soil were made.

#### Spore Dissemination

Due to their light weight, spores of C. personata (C. personatum) were thought to be wind-borne although no convincing evidence to substantiate this claim was presented (73). The distribution of spores from one leaf to another on the same plant or to adjacent plants was reported to be accomplished via rain or implements used in the culture of the crop. Wolf (73) also suggested that seed transmission may be involved in the wide range spread of leafspot to fields not previously grown to peanuts and to widely separated localities. Miller (45) concluded that a viable inoculum of C. arachidicola can be disseminated on the pods of peanut seeds and that shelling reduces the chance of dissemination. In a later work, Wolf (74) presented convincing evidence to prove that the conidia of C. personata (C. personatum) are windborne. He also showed that conidiospores were disseminated by grasshoppers and other insects. Dissemination by grasshoppers after coming in contact with diseased leaves was shown to be external on the

insect body surface, and internal in voided feces. Wolf (74) concluded that seed treatment, however, did not alleviate the leafspot problem. Seed transmission did not occur in the Philippines (55).

Roldan and Querijero (55) tested wind dissemination of C. personata (C. personatum) spores by exposing healthy peanut plants and vaseline-coated slides. After a period of six weeks exposure at a distance of 3-5 meters from diseased plants in the field, nearly all the plants were infected. Windborne conidiospores of C. personatum were caught on vaseline-coated slides placed at a distance of up to 100 meters away from infected plants.

Although in many instances no supporting evidence was made available, most workers on peanut leafspot apparently agree that Cercospora and Cercosporidium conidiospores are mainly dispersed by wind (12, 21, 65, 73, 74). Sreeramulu (65), in India, trapped conidia of C. arachidicola approximately one month after planting. Cercosporidium personatum conidia were trapped one week later. However, sufficient numbers of conidia were not trapped until 30-40 days after emergence of the plants. The spore trapping study was conducted using a Hirst automatic volumetric air sampler, and lasted from 15 February until 4 May 1959. Peak catches of C. arachidicola and C. personatum occurred 10 days before harvest, the peanut crop being sown in February. Lyle (39), in a study of the development of Cercospora leafspot of peanuts in Alabama, noted that the greatest number of conidia of C. arachidicola and C. personata (C. personatum) were trapped on vaseline-coated slides mounted on weather vanes during the period of July 15-31 when moisture was abundant and the highest minimal and maximal temperatures were 22.2 and 34.4 C, respectively. Smith and Crosby (61), in their study of the

aerobiology of two peanut leafspot fungi in Georgia, namely C. arachidicola and Leptosphaerulina crassiasca found that for three years, an average of 42.3 conidia of C. arachidicola per cubic meter of air per day occurred in the period of July 16-August 31. The concentration of C. arachidicola conidia increased throughout the growing season until by the end of the season, the conidiospores were continuously present in the air. Vertical dissemination, which was studied by trapping C. arachidicola conidia by exposing potted peanut plants at different heights, showed that the conidia were present in the air above the field and that the number of conidia had an inverse relationship with exposure height.

Work with other species of Cercospora have long shown that wind is one of the agencies of conidial dispersal (6, 29, 40, 48). Viable conidia of C. beticola Sacc. were trapped from the air near diseased sugar beet fields in Colorado by McKay and Pool (40) who concluded that the conidia are windborne. Canova (8), working in Northern Italy, concluded also that conidia of C. beticola Sacc. are dispersed by wind, although spore release is brought about by water (rain or dew). Conidia of C. zebrina Pass. were trapped from the air near Madison, Wisconsin (6). While no conidia of C. musae Zimm. were caught on spore traps designed to study windborne dissemination in the atmosphere of banana plantations by Meredith in Jamaica (43), Kaiser and Lukezic (29), in their aerobiological studies using a Hirst spore sampler, trapped conidia of C. hayi Calpouzios from the air over a banana plantation in Honduras. Carlson (9) studied the relation of weather factors to dispersal of C. beticola Sacc. in sugar beet fields in South Dakota, U.S.A. Glass rods covered with petrolatum-coated polyethylene strips, and

sugar beet potted plants were used as spore traps. Conidia were trapped on polyethylene strips and potted plants throughout the summer, and most abundantly during rainy periods. Generally, inoculum concentration on coated strips as on plants was higher on the day following a rain than on rainless days.

### Control

The importance of peanut debris in the field as a survival site for the peanut leafspot organisms, and the fact that no hosts other than peanuts were attacked led early researchers to suggest such measures as crop rotation, burial of peanut debris, and seed treatment for the control of the disease (73, 74). Wolf (73), though, suggested crop rotation as a control measure for *Cercospora* leafspot, he did not describe a definite rotation scheme. Based on experimental data, Wolf (74) later concluded that rotation in itself was not an effective measure in the control of peanut leafspot under field conditions. Hemingway (21), recognizing the fact that no evidence of the longevity of soil-borne persistence existed, recommended that for adequate leafspot control in East Africa at least a one-year interval between peanut croppings should elapse. On soils of low biological activity, Hemingway (21) suggested a two-year interval between successive crops. Littrell and Lindsey (36), also observed that disease severity due to *Cercospora* leafspot was much less when peanuts were grown in a three-year rotation with other agronomic crops. In the southeastern U. S., Kucharek (33) reported that crop rotation reduced early season leafspot by 88-93%. Even when no fungicides were applied, a one-year crop rotation resulted in 91% reduction of lesion/leaflet compared to the situation where

peanuts followed peanuts. The effectiveness of crop rotation in reducing losses on peanuts may be influenced by the distance between sites of successive crops and the direction of prevailing winds (58).

The presence of Cercospora conidiospores adhering to the surface of shells and the occurrence of the disease in areas not previously grown to peanuts, led Wolf (73) to suggest steeping the seeds in copper sulfate or formaldehyde. Later experimental evidence (74) showed that seed treatment separately or in conjunction with crop rotation did not preclude leafspot of peanuts. Roldan and Querijero (55) stated that leafspot of peanuts is not seedborne in the Philippines. Hemingway (21) believed that seedborne infection was of minor importance when compared with the large numbers of spores which can persist in peanut debris and soil, and those released from infected volunteer plants. In greenhouse experiments, Miller (45) planted seeds and pods containing seeds in pots watered with a hose so as to spatter potential inoculum. Leafspot readings eight weeks later showed considerable infection on plants from two-month old, non-treated seeds, while plants from non-treated 14-month old seed lots showed only a light infection indicating that the potential inoculum apparently loses its vitality with the passage of time.

Volunteer plants were reported to play a major part in the spread of the disease in certain parts of Africa (21, 58). Although the growth of volunteer plants is drastically reduced during the dry season, some Cercospora lesions could always be found. With early rains, the fungi sporulate profusely and reinfect the foliage of renewed growth of host plants and substantial inoculum will be available when the new crop is planted. Hemingway (21) recommended cultivation of fields carrying any substantial volunteer plants population. For this purpose,

light discing to 3.8-5.0 cm depth was found to be sufficient to kill the plants. Also the use of varieties whose seeds have a dormant period was suggested to be of benefit. Smartt (58) also believes that early destruction of volunteer plants when practiced with other measures will provide a reasonable degree of leafspot control. The same viewpoint had been advocated by others (25).

Because there is almost a consensus among most workers on *Cercospora* leafspot of peanuts that the causal fungi persist in infected peanut residues (25, 55), and based on reported success with fall plowing to 35 cm deep in greatly reducing injury due to *Cercospora beticola* in sugar beets when successive crops were grown (70), Wolf (73) recommended the burial of peanut debris by deep plowing. Hemingway (21) also proposed that all residues remaining on the field should be buried by discing or plowing before the new crop germinates, while all combustible peanut debris should be heaped and burned by the middle of the dry season in East Africa.

Although the morphological and physiological bases of resistance in peanuts to *Cercospora* leafspots have not yet been determined, Hemingway (23) found that the color and leaf thickness, and the size of stomata were related to resistance in certain peanut varieties grown in East Africa. He also reported that differences in the branching pattern of the host were somehow related to resistance. Resistance to *Cercospora* leafspots was reported by Smartt (58) to be correlated with the branching habit with alternately branched varieties showing a greater degree of resistance over sequentially branched forms.

Even though considerations of crop returns to offset the cost of applying fungicides together with the large amounts of water consumed



in spray programs (a critical factor in dry areas), and the persistence of formulation residue against local weather conditions play a major role in fungigation programs (21), curtailment of severe economic losses have been achieved with modern fungicides (4, 20, 60). Prior to 1971, dust and spray formulations of sulfur, copper, and copper plus sulfur were routinely used to suppress *Cercospora* leafspots on peanuts (12, 21, 58). With the advent of organic and systemic fungicides, there was a rapid change from dusting to spraying (62). *Cercospora* and *Cercosporidium* leafspots of peanuts were effectively controlled using a variety of fungicides (10, 50, 67), however, strains of the pathogens tolerant to high dosages of the most effective fungicides in use, were reported (11, 30, 35, 63). Development of fungicide-tolerant strains of other *Cercospora* species were also reported (5, 18).

#### Effect of the Environment on Infection and Disease Development

In the United States, early leafspot of peanuts caused by *C. arachidicola* reaches epiphytotic levels early in the season (26, 44, 75). Miller (45), reporting on observations made in Virginia over a period of six years, showed that *C. personata* (*C. personatum*) occurred only in the latter part of the season, and did not exceed 30% of the infections by harvest time. Observations made in 1947, however, showed that 80% of the lesions from Virginia collected on October 13 were caused by *C. personata* (*C. personatum*). The same author stated that the disease caused by *C. personata* reaches epiphytotic proportions in Virginia about once every four years, while that by *C. arachidicola* causes an epiphytotic in Virginia every year.

It is commonly observed that, under field conditions, heavy infection of peanuts with *Cercospora* leafspot occurs during the rainy season when the air is moist, thus providing the optimum relative humidity required for conidial germination and subsequent penetration of plant tissue (47). Wolf (73), in 1914, found no correlation between temperature and moisture, and the prevalence of leafspot of peanuts in Alabama. In Georgia, Jenkins (26) observed that cool, humid weather during epiphytotic months favored the spread and development of leafspot. He found that, under laboratory conditions, conidia germinated within 3-8 hours when moisture, oxygen, and temperature were optimum. Miller (44), in a review of previous work, reported that among other investigators it is generally agreed that the rapid spread of the leafspot disease may be correlated with periods of heavy rainfall. He also concluded that in the field, infection is less under dry conditions. Cooper and Wells (13) reported that under arid conditions in Israel when infrequent surface irrigation was the only source of water, leafspot infection and defoliation were very light. They also stated that the peanut leafspot fungi are unable to infect readily under low humidity conditions encountered when peanut culture is carried into arid areas by dry farming or irrigation. In Virginia, Miller (45) noticed that the frequency of rainfall rather than the amount during the growing season had the greater influence on the development of leafspot. He further stated that rainfall during the months of September and October was more important for disease development than rainfall during June, July, and August, and that heavy dews were observed to form on the foliage every night in August, September, and October. In areas of high or frequent rainfall, like Virginia - Carolina and Southeast areas, 20-30% losses

due to *Cercospora* leafspots were reported by Miller (44), while only negligible losses occurred in dry-land cultures in Texas. Jensen and Boyle (27) found that a 95% RH or above for nine hours or longer, and a temperature in the mid 70's were required for a heavy infection with *Cercospora* to occur. They also observed that the beginning of a period with such favorable conditions occurred 11 days before symptoms were visible, which is in accordance with Jenkin's finding of 10-21 days incubation period after inoculation. Young et al. (76) reported that leafspot in South Africa was severe when moist conditions with higher temperatures prevailed. However, they stated that under the conditions of their study, it appeared that *Cercospora* leafspot infections occurred at appreciably lower temperatures than were reported by Jensen and Boyle (27).

In most of the southern peanut growing areas, symptoms of early leafspot caused by *C. arachidicola* appear in late June or during July depending on weather conditions (66). By allowing 15-20 days for incubation, the appearance of disease symptoms seems to coincide with the time of ascospore discharge as suggested by Jenkins (26).

## CHAPTER III

### MATERIALS AND METHODS

#### Survival Study

Naturally-infected peanut material, including leaves, petioles, and stems, were collected from growing plants in the field late in the growing season. Leaflets were separated from petioles and the stems were cut into 5-6 cm segments. Leafspot material was placed between cardboard paper in a plant press for a week.

Nylon netting (100 mesh/cm<sup>2</sup>) bags were made by cutting pieces measuring approximately 40 X 30 cm, each piece was folded, the two loose parallel sides were attached with masking tape and secured with staples to make a bag measuring 20 X 15 centimeters.

Six leaflets, two stem segments and two petioles (Figure 1), all with well-developed lesions were selected from the stock peanut tissue stored at room temperature, and spread flat in each bag. The bags were then closed, taped, and stapled.

The study was initiated in the field at the onset of cold weather by mid-December. The first year study (1977-78) was carried out at three different locations in Oklahoma: a field usually planted to peanuts at the Agronomy Research Station (Perkins), at a site at the Plant Pathology Farm (Stillwater) where peanuts had not been grown, and at a site of unknown history at the Caddo Peanut Research Station (Fort Cobb).

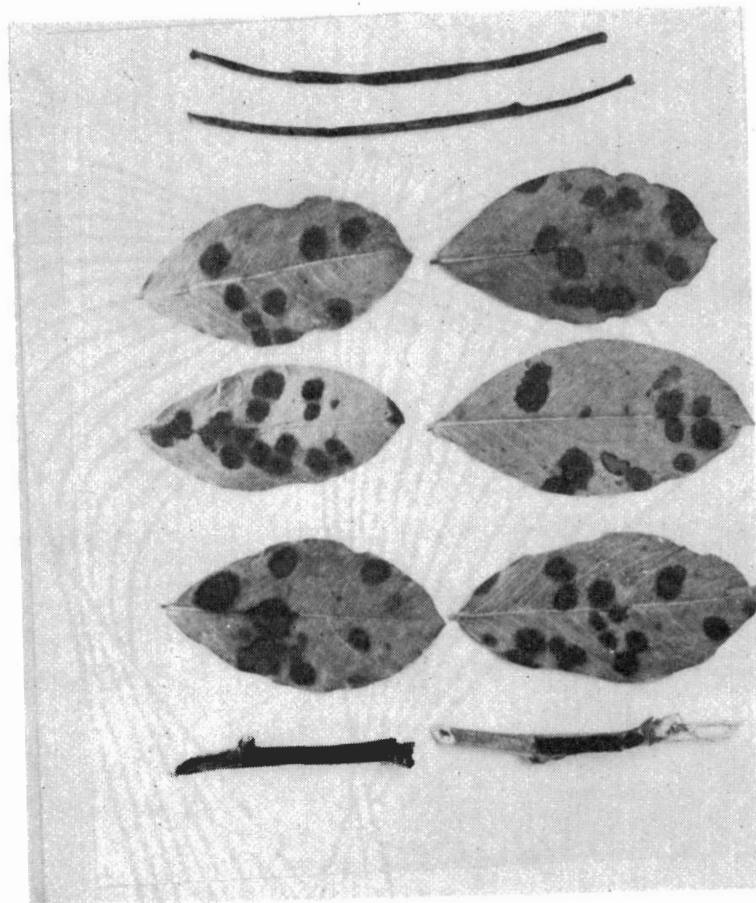


Figure 1. A Sample of *Cercospora arachidicola*-Infected Peanut Tissue used in the Overwintering Study. Each Sample Contained Six Leaflets, Two Petioles, and Two Stem Segments.

The study was repeated the following year (1978-79) at the first two locations at Perkins and Stillwater only. At each location, the ground was tilled with a rototiller to pulverize the soil and remove the weeds and stubble. The ground was then levelled. Samples to be placed on the soil surface were laid down about 40 cm apart, sprinkled with a thin layer of soil, and covered with a wire screen kept in place by wooden stakes or soil to keep the wind from blowing the samples away. For below soil surface burial, a ditch of the required depth was dug with the help of a shovel and hoe, the bed was levelled until a uniform depth was obtained. The bags were laid flat about 40 cm apart on the bottom of the ditch covered with soil and slightly compacted to insure good contact and exclude air pockets. Bouyoucos gypsum blocks (CEL-WFD) to monitor available moisture and soil temperature probes to sense surrounding temperature were buried similarly with the temperature recorders (Tempscribe, Bacharach Instrument Company; Dickson Minicorder, Dickson Company; and Belfort Instrument Company) being kept in a weather box. Temperature was recorded daily on a chart that ran for a week. Moisture data were taken each week on the day the weekly temperature charts were to be changed. A recovery schedule of samples was worked out to retrieve a certain number of bags at random from each depth at intervals of approximately two, three, four, five, and six months. Samples at Fort Cobb were all recovered at one time after about five months exposure to field conditions. Recovered samples were stored in paper bags in a cold room maintained at an average temperature of 1.7-4.4 C.

### Processing Samples

Samples to be processed were washed under running tap water for 15-20 minutes. The contents of each bag were transferred to a clean, ten-centimeter plastic pot with no drainage holes. A double thickness nylon netting (100 mesh/cm<sup>2</sup>) was fitted on the top of each pot with a thick rubber band, and a stream of tap water was left to run in each pot for approximately 10-15 minutes with occasional decanting through the netting to remove soil particles sedimenting to the bottom. When no more soil particles were visible, the peanut tissue was surface sterilized by dipping in 10% clorox (5.25% sodium hypochlorite) solution for 2-3 minutes. After rinsing thoroughly with distilled water, the plant material was partially dried on filter or towel paper before plating onto 2% water agar. The plates were incubated under fluorescent lights at room temperature. Three to four days later, lesions on incubated, infected peanut tissue were examined for Cercospora or Cercosporidium conidia using a stereoscope, and sporulation was rated on the basis of the number of spore-producing stromata. Isolations were made by transferring conidia with an alcohol-sterilized dental root canal file onto peanut yeast dextrose agar (PYDA). Inoculated plates were incubated at room temperature under continuous fluorescent light (Westinghouse Cool White, 40-watt tubes). The lights were located 30 cm above a laboratory shelf where inoculated plates were placed.

### Pathogenicity Testing

To test if the early leafspot fungus was still virulent after going through an overwintering period, representative C. arachidicola isolates from overwintering samples buried in the field at 0, 5, 10,

and 20 cm depths at Perkins, Stillwater and Fort Cobb were tested for pathogenicity. For this purpose, inocula were obtained from 10-14 day-old cultures in a similar manner to that used by Abdou (1), and Smith (59). One to two drops of Amway all-purpose adjuvant were added per 100 ml of sterile distilled water, then each culture plate was flooded with approximately 20 ml of the sterile distilled water and adjuvant. Conidia were knocked loose with the help of a camel hair brush passed gently over the entire culture. The resultant conidial suspension was adjusted to a concentration of approximately 20,000 conidia per ml. Inocula were applied by: a) dipping a camel hair brush into the conidial suspension, and then brushing detached peanut leaflets (Tamnut 74 Cultivar) placed on moist filter paper in plastic or glass plates; b) the spore suspension was applied onto the surface of detached peanut leaflets using a hand atomizer. Four leaflets per plate were used and each treatment was duplicated. Leaflets in control plates were brushed or sprayed with sterile water containing all-purpose adjuvant at the rate of 2-3 drops per 100 ml. The plates were incubated under fluorescent lights for 10-14 days. Every 2-3 days, a small amount of distilled water was added to each plate to moisten the filter paper (two sheets), and prevent the leaflets from drying. Based on symptom expression, the leaflets were visually evaluated two weeks after treatment for infectivity of the tested C. arachidicola isolates.

#### Infection Study

This study was designed to gain information on how, when, and where the primary infection of peanut occurs. To simulate natural conditions in the field, the following possibilities were considered:



1) could peanuts be infected prior to emergence by overwintering inoculum in association with buried peanut debris or by inoculum surviving freely in the soil, 2) does infection occur after emergence by inoculum splashed from infested soil or infected debris onto the foliage of the plant.

For this purpose, plant material, consisting of leaves, petioles, and stems, were heaped into a pile and left to overwinter on location. During May, samples of peanut tissue were collected from the outside, middle, and inside of the pile and stored separately in plastic bags at an average temperature of 1.7-4.4 C in a cold room. Other sources of potential inocula included: a) field soil to a depth of about five cm was sampled from areas around plants showing heavy leafspot infection late in the growing season of 1979. The soil was stored in plastic bags after removal of plant debris and kept in a cold room; b) peanut debris mainly of fallen leaves that were collected from the field after harvest and stored in the cold room before it had a chance to overwinter in the field; c) infected peanut tissue collected from growing plants in the field and stored dry at room temperature; d) spore suspensions of a two-week, well-sporulating culture of C. arachidicola.

The infectivity associated with diseased peanut tissue, whether overwintered, non-overwintered debris, or dried tissue, was tested by: 1) applying a one cm thick layer of fragmented peanut tissue on top of sterilized soil mix composed of one part field soil, two parts builder's sand, and one part perlite in 11.6 cm plastic pots. Four peanut seeds per pot were planted directly or germinated for 48-72 hours between wet towel paper in a plastic bag at room temperature and then planted; 2) infected peanut tissue was coarsely ground in a Waring blender for

14-30 seconds, and then incorporated in the top 2-3 cm of the sterilized soil mix in the pot. Peanut seeds were sowed directly or germinated prior to planting as in (1). Pots were set in a polyethylene chamber illuminated by a bank of fluorescent lights (40-watt, Westinghouse Cool White tubes). The temperature inside the chamber ranged between 23.9-29.4 C. Plastic trays were used to provide subterranean watering to each pot. Additional humidity was provided by a regular household humidifier operated for 2-3 hours every other day or as needed to maintain relative humidity at 95-98% level.

To study the possibility that infection by Cercospora occurs after emergence of the plants, the same experimental set up as for pre-emergence infection was adopted except for the manner in which the plants were watered. In this case, a jet of water was directed towards the soil surface or peanut debris to spatter potential inoculum onto the foliage.

Since there is no convincing evidence that Cercospora conidia can survive in the soil independent of peanut debris, an experiment was carried out to see if a pre-emergence or post-emergence infection of peanuts could be induced by applying a conidial suspension to the soil. Inocula for the conidial suspension were made by either transferring an agar block approximately one cm<sup>2</sup> from a 15-day old, well-sporulating culture of C. arachidicola to a peanut yeast dextrose agar (PYDA) or peanut oatmeal agar (POA) plates previously flooded with one ml sterile distilled H<sub>2</sub>O, and the plate was gently swirled around to insure almost a uniform distribution of the conidia on the underside of the agar block, or the agar block was first transferred aseptically to a test tube containing five ml of sterile distilled H<sub>2</sub>O to which Amway all-

purpose adjuvant at the rate of 1-2 drops per 100 ml had been added, and after shaking for 1-2 minutes, approximately one ml of the conidial suspension was poured onto PYDA or POA plates and swirled around to insure a uniform distribution of the suspension. Inoculated plates were incubated at room temperature under fluorescent lights for 14-21 days. At the end of the incubation period, each plate was flooded with enough sterile distilled H<sub>2</sub>O plus adjuvant (1-2 drops/100 ml) to cover the surface. Conidia were brushed gently with a camel hair brush and the suspension was transferred to a glass beaker and the conidia concentration adjusted to approximately  $30 \times 10^3$  to  $40 \times 10^3$  spores/ml. To infest the soil, 50 ml of spore suspension were added in the vicinity of planted (germinated or sown directly) peanut seeds in 11.6 cm plastic pots. Four seeds were planted per pot. Each treatment was replicated at least ten times and the pots were positioned in the chamber in a completely randomized design. Subterranean watering to insure no splashing was used in the pre-emergence infection study, while in the post-emergence version, watering was accomplished by directing a stream of water to the soil surface or infected peanut material to increase chances of spore splashing onto peanut foliage.

Attempts to recover Cercospora or Cercosporidium spores from the soil were made using a flotation technique developed by Ledingham and Chinn for isolating spores of Helminthosporium sativum (34). Soil samples from the field and portions of the soil mix in pots inoculated with known concentration of C. arachidicola spore suspension were tested for the presence of spores using the flotation method.

Another attempt to test for the presence of viable leafspot inoculum in infested soil was made. Samples of "infested" soil and soil

mix artificially infested with C. arachidicola were washed with distilled water. Elutriates were recovered and left to stand for 20-30 minutes. The supernatant was then sprayed onto four one-month old peanut plants per pot and each treatment was replicated three times. Plants were kept in a polyethylene chamber and were evaluated for leaf-spot development one month after application of treatments.

Search for the Perfect Stages: M. arachidis  
and M. berkeleyii

Jenkins (26) reported the presence in Georgia of the perfect stages of M. arachidicola (M. arachidis) and M. berkeleyii on infected peanut tissue in the field no earlier than May, however, he stated that infected leaf tissue collected in the field in late February-March and incubated under inverted plates led to the recovery of almost pure cultures of Cercospora spp. due to ascospores being shot from perithecia embedded in the peanut leaf tissue.

To investigate the possible existence of the Mycosphaerella stage in Oklahoma, a study was initiated early in the spring (March 1979) at a site where mature peanut plants were not harvested or turned under, and the whole peanut plot was left undisturbed to overwinter under natural conditions. The search involved: a) setting wind-vane type spore traps with vaseline (petroleum-jelly) or silicone-coated slides, the traps were set 30 cm high from the ground. The rationale was that if the perfect stage was present and is the source of primary inoculum, then it would be expected that ascospores would be shot out from mature perithecia, and blown around in air currents. The slides were exposed for 48 hours, after which the slides from five spore traps in the field

were collected, brought into the laboratory, and examined microscopically using a standard fungal stain. b) In conjunction with the spore traps, 6-8 week old, healthy peanut plants grown in 22.9 cm pots in the greenhouse were taken outside for 48 hours to be hardened, and then exposed in the open field so that one pot was located next to each spore trap in the field. Exposure continued for two weeks, after which plants were incubated inside a polyethylene chamber in the greenhouse where relative humidity was kept at approximately 90-95% and temperature ranged between 35-37.8 C. c) Deep-bottomed glass plates containing PYDA were inverted on overwintered peanut debris laying on the ground and kept moist during the period of investigation (March 1979-May 1979) by frequent sprinkling with water. The plates were shielded from direct sunlight, wind and rain by inverted plastic Dow cups (11.4 X 14.6 cm) supported by wooden stakes to allow about 1-2 cm clearance above surface debris to provide for ventilation. The plates were exposed for 48 hours, then collected and examined under the microscope. d) Overwintered samples from the survival study at Stillwater and Perkins were screened for the presence of the perfect stage by incubating washed, surface-sterilized, infected peanut tissue under inverted deep-bottomed glass plates containing PYDA or PDA.

Infected peanut debris was monitored again for the presence of the Mycosphaerella stage in a similar manner to that followed by Jenkins (26). Infected peanut foliage remaining on the ground after harvest was raked into a 1.5 X 1.0 m plot on November 21, 1979, in a field west of Stillwater, and left to overwinter on location. Samples of the overwintering debris were recovered in February and March. The debris was washed thoroughly under running tap water and incubated below inverted

deep-bottomed petri plates containing PYDA. Sampling recovery continued through May 1980 and samples were processed as outlined earlier.

### Spore Trapping

The dissemination and dispersal of a pathogen are important factors for understanding the epidemiology of the disease, therefore, it was necessary to investigate the horizontal movement of Cercospora and Cercosporidium conidia within the peanut field as well as determining conidiospore densities in the air by spore trapping.

The first study was started in June 1979 where three spore traps of the wind-vane type equipped with vaseline or silicone-coated slides were set at a height of 30.0 cm in the peanut plots at the Agronomy Research Station in Perkins, Oklahoma. Three more traps of the same type were also set at another peanut field off SH51, three miles west of Stillwater. The vaseline or silicone-coated slides were exposed for 24-48 hours, depending on weather conditions. After exposure, the slides were either examined directly under the microscope using a standard laboratory stain, or the slides were incubated first in a moist chamber for 12-24 hours to induce germination of the Cercospora spores which made their identification much easier. A stain (lactophenol + methyl blue) was then used and the slides were examined. The number of leafspot conidia in the coated area of the slides (approximately 10 X 22 mm) was recorded for the particular exposure period. Spore monitoring continued for two months until infection of peanuts in the field was well established.

During the growing season 1980, a Kramer-Collins 7-Day Drum Spore Sampler (32) was used to sample the air in an irrigated peanut field at

the Agronomy Research Station in Perkins at the rate of 20 liters per minute once each hour. The trapping surface consisted of a 12.7 mm wide cellophane tape coated with a thin film of vaseline on the exposed surface. The trap was set in the field with the intake orifice 50 cm high from the soil surface. By the end of a week exposure cycle, the drum was replaced by another having a fresh cellophane tape. The exposed tape was cut into 60 mm (24-hour) lengths, and transferred to separate glass microscope slides. Fifteen millimeter portions were marked on the tape with each portion representing an interval of six hours. A few droplets of a mounting medium (lacto-phenol + methyl blue) and cover slips were added to the tape which was then checked for Cercospora and Cercosporidium spores under the microscope using a 200X magnification.

The study started on June 27, 1980, and lasted for 16 weeks until October 16, when the spore trap was dismantled.

## CHAPTER IV

### RESULTS

#### Survival

Overwintering samples were recovered from the field after exposure for various lengths of time. The samples were processed as outlined in the chapter on methods and materials.

Although a schedule to recover the samples from the Agronomy Farm in Perkins and the Plant Pathology Farm in Stillwater was worked out, it was not always feasible to abide by the schedule because of either weather or soil conditions. The soil sometimes was too hard to dig because of freezing or too wet because of rain. With the short exposure periods, most of the buried peanut tissue was still intact, and could be handled with relative ease in contrast to the longer exposure periods where the buried peanut tissue was badly decayed, especially in the case of leaflets where only the necrotic lesions could sometimes be found. Samples at the Caddo Peanut Research Station at Fort Cobb were all recovered at one time after 20 weeks of field exposure.

In the early stages of the investigation, the recovered plant tissue was surface-sterilized for approximately 20-30 sec in 10% clorox, but this resulted in too much contamination which made it difficult to evaluate sporulation of Cercospora and/or Cercosporidium spp. The major contaminants frequently encountered included such fungi as:



Alternaria spp., Colletotrichum spp., Ascochyta spp., Leptosphaerulina spp., and a few other unidentified fungal genera. To reduce the contamination problem, peanut tissue was dipped in 10% clorox for 2-3 minutes.

Although stem segments and petioles withstood degradation processes better than leaflets, soil contaminants including mites, nematodes and fungi was greater.

There were always problems associated with soil temperature and moisture recordings due to failure of the recording equipment. Soil temperature was monitored through a probe buried at either of the designated depths 5, 10, and 20 cm. Recordings of the temperature at these depths were charted on thermograph paper. In spite of the equipment being initially calibrated prior to installation in the field, recalibration was frequently required. Many times the equipment simply did not operate, leading to loss of data for the corresponding period of malfunction. Similarly, problems were encountered with soil moisture readings monitored through gypsum moisture blocks buried at different depths in the soil.

Upon incubation of surface-sterilized overwintering infected peanut tissue, sporulation took place in 2-3 days (Figure 2). If an observation was made any later than that, the sporulating stromata would have been over-grown by fast-growing fungi, e.g. Alternaria spp., which made evaluation of sporulation based on the number of sporulating stromata a very difficult task.

The results of the overwintering studies which were carried out from December 1977 to June 1978 at Perkins, and from January and February 1978 to July 1978 at Stillwater and again from December 1978 until June 1979 at both locations are summarized in Table I for 0, 5, 10, and

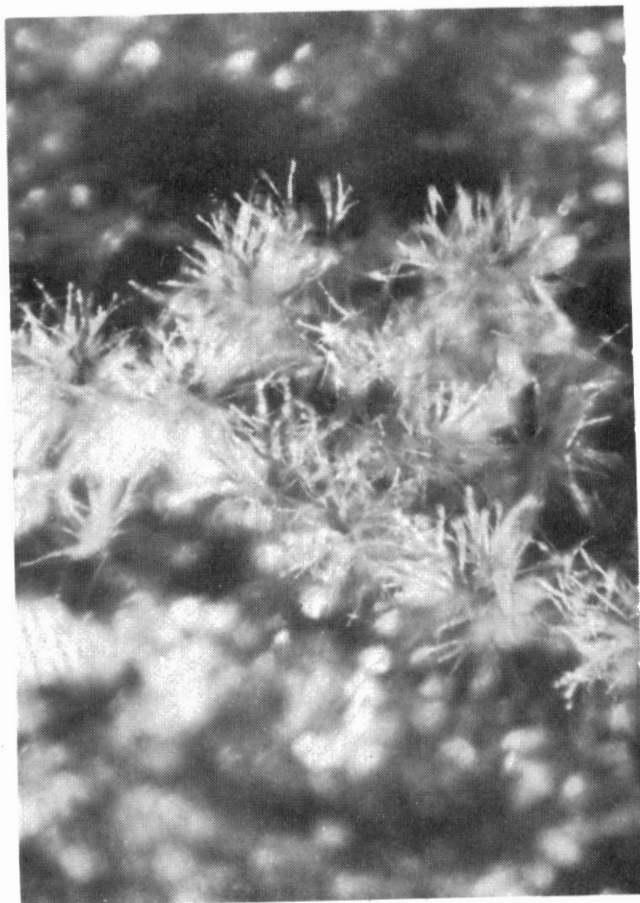


Figure 2. Sporulating Stromata of Cercospora arachidicola in a Leaflet Lesion from a Sample Buried at 10 cm Depth for 92 Days. 50X

TABLE I  
 SPORULATION OF OVERWINTERING CERCOSPORA ARACHIDICOLA-  
 INFECTED PEANUT DEBRIS EXPOSED TO FIELD<sup>a/</sup>  
 AND COLD ROOM CONDITIONS

Date of Study	Exposure Period (weeks)		Average Field Temperature C		Average Rainfall (cm)	Degree of Sporulation <sup>b/</sup>		
	Field	Cold Room	Minimum	Maximum		Leaflet	Petiole	Stem
	Burial Depth: 0 cm							
December '77 - June '78	12.7	2.7	-6.5	-0.4	8.4	1		2
	"	3.7	"	"	"	0		0
	"	5.1	"	"	"	2		3
	18.3	3.0	-2.2	7.0	14.5	2		2
	"	3.0	"	"	"	1		2
	"	4.7	"	"	"	2		1
	21.4	3.4	-0.9	9.0	21.1	0		0
	"	8.0	"	"	"	0		0
	"	9.3	"	"	"	2		4
	25.4	4.8	2.0	11.8	37.1	1		1
	"	9.7	"	"	"	0		2
	"	13.1	"	"	"	0		1
December '78 - June '79	13.4	4.1	-6.5	5.4	3.3	2	0	0
	"	5.6	" "	"	"	3	3	0
	"	26.7	"	"	"	4	0	0
	18.3	7.8	-3.2	9.0	8.8	4	0	0
	"	8.6	"	"	"	0	0	0
	"	20.0	"	"	"	1	0	0
	23.7	11.7	-0.4	12.0	16.1	2	2	0
	"	13.3	"	"	"	0	2	2
	"	13.6	"	"	"	1	3	4
	26.3	8.6	1.0	13.5	19.9	3	0	0
	"	12.6	"	"	"	0	1	2
	"	13.1	"	"	"	4	4	4

TABLE I (Continued)

Date of Study	Burial Depth: 5 cm							
	Exposure Period (weeks)		Average Field Temperature C		% Average Soil Moisture	Degree of Sporulation <sup>b/</sup>		
	Field	Cold Room	Minimum	Maximum		Leaflet	Petiole	Stem
December '77 - June '78	13.0	2.7	-1.1	3.7	71.1	2		2
	"	3.7	"	"	"	0		0
	"	5.1	"	"	"	3		3
	18.7	3.0	2.3	10.8	79.1	3		2
	"	3.0	"	"	"	2		4
	"	4.7	"	"	"	3		0
	21.8	3.4	3.8	12.9	79.7	1		2
	"	8.0	"	"	"	0		1
	"	9.3	"	"	"	4		4
	25.8	4.8	7.0	16.2	82.9	c/		c/
"	9.7	"	"	"	1		4	
"	13.1	"	"	"	2		0	
December '78 - June '79	13.4	4.1	1.0	4.4	68.9	3	3	0
	"	5.6	"	"	"	4	2	0
	"	26.7	"	"	"	2	4	0
	18.3	7.8	3.3	7.8	77.4	4	0	1
	"	8.6	"	"	"	3	0	4
	"	20.0	"	"	"	2	4	3
	23.7	11.7	6.1	11.1	78.6	2	0	4
	"	13.3	"	"	"	0	3	4
	"	13.6	"	"	"	1	4	4
	26.3	8.6	7.5	12.5	80.4	1	2	4
	"	12.6	"	"	"	1	4	4
	"	13.1	"	"	"	0	0	4

TABLE I (Continued)

Date of Study	Exposure Period (weeks)		Average Field Temperature C		% Average Soil Moisture	Degree of Sporulation <sup>b/</sup>		
	Field	Cold Room	Minimum	Maximum		Leaflet	Petiole	Stem
					Burial Depth: 10 cm			
December '77 - June '78	13.0	2.7	2.7	4.6	77.3	3		4
	"	3.7	"	"	"	0		0
	"	5.1	"	"	"	2		3
	18.7	3.0	6.7	10.4	81.9	2		3
	"	3.0	"	"	"	2		3
	"	4.7	"	"	"	2		3
	21.8	3.4	8.2	12.2	82.5	1		3
	"	8.0	"	"	"	c/		2
	"	9.3	"	"	"	3		4
	25.8	4.8	9.4	14.4	84.5	1		4
	"	9.7	"	"	"	2		3
	"	13.1	"	"	"	3		2
December '78 - June '79	13.4	4.1	-0.3	2.8	69.5	3	0	0
	"	5.6	"	"	"	3	4	3
	"	26.7	"	"	"	4	2	0
	18.3	7.8	2.1	6.2	78.0	1	4	4
	"	8.6	"	"	"	2	4	2
	"	20.0	"	"	"	2	4	0
	23.7	11.7	4.9	9.3	82.7	0	1	3
	"	13.3	"	"	"	0	3	4
	"	13.6	"	"	"	0	0	1
	26.3	8.6	6.4	10.9	84.1	1	0	2
	"	12.6	"	"	"	1	4	4
	"	13.1	"	"	"	1	d/	d/

TABLE I (Continued)

Date of Study	Exposure Period (weeks)		Average Field Temperature C		% Average Soil Moisture	Degree of Sporulation <sup>b/</sup>		
	Field	Cold Room	Minimum	Maximum		Leaflet	Petiole	Stem
December '77 - June '78	12.7	2.7	-2.6	-0.9	86.8	2		2
	"	3.7	"	"	"	0		0
	"	5.1	"	"	"	3		3
	18.2	3.0	2.2	5.1	91.5	2		2
	"	3.0	"	"	"	2		3
	"	4.7	"	"	"	3		2
	21.4	3.4	4.4	7.5	92.5	1		4
	"	8.0	"	"	"	1		0
	"	9.3	"	"	"	4		3
	25.4	2.8	7.4	10.6	92.7	0		4
"	7.7	"	"	"	2		4	
"	11.1	"	"	"	2		3	
December '78 - June '79	13.4	4.1	-0.7	1.7	68.0	0	0	4
	"	5.6	"	"	"	3	1	0
	"	26.7	"	"	"	4	2	3
	18.3	7.8	1.8	4.7	76.9	1	0	4
	"	8.6	"	"	"	4	3	c/
	"	20.0	"	"	"	4	3	2
	23.7	11.7	4.7	7.7	81.9	0	0	4
	"	13.3	"	"	"	1	4	1
	"	13.6	"	"	"	2	4	4
	26.3	8.6	6.0	9.1	83.4	1	4	4
"	12.6	"	"	"	3	3	3	
"	13.1	"	"	"	0	d/	d/	

<sup>a/</sup> Location: Agronomy Research Station, Perkins, Oklahoma

<sup>b/</sup> 0 - no sporulating stromata  
 1 - 1 to 5 sporulating stromata  
 2 - 6 to 10 sporulating stromata  
 3 - 11 to 15 sporulating stromata  
 4 - more than 15 sporulating stromata

<sup>c/</sup> contaminated sample

<sup>d/</sup> no tissue was recovered

20 cm depths in Perkins, and Table II for 0, 5, 10, and 20 cm in Stillwater. Table III summarizes the data in Tables I and II, but instead of degree of sporulation, the number of leaflet, petiole, or stem segment samples yielding sporulating stromata are presented as percentages. Graphic presentation of percentage of samples with leaflets, petioles or stem segments bearing sporulating stromata at Perkins and Stillwater is shown in Figure 3. The degree of sporulation of leaflet, petiole, and stem segment from overwintering peanut tissue buried at the Agronomy Research Station in Perkins during the 1978 and 1979 studies are graphically shown in Figures 4 and 5.

Since all of the samples were stored in the cold room at a temperature of 1.7-4.4 C until processed, the storage period in the cold room is indicated as part of the exposure period. The average maximum and minimum temperatures during the exposure interval in the field, as well as the average cumulative rainfall for the 0 cm depth, and the average soil moisture content for 5, 10, and 20 cm depths, as measured by gypsum moisture blocks, are also shown.

The data showed the potential for C. arachidicola to survive is great and seems to be independent of the exposure period (time), temperature, moisture, or depth. Although some C. personatum-infected peanut tissue was included with early leafspot (C. arachidicola)-infected material in the 1978-79 survival study at Perkins and Stillwater, no sporulating stromata characteristic of C. personatum were observed and, not a single C. personatum culture was isolated. Cercospora arachidicola survived at all tested depths of 0, 5, 10, and 20 cm. The only observable effect that could be attributed to depth was that of the degree of tissue decay as a result of microbial activity

TABLE II  
 SPORULATION OF OVERWINTERING CERCOSPORA ARACHIDICOLA-INFECTED PEANUT DEBRIS EXPOSED TO  
 FIELD<sup>a/</sup> AND COLD ROOM CONDITIONS

Date of Study	Burial Depth: 0 cm							
	Exposure Period (weeks)		Average Field Temperature C		Average Rainfall (cm)	Degree of Sporulation <sup>b/</sup>		
	Field	Cold Room	Minimum	Maximum		Leaflet	Petiole	Stem
Feb '78-July '78	11.4	1.7	1.7	14.4	12.9	3	0	0
	11.4	3.6	1.7	14.4	12.9	0	3	2
	27.8	5.7	8.3	19.7	39.7	3	2	3
	27.8	13.1	8.3	19.7	39.7	3	3	4
	27.8	30.1	8.3	19.7	39.7	1	0	1
	27.8	81.4	8.3	19.7	39.7	2	4	4
	Dec. '78 - June '79	2.4	31.4	0.8	14.5	0.6	4	3
8.1		31.0	5.4	18.2	13.8	4	2	c/
13.3		1.0	-6.2	6.0	6.8	3	0	2
13.3		29.0	-6.2	6.0	6.8	0	1	0
15.4		20.7	9.1	21.6	39.5	4	4	4
19.0		6.0	-2.2	10.1	20.0	0	3	0
19.0		29.4	-2.2	10.1	20.0	4	0	4
26.3		18.8	2.1	14.8	45.7	1	0	0
26.3		19.6	2.1	14.8	45.7	0	3	4



TABLE II (Continued)

Date of Study	Burial Depth: 5 cm							
	Exposure Period (weeks)		Average Field Temperature C		% Average Soil Moisture	Degree of Sporulation <sup>b/</sup>		
	Field	Cold Room	Minimum	Maximum		Leaflet	Petiole	Stem
Jan '78-July '78	14.3	1.7	4.2	13.1	62.9	0	4	1
	14.3	3.6	4.2	13.1	62.9	3	4	1
	32.1	13.1	7.2	16.3	76.0	2	0	0
	32.1	30.1	7.2	16.3	76.0	2	1	2
	32.1	81.4	7.2	16.3	76.0	3	0	0
	32.1	81.4	7.2	16.3	76.0	4	0	4
Dec '78-June '79	2.4	36.7	9.6	11.6	91.7	4	4	c/
	8.1	31.4	11.7	14.7	92.0	4	4	4
	13.1	1.0	-0.4	2.7	33.7	4	2	0
	13.1	29.0	-0.4	2.7	33.7	4	4	0
	15.3	20.7	17.1	20.4	88.6	4	3	4
	18.8	6.0	3.6	6.7	52.1	4	0	2
	18.8	29.4	3.6	6.7	52.1	4	4	4
	26.0	18.8	10.4	12.2	57.2	3	4	0
	26.0	19.6	10.4	12.2	57.2	2	4	3

TABLE II (Continued)

Date of Study	Burial Depth: 10 cm							
	Exposure Period (weeks)		Average Field Temperature C		% Average Soil Moisture	Degree of Sporulation <sup>b/</sup>		
	Field	Cold Room	Minimum	Maximum		Leaflet	Petiole	Stem
Jan '78-July '78	14.3	1.7	10.1	12.3	79.0	1	0	4
	14.3	3.6	10.1	12.3	79.0	1	0	1
	32.1	5.7	11.7	14.3	87.0	2	3	3
	32.1	13.1	11.7	14.3	87.0	4	4	3
	32.1	17.1	11.7	14.3	87.0	1	2	2
	32.1	30.1	11.7	14.3	87.0	0	4	1
Dec '78-June '79	2.4	31.4	4.2	5.5	97.7	4	4	3
	8.1	31.0	15.4	16.7	96.9	4	4	3
	13.1	1.0	1.3	1.8	45.3	4	0	3
	13.1	29.0	1.3	1.8	45.3	0	4	0
	15.3	20.7	15.2	16.8	90.3	4	4	0
	18.8	6.0	6.8	7.8	61.5	4	0	0
	18.8	29.4	6.8	7.8	61.5	2	4	0
	26.0	18.8	9.1	18.3	65.0	2	4	0
26.0	19.6	9.1	18.3	65.0	0	4	2	

TABLE II (Continued)

Date of Study	Exposure Period (weeks)		Average Field Temperature C		% Average Soil Moisture	Degree of Sporulation <sup>b/</sup>		
	Field	Cold Room	Minimum	Maximum		Leaflet	Petiole	Stem
Jan '78-July '78	14.3	1.7	9.6	12.1	81.8	3	2	0
	14.3	3.6	9.6	12.1	81.8	2	4	4
	34.8	5.7	10.2	12.6	89.8	2	2	3
	34.8	13.1	10.2	12.6	89.8	3	2	3
	34.8	17.1	10.2	12.6	89.8	0	1	0
	34.8	81.4	10.2	12.6	89.8	1	0	4
Dec. '78-June '79	2.4	36.7	3.6	5.0	93	4	c/	c/
	8.1	31.4	6.5	7.8	97.3	3	4	4
	13.1	1.0	-0.5	0.8	42.0	3	1	0
	13.1	29.0	-0.5	0.8	42.0	0	0	3
	15.3	19.6	8.4	9.6	96.8	4	4	4
	18.8	6.0	2.6	3.9	60.1	2	4	0
	18.8	29.4	2.6	3.9	60.1	4	4	4
	26.0	18.8	5.1	6.2	67.9	1	4	0
26.0	20.7	5.1	6.2	67.9	4	0	4	

<sup>a/</sup> Location: Plant Pathology Farm, Stillwater, Oklahoma.

<sup>b/</sup> 0 - no sporulating stromata  
 1 - 1 to 5 sporulating stromata  
 2 - 6 to 10 sporulating stromata  
 3 - 11 to 15 sporulating stromata  
 4 - more than 15 sporulating stromata

<sup>c/</sup> contaminated sample

TABLE III

NUMBER OF OVERWINTERING PEANUT TISSUE SAMPLES WITH  
 SPORULATING CERCOSPORA ARACHIDICOLA STROMATA  
 OUT OF TOTAL SAMPLES BURIED AT DIFFERENT  
 DEPTHS IN THE SOIL AT PERKINS  
 AND STILLWATER

Depth (cm)	Perkins (Dec. '77-June '78)		Perkins (Dec. '78-June '79)		
	Leaflets	Stem	Leaflets	Petioles	Stem
0	7/12	9/12	9/12	6/12	4/12
5	9/12	8/12	10/12	8/12	9/12
10	10/12	11/12	9/12	8/12	8/12
20	10/12	10/12	9/12	8/12	9/12
Total	36/48	38/48	37/48	30/48	30/48
%	75.0	79.2	77.1	62.5	62.5

TABLE III (Continued)

Depth (cm)	Stillwater (Feb. 1978-July 1978)			Stillwater (Dec. 1978-June 1979)		
	Leaflets	Petioles	Stem	Leaflets	Petioles	Stem
0	5/6	4/6	5/6	6/9	6/9	5/9
5	5/6	3/6	4/6	9/9	8/9	5/9
10	5/6	4/6	6/6	7/9	7/9	4/9
20	5/6	5/6	4/6	8/9	6/9	5/9
Total	20/24	16/24	19/24	30/36	27/36	19/36
%	83.3	66.7	79.2	83.3	75.0	52.0

a/ Numerator - number of samples yielding C. arachidicola sporulating stromata.

b/ Denominator - number of samples buried per depth.

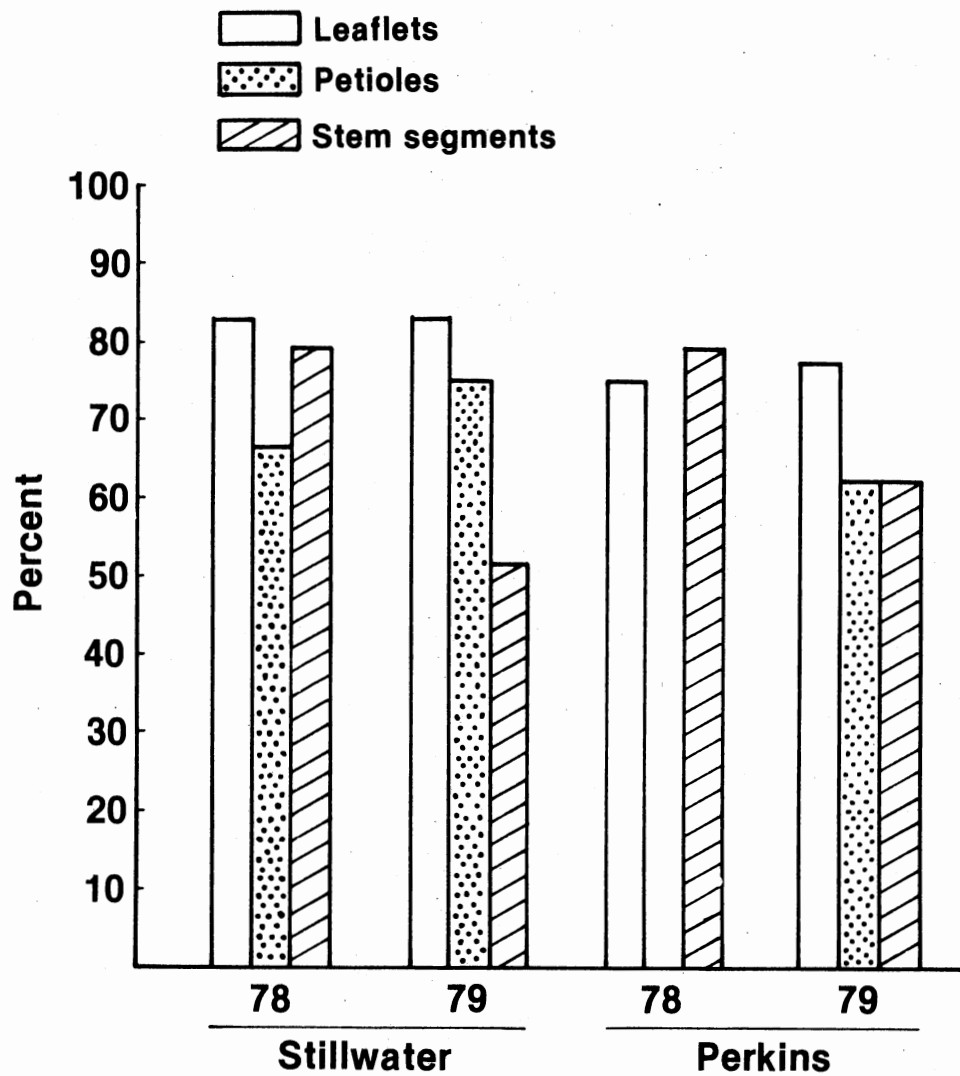


Figure 3. Percentage of Buried Samples with Leaflets, Petioles or Stem Segments Bearing Sporulating Stromata of *Cercospora arachidicola*.

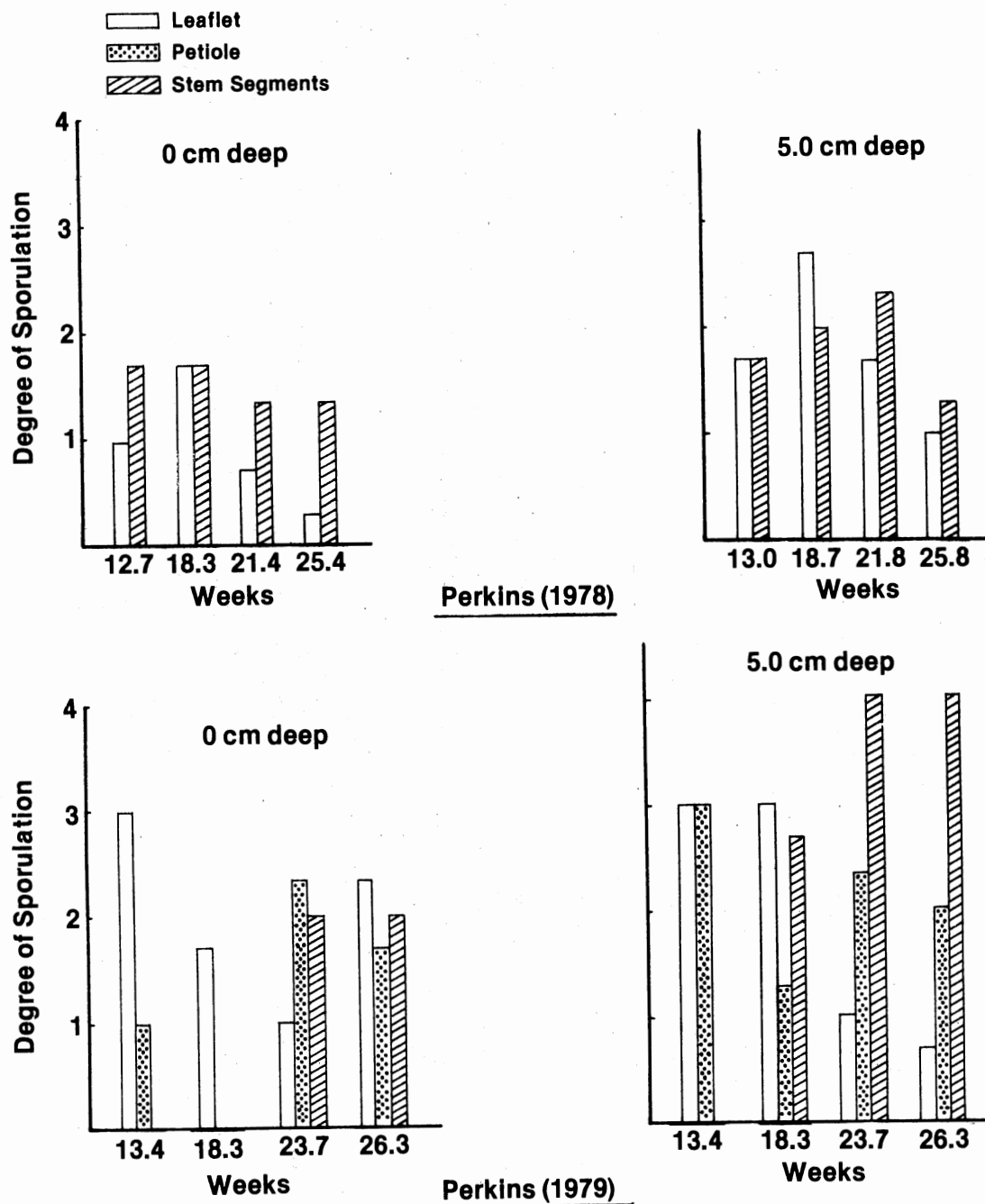


Figure 4. Sporulation of Overwintering *Cercospora arachidicola* Infected Peanut Tissue Samples Recovered from 0.0 and 5.0 cm. (Top) Leaflet and Stem Segments From Perkins Test in 1978. (Bottom) Leaflet, Petiole, and Stem Segments From Perkins Test in 1979.

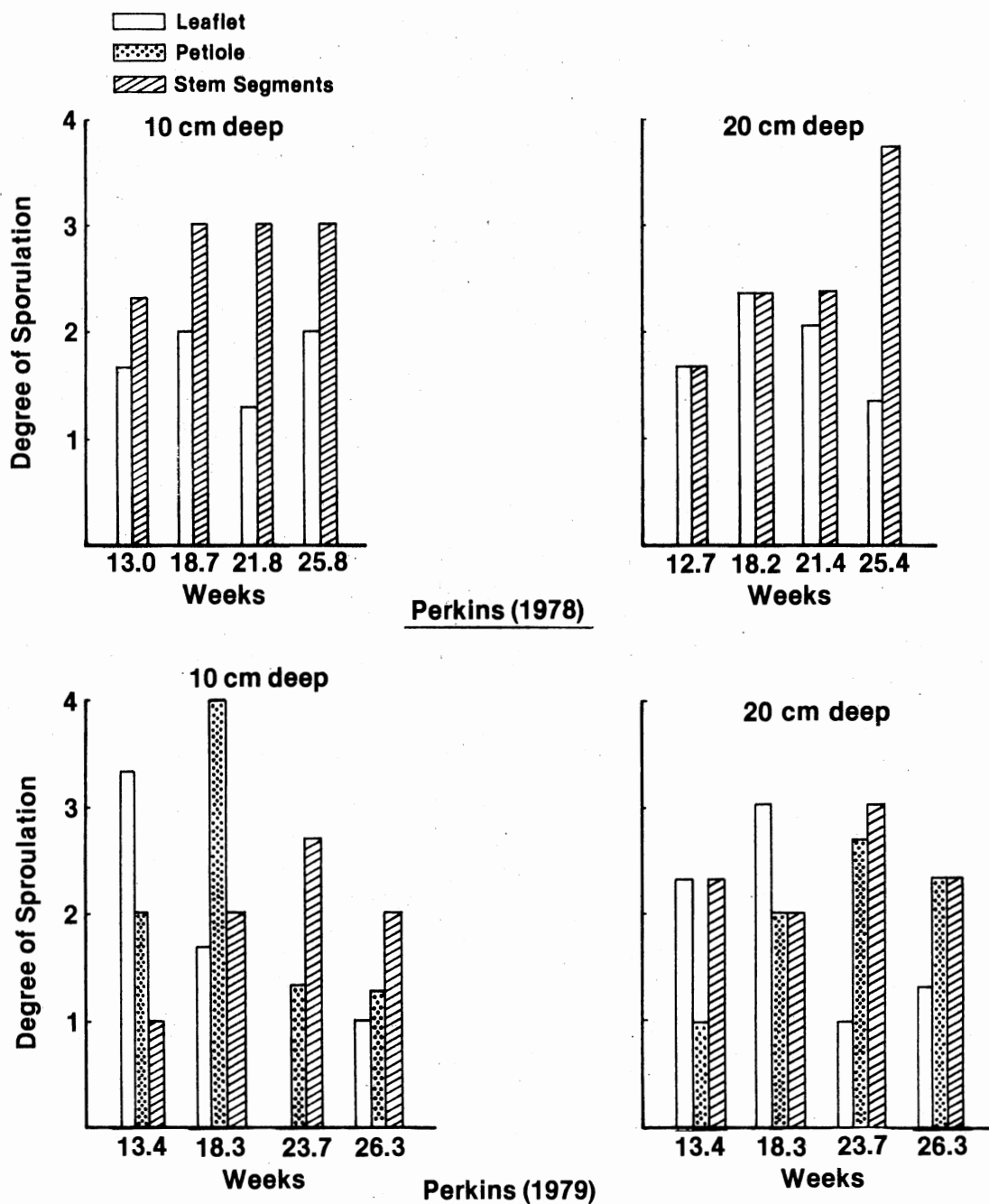


Figure 5. Sporulation of Overwintering *Cercospora arachidicola* Infected Peanut Tissue Samples Recovered From 10.0 and 20.0 cm. (Top) Leaflet and Stem Segments From Perkins Test in 1978. (Bottom) Leaflet, Petiole and Stem Segments From Perkins Test in 1979.



which seemed to increase proportionately with depth and time. In most cases, whenever peanut tissue could be recovered, sporulating stromata developed upon incubation of overwintering tissue under suitable conditions.

Average minimum temperature was low as  $-6.5$  C, and average maximum temperature as high as  $21.6$  C during field exposure combined with exposure to cold room conditions where the temperature was constantly maintained between  $1.7$ - $4.4$  C, did not seem to have any observable effect on the sporulative potential of overwintering infected peanut tissue. Similarly, precipitation and soil moisture content did not seem to drastically affect the sporulation of overwintering Cercospora lesions.

When the number of samples (as percentages) that yielded sporulating stromata (Table III) are taken into account regardless of the sporulation ranking, overwintering (scored as presence or absence of sporulating stromata) was greater in leaflets than in petioles or stem segments in the two studies conducted at the Plant Pathology Farm in Stillwater. Out of a total of 24 samples buried at Stillwater the first year (January and February 1978 - July 1978) with six samples per depth, 20 samples had leaflets with sporulating stromata after they were incubated under suitable conditions, while only 16 samples had petioles with sporulating stromata. Also 19 samples out of 24, had stem segments that resulted in positive C. arachidicola isolations. In terms of overwintering, sporulating stromata from the 24 buried samples, 83.3 percent of leaflets sporulated as compared to 66.7 and 79.2 percent for petioles and stem segments, respectively.

In the second-year study, samples with leaflets, petioles, or stem segments bearing sporulating stromata were 83.3, 75.0, and 52.8 percent

of a total of 36 samples (nine samples/depth) buried at Stillwater (December 1978 - June 1979).

At Perkins (1977-78 study), overwintering samples with leaflets yielding C. arachidicola-sporulating stromata were slightly less than those with stem segments. Only 36 out of 48 samples had leaflets with sporulating stromata, while there were 38 samples that had stem segments with C. arachidicola-producing stromata. No petioles were included with the buried samples in the first-year study (December 1977 - June 1978) at Perkins.

Samples with leaflets having sporulating stromata were more than samples with either petioles or stem segments in the second-year study (December 1978 - June 1979) at Perkins. Sporulating stromata were observed in 77.1, 62.5, and 62.5 percent of the samples for leaflets, petioles, and stem segments, respectively.

If the number of sporulating stromata in the lesion (Table IV) is taken into account, then evaluation of survival solely on basis of percentage of samples yielding sporulating stromata becomes misleading. The degree of sporulation should be taken into account when assessing longevity of the fungus on plant debris. To illustrate the point, the number of samples with leaflets having sporulating stromata (Perkins, December 1977 - June 1978), Table III was 75%, while that for samples with stem segments having sporulating stromata (Stillwater, December 1978 - June 1979) was only 52.0% which would seem to indicate that C. arachidicola survives on leaflets at Perkins better than on stem segments buried at the Plant Pathology Farm in Stillwater. However, within those 52.0% of the samples, there are ten samples with stem segments having lesions with a degree of sporulation of four (more than 15

TABLE IV

NUMBER OF OVERWINTERING PEANUT TISSUE SAMPLES WITH SPORULATING C. ARACHIDICOLA  
STROMATA CLASSIFIED ACCORDING TO THEIR DEGREE OF SPORULATION

Depth (cm)	Degree of Sporulation <sup>a/</sup>																			
	Perkins (Dec. 1977-June 1978)								Perkins (Dec. 1978-June 1979)											
	Leaflet				Stem				Leaflet				Petiole				Stem			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
0	3	4	0	0	3	4	1	1	2	3	2	2	1	2	2	1	0	2	0	2
5	2	3	3	1	1	3	1	3	3	3	2	2	1	2	2	4	1	0	1	7
10	2	5	3	0	0	2	6	3	4	2	2	1	1	1	1	5	1	2	2	3
20	2	5	2	1	0	3	4	3	3	1	2	1	1	1	3	3	1	1	2	5
Total <sup>b/</sup>	9	17	8	2	3	12	12	10	12	8	8	9	3	6	8	12	3	5	5	17

TABLE IV (Continued)

Depth (cm)	Degree of Sporulation <sup>a/</sup>																							
	Stillwater (Feb. 1978-July 1978)												Stillwater (Dec. 1978-June 1979)											
	Leaflet				Petiole				Stem				Leaflet				Petiole				Stem			
	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
0	1	1	3	0	0	1	2	1	1	1	1	2	1	0	1	4	1	1	3	1	0	1	1	3
5	0	2	2	1	1	0	0	2	2	1	0	1	0	1	1	7	0	1	1	6	0	1	1	3
10	3	1	0	1	0	1	1	2	2	1	2	1	0	2	0	5	0	0	0	7	0	1	3	0
20	2	1	2	0	1	3	0	1	0	0	2	2	1	1	2	4	1	0	0	5	0	0	1	4
Total <sup>b/</sup>	6	5	7	2	2	5	3	6	5	3	5	6	2	4	4	20	2	2	4	19	0	3	6	10

<sup>a/</sup> I = 1 to 5 sporulating stromata  
 II = 6 to 10 sporulating stromata  
 III = 11 to 15 sporulating stromata  
 IV = more than 15 sporulating stromata

<sup>b/</sup> Number of samples (leaflet, petiole or stem) within a sporulation degree from all depths (0, 5, 10, and 20 cm).

sporulating stromata), Table IV, while there are only two samples out of 36 buried in Perkins (December 1977 - June 1978) with leaflets having a degree of sporulation of four. In order to make such comparisons easier to comprehend, an attempt to quantify the results of the survival study was made. Since the degree of sporulation was based on a scale of 0-4, where 0=no sporulation and I, II, III, and IV are equal to 1-5, 6-10, 11-15, and >15 sporulating stromata per lesion, respectively, it was decided to assign the midrange value to the corresponding degree of sporulation. Therefore, the midrange values (signifying number of sporulating stromata) 3, 8, and 13 were assigned to the sporulation ranks I, II, and III in order. Sporulation degree IV had no range, and hence no midrange value, but since it denoted more than 15 sporulating stromata, the value 16 was arbitrarily assigned to it. Instead of having ranks of sporulation, the number of samples within each rank was totaled (Table V). Then the rank total was multiplied by the midrange value giving the total number of sporulating stromata which, upon dividing by the number of samples with spore-producing stromata, gives an average value of the number of sporulating stromata per sample. Based on these values (number of sporulating stromata per lesion), it appears there is no marked difference in the overwintering of C. arachidicola at the two experimental locations (Perkins and Stillwater), nor is there a difference in the longevity of the fungus on different plant portions, namely leaflets, petioles, and stem segments.

Samples from the survival study at Fort Cobb were all recovered after exposure in the field for 20 weeks. Temperature and precipitation were not monitored at this location. Only two samples from each depth (0, 5, 10, and 20 cm) were processed. Results of sporulation of over-

TABLE V

COMPARISON OF PERCENT SAMPLES YIELDING SPORULATING  
STROMATA AFTER OVERWINTERING IN THE FIELD  
AT DIFFERENT DEPTHS AND THE NUMBER OF  
SPORULATING STROMATA PER SAMPLE

Degree of Sporulation	Samples with Sporulating <i>C. arachidicola</i> stromata				
	Perkins (Dec. '77-June '78)		Perkins (Dec. '78-June '79)		
	Leaflets	Stem Segments	Leaflets	Petioles	Stem Segments
I	9 (27) <sup>a/</sup>	4 (12)	12 (36)	3 (9)	3 (9)
II	17 (136)	12 (96)	8 (64)	6 (48)	5 (40)
III	8 (104)	12 (156)	8 (104)	8 (104)	5 (65)
IV	2 (32)	10 (160)	9 (144)	13 (208)	17 (272)
Total	36 (299)	38 (424)	37 (348)	30 (369)	30 (386)
%	75.0	79.2	62.5	62.5	83.3
No. of Sporulating Stromata/ sample	8.3	11.1	9.4	12.3	12.9

TABLE V (Continued)

Sample with Sporulating <i>C. arachidicola</i> stromata						
Degree of Sporulation	Stillwater (Feb. '78-July '78)			Stillwater (Dec. '78-June '79)		
	Leaflets	Petioles	Stem Segments	Leaflets	Petioles	Stem Segments
I	6 (18) <sup>a/</sup>	2 (6)	5 (15)	2 (6)	2 (6)	0 (0)
II	5 (40)	5 (40)	3 (24)	4 (32)	2 (16)	3 (24)
III	7 (91)	3 (39)	5 (65)	4 (52)	4 (52)	6 (78)
IV	2 (32)	6 (96)	6 (96)	20 (320)	19 (304)	10 (160)
Total	20 (181)	16(181)	19 (200)	30 (410)	27 (378)	19 (262)
%	66.7	66.7	79.2	83.3	75.0	52.0
No. of Sporulating Stromata/ Sample	9.0	11.3	10.5	13.7	14.0	13.8

( )<sup>a/</sup> = The product of number of samples with positive *C. arachidicola* isolations X midrange value of sporulation degree.

wintering peanut debris after 2-3 days incubation in moist chambers are shown in Table VI. Sporulative C. arachidicola stromata were observed on leaflets, petioles, or stem segments exposed to field conditions for a maximum period of 20 weeks and storage in the cold room for, at least, an additional period of 89.3 weeks. Depths of 0, 5, 10, and 20 cm did not seem to affect the survivability of the fungus.

### Spore Trapping

#### Conventional Wind-vane Traps

The number of conidia of peanut leafspot fungi trapped from June 27 until August 28, 1979 in peanut fields at Perkins and Stillwater on vaseline-coated cellophane tapes mounted on glass rods (Figure 6) and on vaseline-smearred microscope slides (Figure 7) are shown in Table VII. The total number of conidia from the three traps located at Perkins was between 3 and 34 with the highest spore density occurring during the fifth, seventh, sixth, and fourth weeks, in descending order.

The data from the Stillwater study show that the highest total number of spores from the three traps was 80, and was observed the first week starting June 27, 1979. Seventy-two, 53, 42, and 17 leafspot spores were recorded on week 5, 4, 3, and 2, respectively.

Generally, more Cercospora and Cercosporidium conidia were caught on wind-vane spore traps in the peanut field located at Stillwater than at Perkins. For the nine weeks period of investigation, a total of 302 conidia were caught on the three traps at Stillwater, while only 123 conidia were trapped in the peanut field at Perkins. The same trend of more spores at Stillwater was observed weekly except on the sixth



TABLE VI

SPORULATION OF OVERWINTERING CERCOSPORA ARACHIDICOLA-  
INFECTED PEANUT DEBRIS EXPOSED TO FIELD<sup>a/</sup>  
AND COLD ROOM CONDITIONS

Depth	Sample No.	Exposure (weeks)		Degree of Sporulation		
		Field	Cold Room	Leaflet	Petiole	Stem
0	1	20	89.3	4	0	4
	2	20	89.8	4	0	4
5	1	20	89.3	3	0	4
	2	20	89.8	1	4	0
10	1	20	89.3	3	3	0
	2	20	89.8	4	2	4
20	1	20	89.3	1	4	3
	2	20	89.8	0	2	0

<sup>a/</sup> Location: Caddo Peanut Research Station at Fort Cobb, Oklahoma  
(January 9-May 29, 1978).

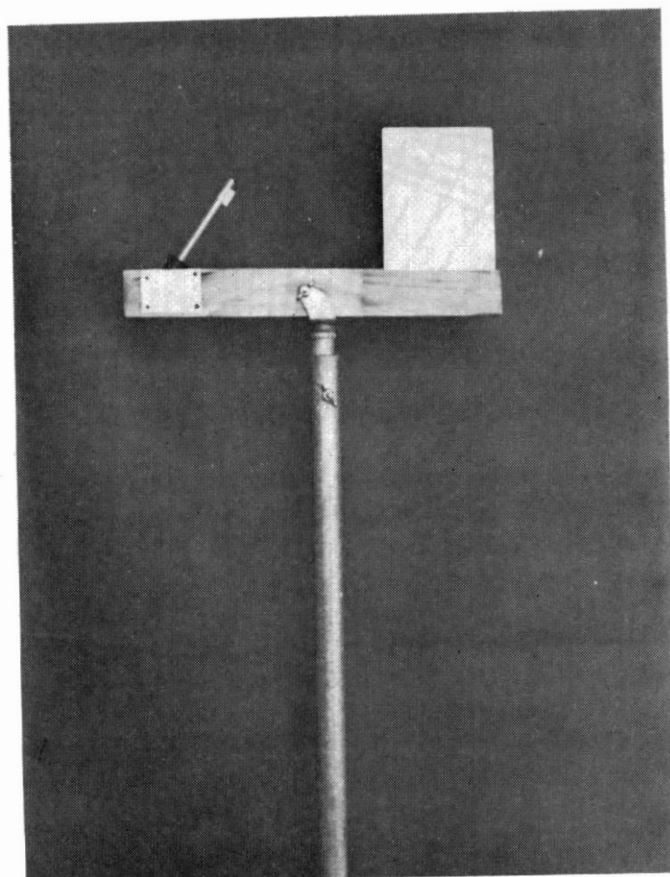


Figure 6. A Conventional Windvane-type Spore Trap Where the Trapping Surface Consists of a Vaseline-coated Tape Mounted on a Glass Rod.

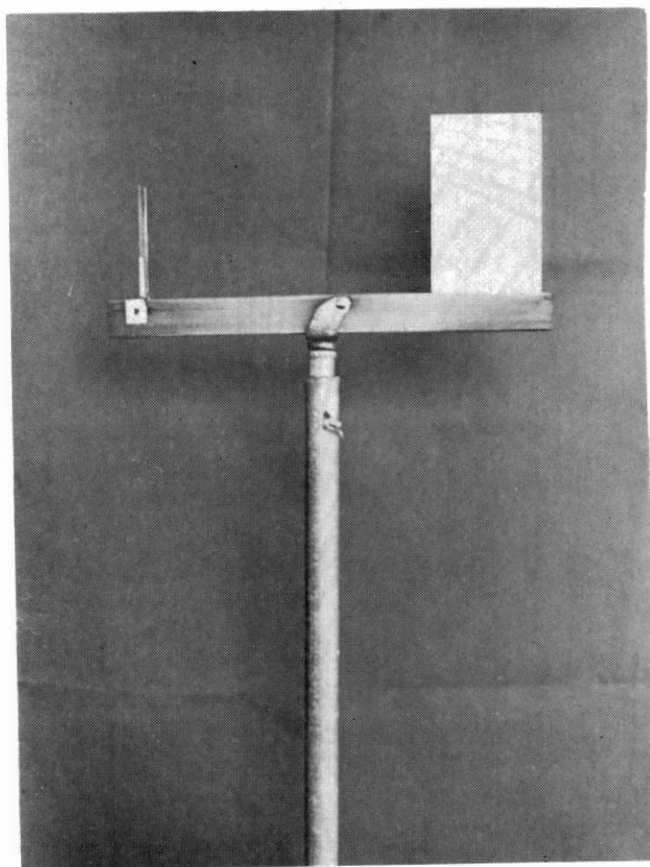


Figure 7. A Conventional Windvane-type Spore Trap Where the Trapping Surface Consists of a Partially Vaseline-coated Glass Slide.

TABLE VII

SPORE DENSITIES IN PEANUT FIELDS FROM JUNE 27 - AUGUST 28  
(1979) DETERMINED BY USING WIND-VANE TYPE TRAPS

Week	Perkins				Stillwater			
	Trap No.			Total	Trap No.			Total
	1 <sup>a/</sup>	2 <sup>b/</sup>	3 <sup>b/</sup>		1 <sup>a/</sup>	2 <sup>b/</sup>	3 <sup>b/</sup>	
1	5	0	0	5	33	31	16	80
2	0	2	4	6	3	7	7	17
3	3	0	2	5	11	9	22	42
4	9	0	5	14	10	3	40	53
5	18	6	10	34	33	18	21	72
6	15	0	1	16	4	3	3	10
7	0	29	4	33	8	6	0	14
8	0	2	1	3	0	0	6	6
9	0	3	4	7	4	0	4	8
Total	50	42	31	123	106	77	119	302

<sup>a/</sup> spores trapped on vaseline-smearred slide.

<sup>b/</sup> spores trapped on vaseline-coated cellophane tape on a glass rod.

and seventh weeks when 16 and 33 conidia were recorded at Perkins, while only 10 and 14 conidia were observed during the same period at Stillwater. The variation in the number of conidia at the two locations is graphically presented in Figure 8.

An attempt was made to relate spore densities during the nine weeks of investigation to the weather conditions during the same period. This is shown in Figure 9. Except for the first week where a high spore reading was made at Stillwater, increase in spore densities was gradual from week two up to week five, then followed a period of leveling off. At Perkins, not much increase in spore concentration during the first three weeks was observed. The major period of increase in spore density at Perkins occurred from July 17 until August 13 (week 4 to week 7) followed by a leveling off period during the last two weeks of study.

In a separate study which was designed to investigate the Mycophae-  
rella stage of the peanut leafspot fungi, five spore traps were set up on April 19, 1979, at a small peanut plot where the tops of plants were cut by the end of the growing season, and debris was left on the ground to overwinter. The traps were used in the hope of catching ascospores of the Mycosphaerella stage. Together with many other spores trapped, conidiospores of C. arachidicola were frequently observed and their numbers were, therefore, noted.

After five weeks of investigation, the results (Table VIII) of trapping on vaseline-coated slides exposed for a minimum of 48 hours showed the presence of conidia of C. arachidicola as early as April 19 before any peanut crop was planted anywhere in Oklahoma. A total of 159 spores were collected over the span of five weeks.

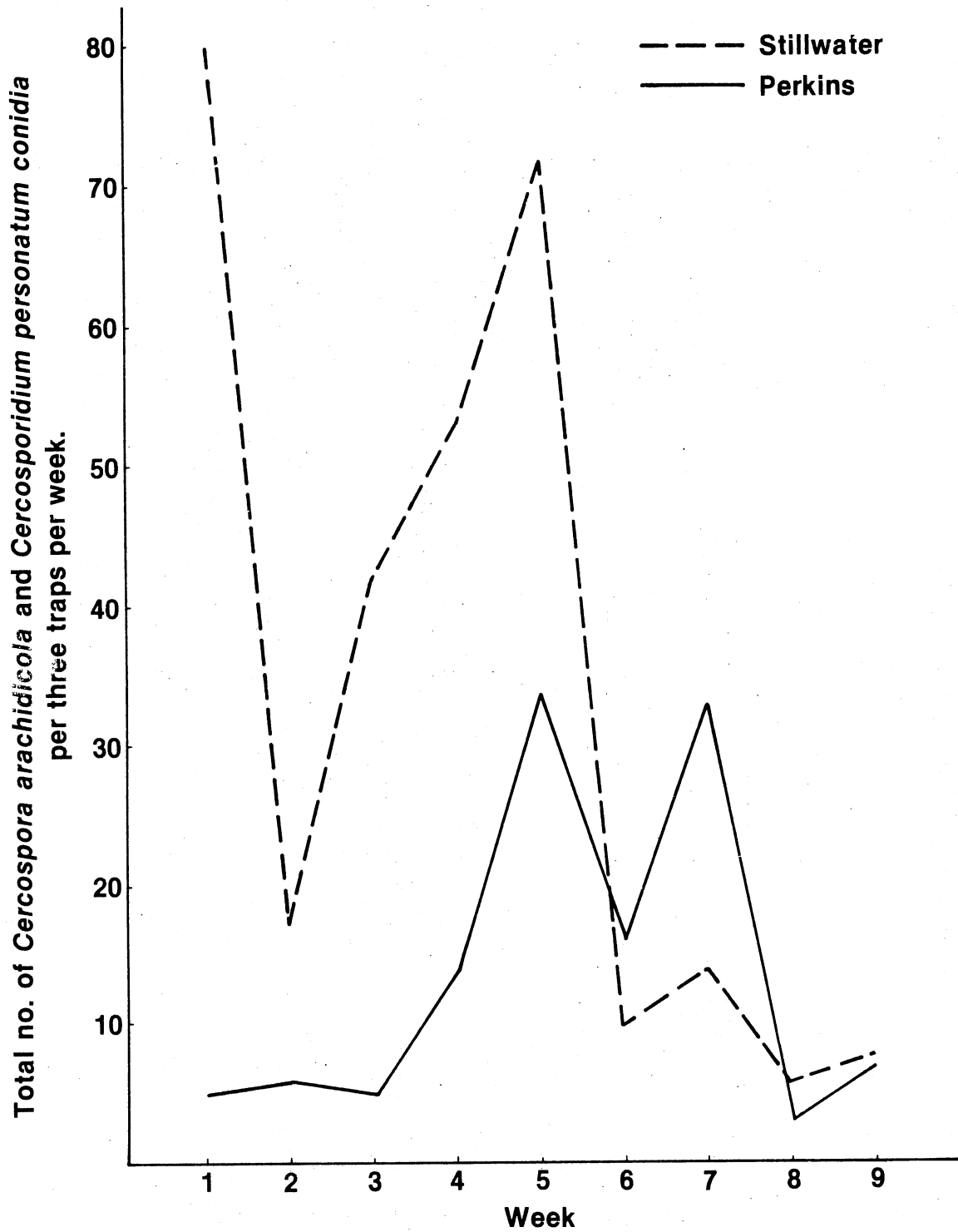


Figure 8. Total Number of Peanut Leafspot Spores Caught on Three Wind Vane Spore Traps Where Three Slides per Trap per Week Were Exposed for 48, 48, and 72 Hours at Stillwater and Perkins (June 27 - August 28, 1979).

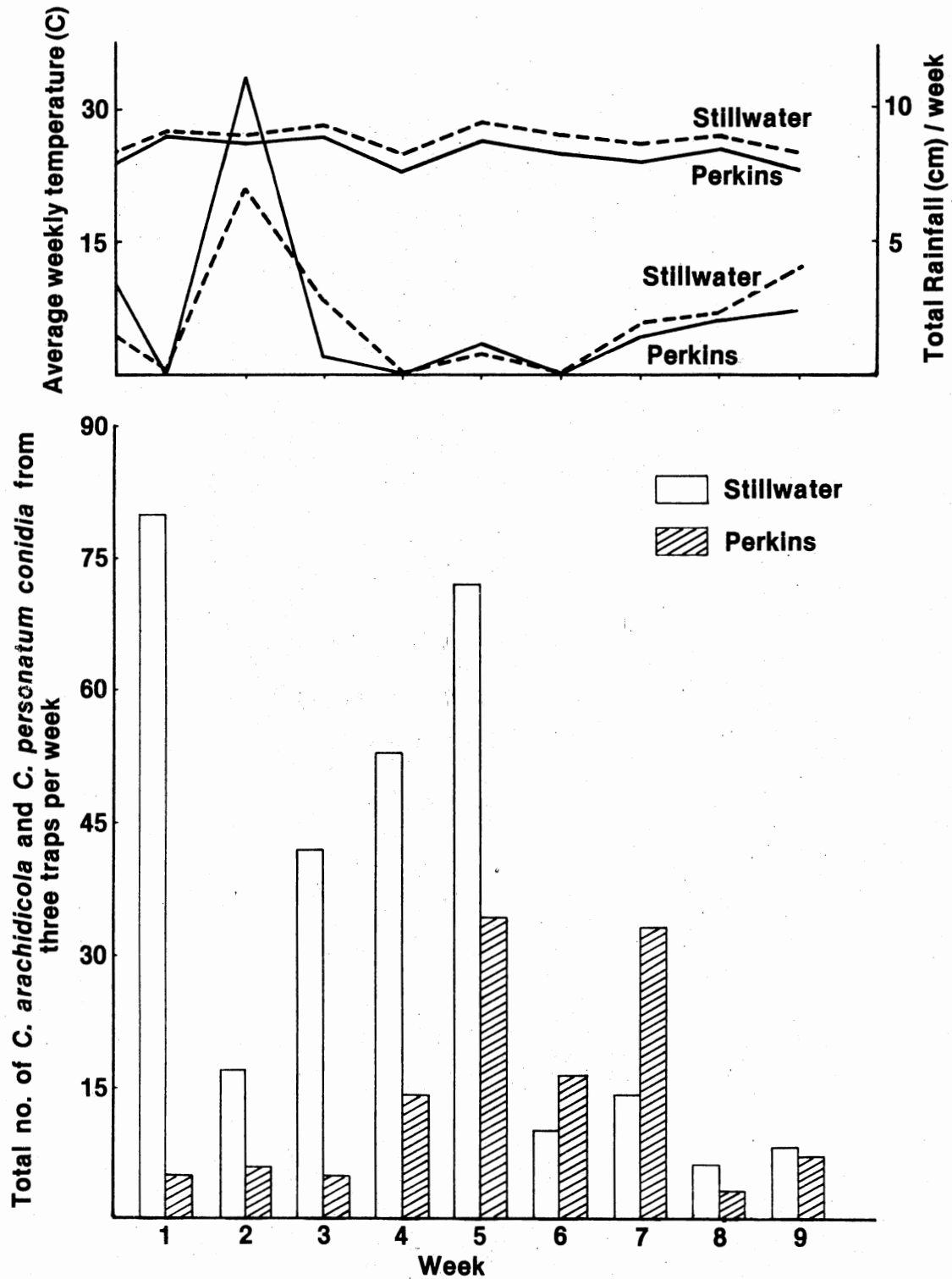


Figure 9. Mean Temperature, Rainfall, and Number of Trapped *Cercospora arachidicola*, and *Cercosporidium personatum* Spores per Week at Stillwater and Perkins from June 27 - August 28, 1979.

TABLE VIII

SPORE COUNTS FROM WIND VANE-TYPE TRAPS DURING NON-CROPPING PERIOD AT AN ISOLATED FARM PLOT<sup>a/</sup> GROWN TO PEANUTS THE PREVIOUS SEASON

Week	Trap <sup>b/</sup>					Total	Average per trap
	1	2	3	4	5		
I	4	0	8	7	5	24	4.8
II	3	4	4	2	0	13	2.6
III	0	59	2	0	1	62	12.4
IV	3	2	0	2	3	10	2.0
V	1	16	12	8	12	50	10.0
Total	11	81	26	19	22	159	
Average per week	2.2	16.2	5.2	3.8	4.4		

<sup>a/</sup> Location: Two miles west of Stillwater on Lakeview Road (4/19-5/24, 1979).

<sup>b/</sup> Trapping surface mounted on a slide.



### Kramer-Collins Seven Day Sampler

Spore concentrations per unit volume of air per unit interval of time could not be determined using conventional wind-vane traps. However, the use of the Kramer-Collins spore trap (Figure 10) made it feasible to determine spore density in relation to time and volume of air.

The data in Table IX show that as early as June 27, 1980, the day the spore trap was set in the field at Perkins, a total of 14.6 C. arachidicola spores per cubic meter of air were caught. Figure 11 shows that the total number of spores trapped on the tape during the first week (June 27 - July 3) was 72.9 spores per cubic meter. During the same period, no precipitation occurred, and the mean air temperature for the whole week was 31.8 C. The minimum and maximum temperatures for the same period ranged between 21.7 and 41.1 C. However, a week prior to the day the study was initiated, a total of 10.2 cm of rainfall was received in the area, and the mean temperature for the same period was 25.9 C. Since the planting of the peanut crop on two different dates (May 14 and June 7), the field received 1.9 cm of irrigation water per week. With no measurable precipitation, and mean weekly temperatures of 31.1 and 32.2 C, the spore load per cubic meter of air during the second and third week was 58.5 and 27.1, respectively. While temperature dropped to 29.3 C in the fourth week, and with no rainfall, spore density climbed slightly to 62.6 spores per cubic meter. Although a 0.1 cm of rain was received in the fifth week, spore density dropped appreciably to a low of 4.2 conidia. On the sixth week with temperature rising to an average of 31.4 C, the number of trapped

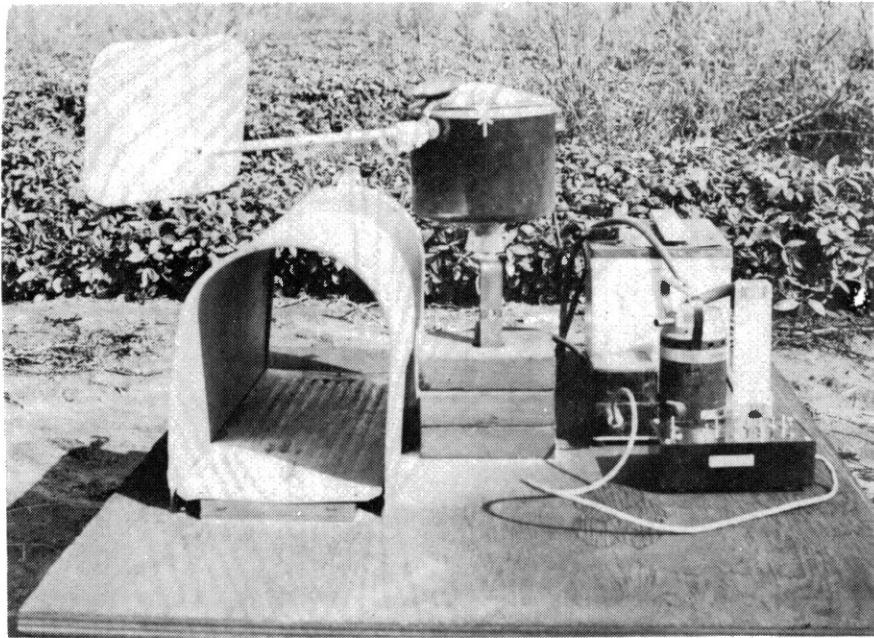


Figure 10. Kramer-Collins 7-day Spore Sampler as Was Set in the Field. Intake Slot is 50 cm High Above the Ground.

TABLE IX  
 TEMPERATURE (C), RAINFALL (CM), AND NUMBER OF CERCOSPORA  
ARACHIDICOLA AND CERCOSPORIDIUM PERSONATUM SPORES  
 TRAPPED AT PERKINS ON A KRAMER-COLLINS SPORE  
 SAMPLER FROM JUNE 27-OCTOBER 16, 1980

Week	Date	No. of Spores Trapped on tape per period <sup>a,b/</sup>				C.a.c./	C.p.d./	Total Spores per day per m <sup>3</sup>	Temperature (C)			Rainfall (cm)		
		I	II	III	IV				Min.	Max.	Mean			
01	June	27	0	2	3	2	7	0	14.6	22.2	39.4	30.8	0.00	
		28	0	5	1	0	6	6	0	12.5	24.4	41.1	32.8	0.00
		29	0	1	0	1	2	2	0	4.2	--	--	--	0.00
	July	30	4	0	1	2	7	7	0	14.6	24.4	41.1	32.8	0.00
		1	1	0	0	0	1	1	0	2.1	22.8	38.9	30.8	0.00
		2	1	1	2	6	10	10	0	20.8	22.8	40.0	31.4	0.00
	3	2	0	0	0	2	0	4.2	23.9	40.5	32.2	0.00		
02		4	1	1	0	0	2	0	4.2	26.1	40.0	33.1	0.00	
		5	0	1	1	1	3	0	6.2	22.2	38.9	30.1	0.00	
		6	0	1	6	6	13	13	0	27.1	21.7	37.8	29.7	0.00
		7	1	2	1	0	4	4	0	8.3	22.8	38.3	30.1	0.00
		8	2	0	0	0	2	2	0	4.2	23.9	38.3	31.1	0.00
		9	0	0	0	3	3	3	0	6.2	23.9	38.3	31.1	0.00
03		10	0	1	0	0	1	0	2.1	23.9	40.0	31.9	0.00	
		11	1	0	0	0	1	0	2.1	22.8	39.4	31.1	0.00	
		12	1	0	0	0	1	1	0	2.1	23.9	39.4	31.7	0.00
		13	0	2	1	0	3	3	0	6.2	23.9	40.5	32.2	0.00
		14	0	0	0	0	0	0	0	0.0	23.9	42.8	33.3	0.00
		15	1	0	0	3	4	4	0	8.3	24.4	40.5	32.5	0.00
04		16	0	0	0	0	0	0	0.0	24.4	40.5	32.5	0.00	
		17	1	0	2	1	4	4	0	8.3	23.3	41.7	32.5	0.00
		18	0	0	1	2	3	3	0	6.2	21.1	41.7	31.4	0.00
		19	1	0	2	1	4	4	0	8.3	23.9	41.1	32.5	0.00
		20	0	0	1	2	3	3	0	6.2	22.2	40.0	31.1	0.00
		21	2	1	1	0	4	4	0	8.3	21.7	38.9	30.3	0.00
05		22	0	0	2	1	3	0	6.2	21.1	37.2	29.2	0.00	
		23	0	6	6	1	13	13	0	27.1	15.5	35.5	25.5	0.00
		24	0	0	0	0	0	0	0	0.00	15.5	35.0	25.3	0.00
		25	2	0	0	0	2	2	0	4.2	20.0	36.7	28.3	0.00
05		26	0	0	0	0	0	0	0.0	21.7	37.2	29.4	0.00	
		27	0	0	0	0	0	0	0.0	17.8	33.9	25.8	0.13	
		28	0	0	0	0	0	0	0	0.0	17.8	36.1	26.9	0.00
		29	0	0	0	0	0	0	0	0.0	21.1	40.5	30.8	0.00
		30	0	0	0	0	0	0	0	0.0	22.2	42.2	32.2	0.00
		31	0	0	0	0	0	0	0	0.0	22.2	41.1	31.7	0.00

TABLE IX (Continued)

Week	Date	No. of Spores Trapped on tape per period <sup>a, b/</sup>				C.a.c./	C.p.d./	Total Spores per day per m <sup>3</sup>	Temperature (C)			Rainfall (cm)	
		I	II	III	IV				Min.	Max.	Mean		
06	Aug.	1	0	0	0	0	0	0.0	25.0	41.1	33.0	0.00	
		2	2	1	0	1	4	0	8.3	21.7	41.7	31.7	0.00
		3	0	0	0	1	1	0	2.1	21.1	40.5	30.8	0.00
		4	0	9	30	10	49	0	102.1	25.0	37.2	31.1	0.00
		5	0	0	0	0	0	0	0.0	22.8	37.2	30.0	0.00
		6	0	0	0	0	0	0	0.0	24.4	38.3	31.4	0.00
		7	0	0	0	0	0	0	0.0	22.8	37.8	30.3	0.00
07		8	0	0	0	1	1	0	2.1	21.1	38.3	29.7	0.00
		9	0	0	0	0	0	0	0.0	20.5	38.3	29.4	0.00
		10	0	0	0	0	0	0	0.0	21.1	38.9	30.0	0.00
		11	1	0	0	0	1	0	2.1	19.4	38.3	28.9	0.00
		12	0	0	0	0	0	0	0.0	18.3	36.7	27.5	0.00
		13	0	0	0	0	0	0	0.0	21.7	38.9	30.3	0.00
		14	0	0	0	0	0	0	0.0	22.8	37.2	30.0	0.00
08		15	0	0	0	0	0	0.0	23.3	38.9	31.1	0.00	
		16	0	0	0	0	0	0	0.0	24.4	36.7	30.5	0.00
		17	0	0	0	0	0	0	0.0	23.9	36.7	30.3	0.00
		18	0	0	0	3	3	0	6.2	19.4	38.3	28.9	0.25
		19	0	0	0	0	0	0	0.0	23.3	37.2	30.3	0.00
		20	0	0	0	0	0	0	0.0	25.0	37.8	31.4	0.00
		21	0	0	0	0	0	0	0.0	19.4	39.4	29.4	2.36
09		22	0	0	0	0	0	0.0	17.2	34.4	27.2	1.35	
		23	0	0	0	0	0	0	0.0	18.9	35.5	25.8	0.00
		24	1	0	0	0	1	0	2.1	21.1	35.0	28.0	0.00
		25	0	0	0	0	0	0	0.0	13.3	38.3	25.8	0.00
		26	0	0	0	0	0	0	0.0	17.8	36.7	27.2	0.00
		27	0	0	0	0	0	0	0.0	18.3	37.2	27.8	0.00
		28	0	0	0	1	1	0	2.1	18.9	37.2	28.0	0.00
10		29	0	0	0	0	0	0.0	17.8	33.9	25.8	0.00	
		30	0	0	0	0	0	0	0.0	22.2	37.2	29.7	0.00
		31	0	0	0	0	0	0	0.0	24.4	35.0	29.7	0.00
		Sept. 1	0	0	0	0	0	0	0.0	26.7	35.0	30.8	0.00
		2	0	0	0	0	0	0	0.0	20.0	37.8	28.9	1.85
11		3	0	0	0	0	0	0.0	19.4	31.1	25.3	0.13	
		4	5	1	1	2	10	0	20.8	18.9	34.4	26.7	0.00
		5	2	7	5	10	24	0	50.0	18.3	36.1	27.2	0.00
		6	1	4	1	1	7	0	14.6	21.1	36.1	28.6	0.00
11		7	2	10	9	5	25	1	54.2	18.9	36.7	27.8	0.00
		8	0	0	5	3	8	0	16.7	19.4	35.0	27.2	0.00
		9	1	3	1	0	5	0	10.4	17.8	35.0	26.4	0.00
		10	0	5	0	0	5	0	10.4	15.5	36.7	26.1	0.00
		11	8	5	0	0	12	1	27.1	16.1	30.5	23.3	0.00

TABLE IX (Continued)

Week	Date	No. of Spores Trapped on tape per period <sup>a, b/</sup>				C.a. <sup>c/</sup>	C.p. <sup>d/</sup>	Total Spores per day per m <sup>3</sup>	Temperature (C)			Rainfall (cm)		
		I	II	III	IV				Min.	Max.	Mean			
12	Sept.	12	1	2	3	1	6	1	14.6	20.5	35.0	27.8	0.00	
		13	0	4	6	1	11	0	22.9	20.0	36.7	28.3	0.00	
		14	2	8	4	0	14	0	29.2	19.4	37.8	28.6	0.00	
		15	4	3	4	0	11	0	22.9	18.3	37.2	27.8	0.00	
		16	5	5	0	0	6	4	20.8	20.5	37.2	28.9	0.00	
		17	1	1	0	0	2	0	4.2	6.1	37.8	21.9	0.00	
		18	3	14	5	10	9	23	66.7	7.8	26.7	17.2	0.00	
13		19	6	6	1	2	12	3	31.2	15.5	33.3	24.4	0.00	
		20	3	2	0	0	2	3	10.4	21.1	35.5	28.3	0.00	
		21	0	1	1	1	3	0	6.2	20.0	36.7	28.3	0.00	
		22	2	1	8	2	11	2	27.1	24.4	37.2	30.8	0.00	
		23	0	4	2	4	7	3	20.8	14.4	31.1	22.8	0.00	
		24	0	6	5	3	13	1	29.2	11.7	22.8	17.2	0.00	
		25	0	0	0	0	0	0	0.0	14.4	28.9	21.7	1.90	
14		26	0	0	0	0	0	0	0.0	11.1	26.1	18.6	0.08	
		27	0	0	0	0	0	0	0.0	10.0	18.9	14.4	1.93	
		28	0	0	0	0	0	0	0.0	9.4	13.9	11.7	1.42	
		29	0	0	0	0	0	0	0.0	11.1	15.5	13.3	0.00	
		30	0	0	0	2	2	0	4.2	11.1	17.8	14.4	0.00	
		Oct.	1	1	1	0	0	2	0	4.2	11.7	29.4	20.5	0.00
			2	17	9	5	1	28	4	66.7	5.5	33.3	19.4	0.00
15		3	3	0	2	4	7	2	18.7	3.9	20.5	12.2	0.00	
		4	1	1	2	0	4	0	8.3	7.8	25.5	16.7	0.00	
		5	1	3	1	4	9	0	18.7	7.2	18.9	13.0	0.00	
		6	7	5	10	4	26	0	54.2	12.2	18.9	15.5	0.00	
		7	2	0	0	0	1	1	4.2	10.0	19.4	14.7	0.00	
		8	4	0	0	0	4	0	8.3	11.1	28.3	19.7	0.00	
		9	2	9	1	0	8	4	25.0	11.7	33.9	22.8	0.00	
16		10	3	5	7	4	18	1	39.6	13.3	33.3	23.3	0.00	
		11	2	1	2	1	3	3	12.5	5.0	24.4	14.7	0.00	
		12	0	1	2	0	3	0	6.2	3.9	26.1	15.0	0.00	
		13	0	0	0	1	1	0	2.1	11.7	25.5	18.6	0.00	
		14	9	11	3	9	28	4	66.7	13.9	29.4	21.7	0.00	
		15	5	0	0	0	5	0	10.4	11.4	29.4	21.9	0.38	
		16	0	0	0	0	0	0	0.0	15.0	26.7	20.8	0.15	
Total		128	172	157	125	521	61							

<sup>a/</sup> Period I = 10:30 AM - 16:30 PM

Period II = 16:30 PM - 22:30 PM

Period III = 22:30 PM - 4:30 AM

Period IV = 4:30 AM - 10:30 AM

<sup>b/</sup> Air was sampled at 20 liters per minute once each hour.

<sup>c/</sup> C.a. = Cercospora arachidicola

<sup>d/</sup> C.p. = Cercospora personatum

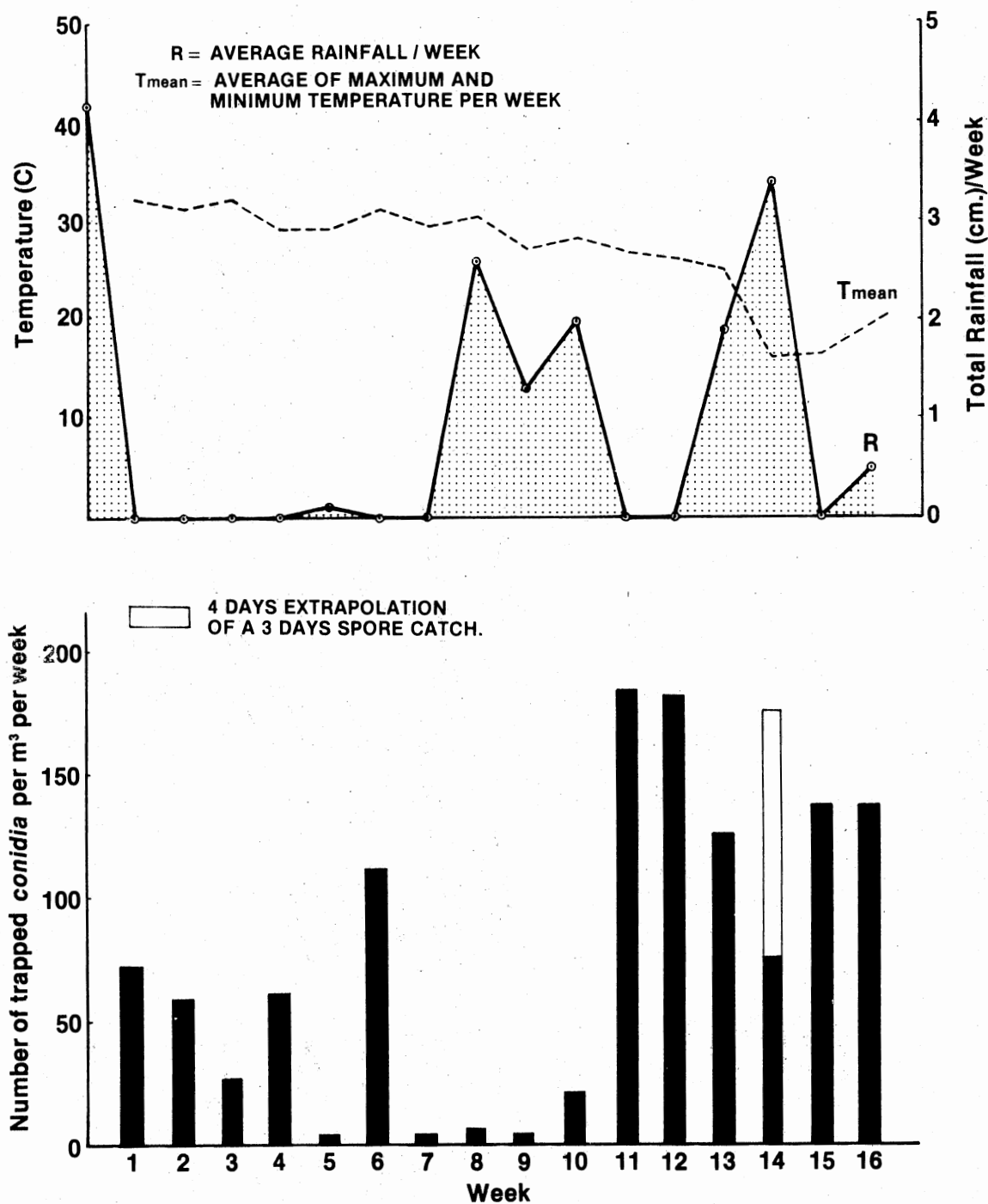


Figure 11. Mean Temperature, Rainfall, and Number of *Cercospora arachidicola*, and *Cercosporidium personatum* Spores Trapped per Week at Perkins From June 27 - October 16, 1980.

conidia, however, reached a high of 112.4 spores per cubic meter of air. With the exception of the eighth week, temperatures were following a downward trend which began during the seventh week and continued throughout the study period which lasted sixteen weeks. Meanwhile, 2.6, 1.3, and 2.0 cm of rain were received during the eighth, ninth, and tenth weeks, respectively. The number of trapped spores during the period from week 7 until week 10 were 4.2, 6.3, 4.2, and 20.8, in order.

Spore densities increased tremendously starting the eleventh week where the highest number of conidia per cubic meter of air (183.3) was trapped. The next highest spore catch (181.2) was scored on the twelfth week. During the following four weeks, the number of trapped conidia were 125.0, 175 (based on extrapolation of a three days catch), 137.5, and 137.5 in successive order. Amounts of 1.9, 3.4, and 0.5 cm of rainfall were recorded on week 13, 15, and 16, respectively. The mean weekly temperature of the last six weeks of the spore trapping study ranged between 16.0 and 26.7 C.

As best as could be determined from microscopic examination of exposed cellophane tapes, the majority of the trapped conidia throughout the study were of the C. arachidicola type (Figure 12). Early leaf-spot spores (C. arachidicola) comprised 89.5% of the total number of spores trapped over the 16-week period of study (Table IX). Late leaf-spot (C. personatum) spores (Figure 13) were not observed until September 7, 1980, more than ten weeks after the study was initiated. By the end of 16 weeks of spore trapping, a total of 61 late leafspot conidia ( $127.1 \text{ conidia/m}^3$ ) were recorded with the highest number (23 spores) occurring on September 18. Cercosporidium personatum spore count during week 12 beginning on September 12 was 28 conidiospores (58.3

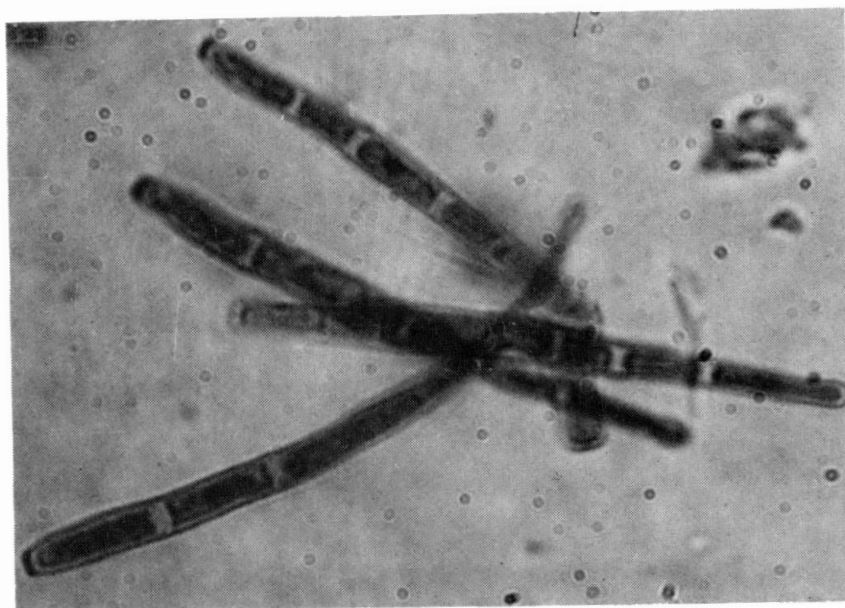


Figure 12. Cercospora arachidicola Spores Trapped on Vaseline-coated Adhesive Tape Mounted on the Drum of a Kramer-Collins 7-day Sampler. 1000X





Figure 13. Cercosporidium personatum Spores Trapped on Vaseline-coated Adhesive Tape Mounted on the Drum of a Kramer-Collins 7-day Sampler. 1000X.

spores/m<sup>3</sup>) which coincided with a high concentration of C. arachidicola spores during the same week (122.9 spores/m<sup>3</sup>). Figure 14 shows the distribution of early leafspot as well as late leafspot over the period of 16 weeks of spore trapping at Perkins.

#### Diurnal Periodicity

To see if there was diurnal periodicity affecting the concentration of C. arachidicola and C. personatum conidia in the air, the number of conidia per week per m<sup>3</sup> were grouped into four, six-hour intervals per day (Figure 15 - where period I represents the time period from 10:30 a.m. - 16:30 p.m.; period II, from 16:30 p.m. - 22:30 p.m.; period III, from 22:30 p.m. - 4:30 a.m.; and period IV, from 4:30 a.m. - 10:30 a.m.).

Generally, more conidia were trapped during the second and third periods than the first and fourth periods. Table IX shows that after 16 weeks of investigation, the highest number of conidia per cubic meter (358.3) was trapped in the second period compared to 327.1, 266.7 and 260.4 spores caught during the third, first, and fourth periods, respectively.

However, spore counts in the last six weeks, where nearly 70% of the total number of conidia were trapped, show that more conidia were observed during the second than during any of the other three periods. While 287.5 conidia/m<sup>3</sup> were recorded during the second six hours, only 214.6, 200, and 158.3 conidia/m<sup>3</sup> were trapped during the first, third, and fourth periods.

#### Pathogenicity Tests

In the early stages of the investigation, isolates of C. arachidi-

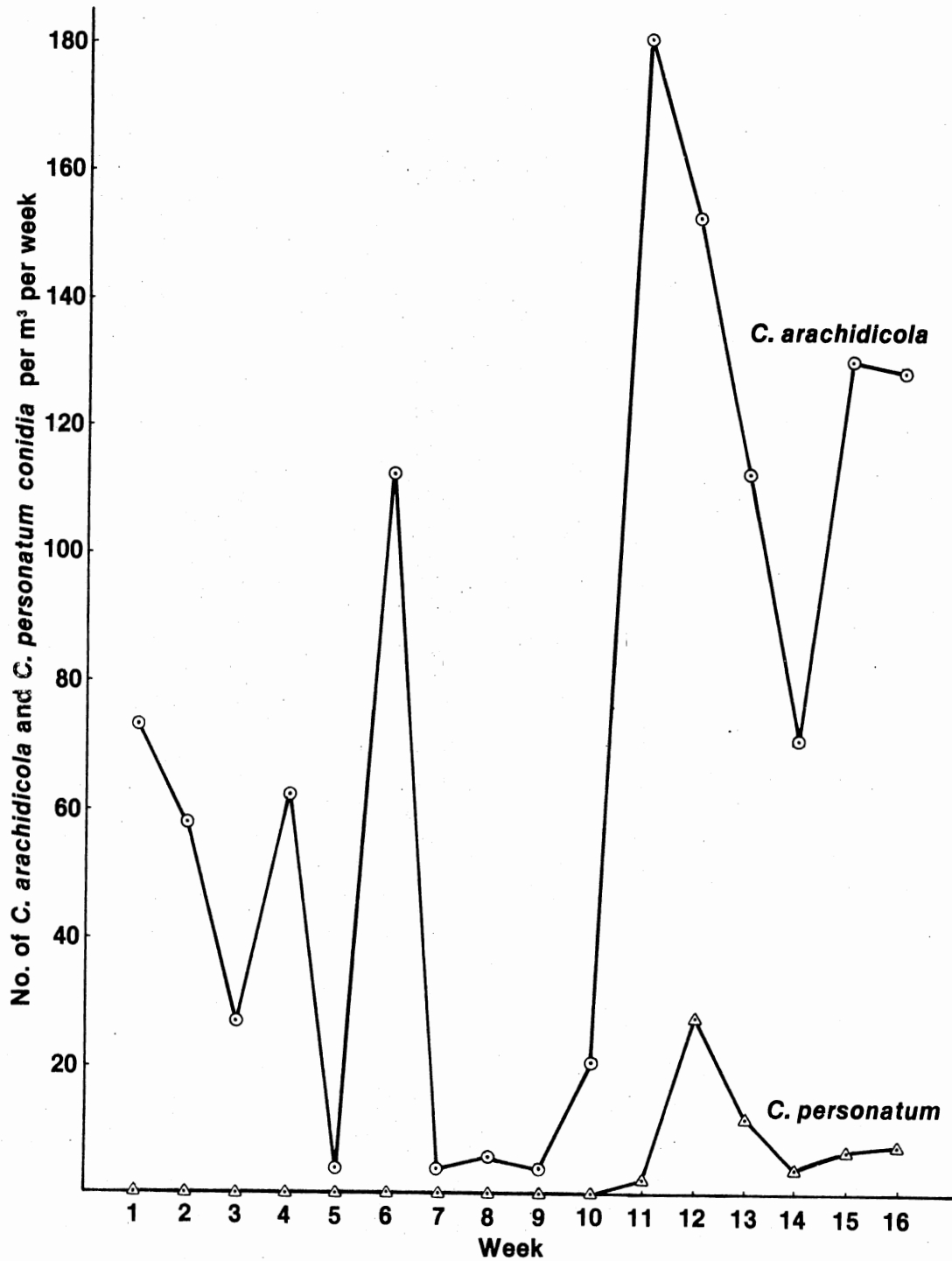


Figure 14. Weekly Counts of *Cercospora arachidicola* and *Cercosporidium personatum* Spores Trapped in a Peanut Field at Perkins From June 27 - October 16, 1980.

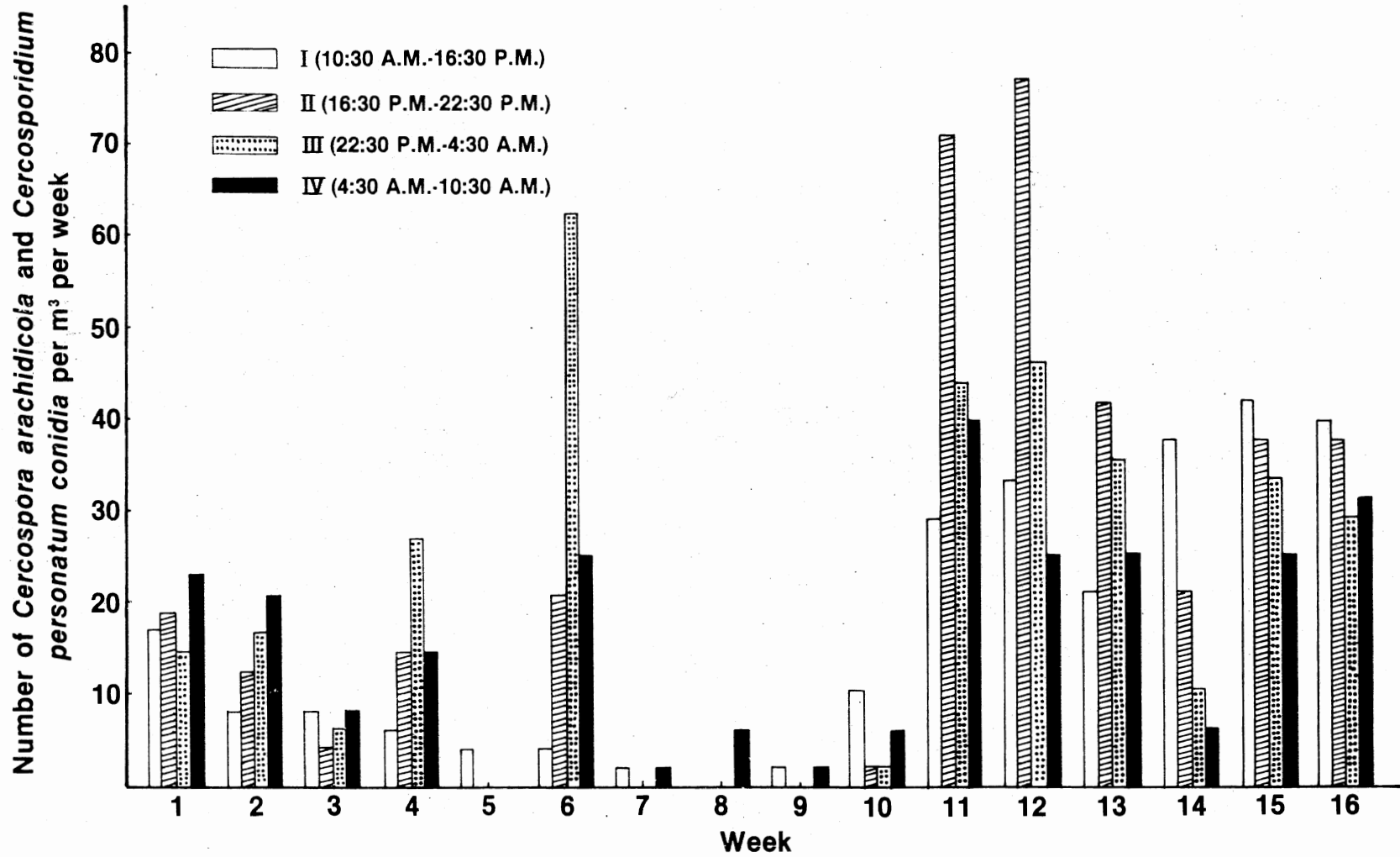


Figure 15. Daily Distribution of *Cercospora* spp. Spores per m<sup>3</sup> of Air Sampled 50 cm Above the Soil in a Peanut Field (Computed on Weekly Basis).

cola recovered from overwintering samples at the three experimental sites (Stillwater, Perkins, and Fort Cobb) were tested for pathogenicity using detached peanut leaflets incubated on moist filter paper, and inoculated by brushing. The results (Table X) show that all ten isolates from Perkins were pathogenic. No matter what plant portion the isolate came from, nor what depth was the sample buried at, the tested C. arachidicola cultures equally produced infection on detached leaflets of peanut cultivar 'Tamnut 74'. Pathogenic reactions of the isolates from Perkins were similar in all three tests.

The four isolates from Stillwater were pathogenic in the first and second tests, while in the third test only three of the isolates produced infection. Isolate S-6-10 (Stem-6-10), on the other hand, failed to incite a pathogenic reaction in the third test.

Only two isolates from those recovered from Fort Cobb were tested. The results of the three tests showed that both isolates were pathogenic.

In additional pathogenicity tests, a hand atomizer was used to spray inoculum on detached peanut leaflets. All isolates, including S-6-10, gave positive pathogenic reactions. More lesions per leaflet were produced when inoculum was sprayed onto the leaf tissue than when it was applied with a brush.

#### Search for the Perfect Stage

(*Mycosphaerella* spp.)

Examination of peanut tissue buried at different depths for various lengths of time failed to detect perithecia. Incubation of washed, surface-sterilized leaflets, petioles, and stem segments under inverted

TABLE X

REACTION OF TAMNUT 74 PEANUT DETACHED LEAFLETS TO BRUSHING  
 INOCULATION BY C. ARACHIDICOLA ISOLATES RECOVERED  
 FROM OVERWINTERING PEANUT TISSUE<sup>a/</sup>

Location	Isolate <sup>b/</sup>	Pathogenicity Reactions <sup>c/</sup>		
		1st Test	2nd Test	3rd Test
Perkins	S-2-0	+	+	+
	P-6-0	+	+	+
	L-7-0	+	+	+
	L-3-10	+	+	+
	S-5-10	+	+	+
	L-7-10	+	+	+
	P-9-10	+	+	+
	S-12-10	+	+	+
	S-8-20	+	+	+
	S-12-20	+	+	+
	Control	-	-	-
Stillwater	S-1-0	+	+	+
	P-3-0	+	+	+
	L-7-5	+	+	+
	S-6-10	+	+	-
	Control	-	-	-
Fort Cobb	S-1-0	+	+	+
	L-2-20	+	+	+
	Control	-	-	-

<sup>a/</sup> Four leaflets per plate in duplicate were inoculated.

<sup>b/</sup> Letter in each isolate designation refer to plant part used for isolation: L = leaflet, P = petiole, S = stem segment  
 First digit refers to sample number; second digit refers to depth in centimeters.

<sup>c/</sup> (+) sign means isolate was pathogenic.

deep-bottomed plates containing PYDA did not result in any ascospores being shot onto the medium and hence no Cercospora or Cercosporidium cultures were obtained this way.

Attempts to catch peanut leafspot spores using wind-vane traps were initiated early in March 1979. This was undertaken to, hopefully, show the presence of airborne ascospores supposedly shot from mature perithecia. The study, which continued until late May 1979, failed to show any Mycosphaerella ascospores fitting the description originally given by Jenkins (26). Spores frequently encountered were those of Alternaria spp., and Helminthosporium spp., however, by April 19, spores of C. arachidicola were being caught on the exposed slides even though no peanuts were yet planted. The results are shown in Table VIII and are discussed under the section on spore trapping.

Potted peanut plants set in the vicinity of spore traps were exposed by mid-April for two weeks and were later incubated in a polyethylene chamber in the greenhouse. The plants were observed for three weeks, and except for one plant showing a slight Cercospora infection on a few leaflets that was later confirmed by sporulation in some of the incubated lesions, the rest of the plants did not exhibit any leaf-spot infection.

Exposure of open, inverted, PYDA plates on top of levelled peanut debris lying on the ground and protected from direct sunlight and rain by inverted Dow plastic cups resulted in no Cercospora or Cercosporidium cultures after 48 hours exposure, although the peanut debris in this case was kept moist by frequent sprinkling with water throughout the period the debris was monitored for the presence of Mycosphaerella ascospores from March 1979 until May 1979. Each time, six plates were

exposed and this was repeated five times during the period of the investigation.

The last attempt to look into the possible existence of the perfect stage (Mycosphaeralla spp.) was investigated from February 1980 until May 1980. Peanut debris was raked into one area of the field after the crop had been harvested by mid-November 1979, and covered with a 1.3 cm<sup>2</sup> mesh plastic net. Recovery of peanut tissue from the field was made every three weeks. The tissue was washed, surface-sterilized and incubated under inverted plates containing PYDA. Examination of the plates under the stereoscopic microscope was made after two weeks of incubation, but no Cercospora or Cercosporidium cultures were identified, leading to the implication that no Mycosphaerella ascospores were shot from the lesions onto the medium.

#### Infection Study

Potential inoculum sources tested in this study were: 1) peanut debris that was left to overwinter on the ground or buried at different depths, 2) peanut debris collected from the field just after harvest, 3) dried, infected peanut tissue collected at the end of the growing season and stored at room temperature, 4) soil collected from around heavily-infected peanut plants in the field and presumed to be naturally infested with C. arachidicola propagules, 5) autoclaved soil mix artificially infested with a spore suspension of C. arachidicola. Treatments were applied either at the time of planting of germinated or non-germinated seeds, or after emergence of peanut plants above the soil when they were about 10 cm high. Emerging plants were continuously observed and any plant part suspected of having C. arachidicola



infection was collected, incubated in a moist chamber and examined for sporulation. Final evaluation of infection was made one month after application of treatments. The results of the infection study are shown in Tables XI and XII.

In the tests on pre-emergence infection (Table XI), germinated seeds planted in an autoclaved soil mix infested with C. arachidicola spore suspension resulted in 17 plants out of a total of 53 plants (32.1%) in the first test, 27 plants out of 37 (73%) in the third test, and 25 plants out of 40 (62.5%) in the fourth test, being infected. While germinated seeds planted in a soil mix infested with dried, infected tissue developed infection on ten plants out of a total of 35 (28.65%) in the third test, none of the other potential inoculum sources tested resulted in the development of infected plants whether peanut seeds were initially germinated or sown directly.

In the set of experiments on post-emergence infection (Table XII), 40 plants out of 53 (75.5%) in the first test, 38 plants out of 50 (76.0%) in the second test, and 25 plants out of 54 (46.3%) in the third test, were infected as a result of infesting a soil mix with a spore suspension of C. arachidicola.

Peanut debris-infested soil resulted in infection of 14 plants out of a total of 52 (26.9%) in the first test, while in the second test, none of the plants developed an infection. When dried, infected peanut tissue was used, 31 plants out of 52 (59.6%), and 31 plants out of 56 (55.4%) were infected in the first and third tests, respectively.

Overwintered debris included in the first and third tests, and field-infested soil used in the second and the third tests failed to induce infection on any of the plants. Similarly, in the control

TABLE XI  
 NUMBER OF PEANUT (COMET CULTIVAR) PLANTS INFECTED  
 WITH CERCOSPORA ARACHIDICOLA FROM DIFFERENT  
 INOCULUM SOURCES APPLIED PRIOR TO  
 EMERGENCE

Treatment <sup>a/</sup>	Number of Infected Plants			
	First Test <sup>b/</sup>	Second Test <sup>c/</sup>	Third Test <sup>d/</sup>	Fourth Test <sup>e/</sup>
Artificially-infested soil	17/53	0/55	27/37	25/40
Field peanut debris	0/51	0/57	---	0/40
Dry infected tissue	0/58	---	10/35	0/40
Overwintered tissue	0/55	---	0/39	---
Field "infested" soil	---	0/53	0/40	---
Control	0/58	0/57	0/40	0/40

<sup>a/</sup> applied before emergence. Watering from bottom to avoid splashing.

<sup>b/</sup> seeds germinated, then planted.

<sup>c/</sup> seeds sown directly.

<sup>d/</sup> seeds germinated, then planted.

<sup>e/</sup> seeds germinated, then planted.

TABLE XII

NUMBER OF PEANUT (COMET CULTIVAR) PLANTS INFECTED WITH  
CERCOSPORA ARACHIDICOLA FROM DIFFERENT INOCULUM  
 SOURCES APPLIED AFTER EMERGENCE

Treatment <sup>a/</sup>	Number of Infected Plants		
	First Test <sup>b/</sup>	Second Test <sup>c/</sup>	Third Test <sup>d/</sup>
Artificially-infested soil	40/53	38/50	25/54
Field peanut debris	14/52	0/55	---
Dry infected tissue	31/52	---	31/56
Overwintered debris	0/50	---	0/57
Field infested soil	---	0/50	0/56
Control	0/55	0/57	1/59

<sup>a/</sup> applied after emergence. Watering with hose from top.

<sup>b/</sup> seeds germinated, then planted.

<sup>c/</sup> seeds sown directly

<sup>d/</sup> seeds germinated, then planted.

treatments and except for one plant out of 59 (1.7%) in the third test being infected, none of the plants in the first and second tests sustained any C. arachidicola infection.

In conjunction with the experiments on infection, an attempt to test naturally "infested" field soil, as well as artificially infested soil mixes for the presence of conidia was made in accordance with Ledingham and Chinn (34) spore flotation technique originally developed for isolation of Helminthosporium sativum spores. Five field soil and five artificially infested soil mix samples were tested using the flotation technique but results were unsatisfactory. No conidia were observed in any of the field soil samples collected at the end of growing season from areas around peanut plants heavily infected with *Cercospora* leafspot. With artificially infested soil mixes, samples were tested one week and two weeks after the addition of spore suspension inocula. Occasionally, a few conidia were observed when aliquots of the emulsion were examined under the microscope, but generally the flotation method was unsatisfactory in recovering *Cercospora* spores from naturally or artificially-infested soils.

## CHAPTER V

### DISCUSSION

Cercospora arachidicola, responsible for the early infection of peanuts, apparently survives the winter in a mycelial form. Stromata (sclerotia-like bodies) which are modified mycelia seem to remain dormant in overwintering infected necrotic lesions on different plant parts. Examination of washed tissue recovered after exposure to field conditions revealed no observable sporulation of C. arachidicola in the lesions. However, if favorable conditions were provided, as when the lesions were incubated in a moist chamber, the stromata were observed to bear conidiophores and conidia, frequently in abundance (Tables I, II, and VI). Isolates recovered from sporulating stromata after undergoing overwintering conditions, and incubation in a moist chamber, proved to be pathogenic when inoculated onto healthy detached peanut leaflets (Table X). Overwintering of C. arachidicola as dormant stromata as shown in this study, is in agreement with similar findings previously reported in the literature (55, 56, 73).

No sporulating stromata characteristic of C. personatum were observed on incubated peanut tissue after exposure to field and cold room conditions. Failure to observe C. personatum could be due to the fact that only a small amount (about 10%) of peanut tissue infected with this organism was mixed with other C. arachidicola-infected peanut

material in the 1978-79 study. It is also possible that C. personatum did not survive well under conditions of this experiment.

The sexual stage, Mycosphaerella arachidicola (M. arachidis), reported by Jenkins (26) from Georgia and found to play a major role in the initiation of primary infection on peanuts early in the season, has not been found and probably is non-existent in Oklahoma. Absence of the sexual stage could be due, in part, to unfavorable environmental conditions in Oklahoma. Jenkins stated that perithecial formation is influenced by temperature and moisture, and that unless the leaflets are kept wet during spermatial release, no perithecia will be formed. Since Jenkins reported his findings about M. arachidicola in 1938, only one further incidence of the Mycosphaerella stage has been reported by Frezzi (17) from Argentina.

Although no detailed mycological investigation of Mycosphaerella spp. was conducted in this study, the failure to detect the presence of the sexual stages of peanut leafspot fungi through the indirect methods employed, and the finding that C. arachidicola per se could overwinter as dormant stromata which can retain their sporulative potential for as long as six months of field exposure and 19 months storage in the cold room strongly suggest that the role of the Mycosphaerella stage in the initiation of primary infection of peanuts early in the season is not significant, at least in Oklahoma. Insignificance of ascospores of Mycosphaerella spp. in primary infection of peanuts, is a view that had also been expressed by Smith (62) and Wolf (73).

Survival of C. arachidicola in leafspot-infected tissue lying on the surface or buried in the ground, was not apparently influenced by depth of burial. Samples placed on the surface (0.0 cm deep), or

buried in the ground at depths of 5, 10, and 20 cm at Stillwater, Perkins, and Fort Cobb (Tables I, II, and VI) yielded sporulating stromata upon incubation, with no observable effects on survivability due to depth. Most of the infected peanut tissue was still intact 2-3 months after exposure to field conditions. However, with longer time periods, more deterioration of tissue occurred, especially in leaflets, which were reduced to mere necrotic lesions by the end of field exposure period. Persistence of lesions in comparison with adjacent leaf tissue was also observed by others (26, 73).

Length of field exposure period (Tables I, II, and VI) did not seem to affect survivability of C. arachidicola either. The shortest interval of field exposure a sample on the surface or in the ground underwent at Perkins was 12.7 weeks (3 months), compared to 2.4 weeks (0.6 month) at Stillwater, while the maximum exposure periods were 26.3 weeks (6.1 month), and 34.8 weeks (8.1 month) at Perkins and Stillwater, respectively. All samples at Fort Cobb were exposed to field conditions for 20 weeks (4.7 month).

Under cold room conditions, the minimum and maximum exposure periods for samples recovered from Perkins were 2.7 weeks (0.6 month), and 26.7 weeks (6.2 month), compared to a minimum and maximum cold room exposure periods of 1.0 weeks, and 81.4 weeks (19.0 month), respectively, for samples recovered at Stillwater. Samples recovered from Fort Cobb were stored at the cold room for a period of 89.3-89.8 weeks (20.8-20.9 month).

No temperature effects on the survival of early leafspot fungus in overwintering peanut tissue could be detected (Tables I and II). During overwintering periods in the field, the average minimum and maximum

temperatures experienced were -6.5 and 16.2 C, respectively, at Perkins, compared to an average minimal and maximal temperatures of -6.2 and 21.6 C, respectively, at Stillwater.

Moisture as rainfall in centimeters or percent soil content apparently played no direct part in overwintering of the fungus. Cercospora arachidicola survived a low average rainfall (during a whole overwintering period) of 3.3 cm, and a high of 37.1 cm received at Perkins. At Stillwater, a low average rainfall of 0.6 cm, and a high of 45.7 cm were received. Average percent soil moisture levels, on the other hand, ranged between 68.9 and 92.5 at Perkins, and 33.7 to 97.7 at Stillwater.

While the environmental factors studied (depth, time, temperature, and moisture) did not seem to affect the survivability of the peanut leafspot fungus, there is apparently an effect due to environment on the degree of decomposition of infected tissue, especially leaflet material. It was commonly observed that as burial depth and time interval increased, the deterioration of the leaflet tissue was greater. Since most of the peanut debris after harvest is composed of leaf material, early deep plowing may aid in the degradation of infected peanut tissue, thus reducing inoculum levels available the following season. It is also important to eradicate any volunteer peanut plants that may emerge in the spring. A few infected volunteer plants were observed on several occasions at Perkins in April and May. The role of volunteer plants in the epidemiology of peanut leafspot had been discussed by Hemingway (21) and Smartt (58).

Overwintering may not solely be through dormant stromata on or in infected peanut debris, since decomposed or deteriorating plant tissue of legume and non-legume species were found by Pyzner (51) to be



infectable by C. arachidicola and C. personatum under experimental conditions. He also reported that Stylosanthes biflora (pencil-flower) which was confirmed by him to be a highly susceptible host for C. arachidicola, was commonly found in several native grass pastures near Stillwater, and could probably constitute 20-30% of the vegetation of a pasture without being distinctly visible.

The presence of pencil-flower in the immediate proximity of peanut fields, together with the ability of leafspot fungi to invade decomposed or deteriorating tissue of several non-legume and legume species may be involved in the overwintering of Cercospora and Cercosporidium spp. under field conditions.

Cercospora arachidicola spores occurred in the air over an area where overwintering peanut debris was lying on the ground as early as April 19 (1979) at Stillwater nearly one month before the recommended date of planting for peanuts. Whether an infection could have been induced if peanuts were there, is hard to ascertain. However, enough leafspot inoculum was present in the air, and most probably it came from overwintering debris. Under field situations, whenever favorable conditions near overwintering debris prevail, dormant stromata would be expected to start sporulating. Conidia produced under those conditions could be airborne, and would normally be disseminated with air currents to adjacent plants. The sporulating stromata may also come in direct contact with the lower leaflets of plants after emergence.

In the spore trapping studies at Stillwater and Perkins in 1979, and at Perkins in 1980, C. arachidicola conidia were caught on June 27, one day after setting the traps (Tables VII and IX). In 1980, Cercospora leafspot symptoms were observed on the lower leaves of some

peanut plants in an irrigated plot as early as July 11 (when the plants were 56 days old). Allowing two weeks for incubation of the fungus as had frequently been reported (17, 21, 26, 55, 71), and 7-10 days for germination of seeds and emergence, initial infection would most probably have taken place approximately 35 days after emergence.

Quantitative studies on spore density of C. arachidicola in the air, 50 cm above an irrigated peanut field at Perkins from June 27 - October 16, 1980 (Table IX) showed that, in general, more conidia were present in the air during periods following a rain than during rainy periods. Carlson (9) similarly observed that the concentration of C. beticola spores tend to increase on the day following a rain than during days without rain. The highest concentration of Cercospora and Cercosporidium spores trapped at Perkins was during a two consecutive weeks period (September 5-11) following a three weeks period (August 15 - September 4) where a total weekly rainfall of 2.61, 1.35, and 1.98 cm were received, respectively. During this period of high concentration of conidia, temperature averages for week 11 and 12 were 26.7 and 25.8 C, in order.

More conidia were trapped from air during the second period (16:30 p.m. - 22:30 p.m.) than during any other period. This trend was maintained throughout the study and was especially noticeable during the last six weeks of the study where nearly 70% of the total conidia trapped were observed.

The first Cercosporidium spore was detected on the tape at Perkins on September 7, sixty-nine days after spores of C. arachidicola were first observed. The occurrence of Cercosporidium conidia this late in the growing season seems to be unusual in light of reported dates of

incidence of late leafspot conidia elsewhere. Lyle (39) reported that the greatest numbers of C. arachidicola and C. personatum conidia were trapped in Alabama during July 15-31, while Littrell (38) reported that late leafspot was first detected in southern Georgia on July 19. In north Florida, Shokes et al. (57) indicated that late leafspot occurred as early as 50 days after planting. Cercosporidium personatum conidia were reported to occur in some areas in India, 5 weeks after emergence (65). Although the number and frequency of late leafspot (C. personatum) conidia increased slightly toward the end of the study period, their concentration remained much lower than C. arachidicola conidiospores. Frequent examination of infected peanut plants at Perkins and Stillwater over a period of three years (1977-1979) did not show any characteristic late leafspot infection due to C. personatum. However, there are indications that late leafspot infection does occasionally occur, though very slightly in peanut fields at Stillwater and Perkins. Melouk (42) was able to collect a few leaflets with C. personatum infection at Stillwater during September, 1979. A small number of leaflets with a few characteristic late leafspot lesions were also spotted by Pyzner (52) at Perkins in the fall of 1978.

The increase in number of C. arachidicola spores towards the latter part of the growing season may not be directly related to effects of increased rainfall and falling air temperatures, but rather to the existence of more diseased peanut tissue due to more secondary infections. With more infections occurring, more inoculum is produced in the lesions and the probability of more conidia becoming airborne is much greater. It would be interesting to see what effects temperature and moisture have on sporulation by actually monitoring temperature and relative

humidity under the canopy of plants. Measurements of leaf wetness may even be more meaningful.

Artificial infection with C. arachidicola or C. personatum under experimental conditions could be successfully accomplished using whole potted plants (3, 45, 53, 55, 56), detached leaflets on moist filter paper in petri plates (51), or detached leaves with petioles immersed in Hoagland solution (41). Inoculum is usually applied by stroking leaflets with a camel-hair brush dipped in a conidial suspension, or spraying the foliage with the suspension using a manual or an electric atomizer. Whatever the inoculation method used, whole plants or detached peanut leaves should be maintained at a high humidity level, at least, for a period of 48-96 hours (45, 53, 56).

The investigator attempted to find out if infection could occur while peanuts were emerging (pre-emergence infection). Watering was provided from the bottom so that no inoculum would be spattered on the foliage, and plants were kept in a humid atmosphere in a polyethylene chamber.

Overwintered debris (collected after six months of field exposure), and field "infested" soil sampled around heavily-infected peanut plants, consistently induced no infection (Tables XI and XII) whether plants were watered from the bottom or from the top where a stream of water was directed to the surface of potting mix to splash potential inoculum. Failure to induce infection could not be ascribed to lack of inoculum because, in the case of overwintered debris, sporulation was observed when samples of overwintered debris were washed, surface-sterilized and incubated in moist chambers. However, in the case of field "infested" soil, there was no way of assuring that inoculum was present. Attempts

to recover spores using the Ledingham and Chinn (34) flotation technique were unsuccessful. Similarly, water elutriate of field "infested" soil atomized onto the foliage of healthy peanut plants failed to produce any infection.

Heavily-infected peanut debris collected after harvest and composed mainly of defoliated leaves was non-infective when applied before emergence (Table XI) whether the peanut seeds were germinated prior to planting or sown directly. The same treatment with splashing in the post-emergence tests (Table XII), resulted in only a small number of plants being infected.

The results on the pre-emergence infection (Table XI) show some evidence that where enough inoculum is present in the vicinity of germinated seeds, e.g. in the case of artificially infested soil, lesions that were confirmed to bear sporulating *C. arachidicola* stromata could develop on cotyledons as early as 10-14 days after planting. Leafspot symptoms were frequently observed on the lower leaflets coming in contact with soil surface.

Necrotic lesions were also observed on cotyledons of plants where dried infected tissue, field peanut debris, and overwintered peanut debris were used, but no sporulation was noticed upon incubation except in one test where dry, infected tissue produced disease symptoms on either cotyledons or lower leaflets in a small number of the plants treated (Table XI, Test 3).

In the tests on post-emergence infection (Table XII), artificial infestation of a soil mix in which germinated and non-germinated seeds were planted, consistently resulted in the development of leafspot symptoms on the foliage. Such an infection would be expected if viable

inoculum was being splashed from the surface of infested soil. Splashing seems to be necessary to incite leafspot infection on the foliage. In pre-emergence tests where no splashing occurred, C. arachidicola lesions mostly developed on the cotyledons and occasionally on the lower leaflets coming in direct contact with artificially infested soil.

Where dry infected peanut tissue was used as a potential inoculum source, typical leafspot lesions were also consistently observed on the foliage. However, field peanut debris collected after harvest did not often produce disease symptoms when used as an inoculum source, although sporulating stromata were observed when samples of field debris were incubated in moist chambers.

Overwintered debris, similarly, failed to induce leafspot infection on healthy peanut plants in any of the tests. Here again, sporulation was checked and lesions were found to bear sporulating stromata, though not as profusely as in the case of hand-picked infected tissue that was stored dry for later use.

The partial and complete failure of field peanut debris and overwintered debris, respectively, to reproduce infection on peanut foliage could not satisfactorily be explained. Although the soil mix was autoclaved for 6-8 hours, sterilization was never complete. There was also the possibility that fast growing saprophytes were reintroduced with different forms of infected peanut tissue used as sources of inoculum of C. arachidicola. Competition with these saprophytes for food material in field debris and decomposed or decomposing overwintered tissue may be one reason for the inability of Cercospora to grow and infect under the conditions of these experiments.

## CHAPTER VI

### SUMMARY

1. Cercospora arachidicola in infected lesions on peanut leaflets, petioles and stem segments placed on the soil surface or buried at different depths was found to overwinter through the non-cropping period in Oklahoma as dormant stromata.

2. Although some C. personatum-infected leaf material was mixed with C. arachidicola-infected peanut tissue in the 1978-79 study at Perkins and Stillwater, no C. personatum sporulating stromata were observed. All cultures isolated, were identified as C. arachidicola.

3. No measurable effects on survivability of the early leafspot fungus due to depth of burial, field and cold room exposure, temperature or moisture, could be detected. Cercospora arachidicola survived a wide range of temperature, moisture, and depth of burial conditions. Sporulation was observed on recovered samples after being buried in the field for more than eight months. Stromata on samples stored in the cold room for 19 months were still capable of sporulation when incubated in moist chambers.

4. Isolated cultures of C. arachidicola recovered from overwintered peanut tissue produced pathogenic reactions upon inoculation of detached peanut leaflets.

5. The ascosporic stage (Mycosphaerella spp.) could not be

detected in Oklahoma, and may not play a major role in the initiation of primary infection on peanuts early in the season.

6. The earliest C. arachidicola could be observed was April 19 on vaseline-coated slides. The traps were set in an isolated peanut plot where infected debris was left to overwinter under natural conditions.

7. In an irrigated peanut field, C. arachidicola conidia were observed on vaseline-coated tapes when the plants were 41 days old. However, Cercosporidium conidia were first observed on September 7, almost four months after planting.

8. Less conidia were observed during a rainy period than during a period immediately following a rain. Under conditions of drought and high temperatures, low concentrations of Cercospora and Cercosporidium conidia were usually observed.

9. More Cercospora and Cercosporidium conidia were trapped between 16:30 p.m. - 22:30 p.m. than during any other period. This trend was visible throughout the study, and especially during the last six weeks.

10. Under experimental conditions, pre-emergence infection occurred mostly on the cotyledons and frequently on the lower leaflets when an available source of conidia was present in the vicinity of germinating peanut seeds.

11. Leafspot infection occurs mostly after emergence. Splashing is necessary to disperse potential inoculum from infested soil, infected peanut tissue, and other infection loci (cotyledons and lower leaflets) occurring during the pre-emergence stage.



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