LEAFSPOT REACTIONS OF CERCOSPORA ARACHIDICOLA AND CERCOSPORIDIUM PERSONATUM ON PEANUTS AND OTHER SELECTED PLANT SPECIES

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CHAPTER I

INTRODUCTION

The peanut is an important food and oil crop in the warmer areas of six continents, and it is a major field crop on sandy soils in southern sections of the United States, especially in the Southeast (4).

Cercospora leafspots are probably the most serious diseases of peanuts on a world wide basis and also the most studied. The annual estimated economic losses from Cercospora leafspots in the United States, where chemical control measures are generally used, averaged about 10% from 1951 to 1960. In many other areas of the world losses are considerably higher (4, 31).

In 1933 Woodroof (73) established that two Cercospora species were present in the United States. Cercospora arachidicola Hori was the most common species. Cercospora personata (Berk. and Curt.) Ellis and Everhart was found to be sporadic in its appearance, however, when it was present, it was probably the most destructive. A third Cercospora, Cercospora canescens Ellis and Martin, has been reported as an infrequent pathogen on peanut in northern Nigeria (31).

Jenkins (32) described the ascomycetous stage of C. arachidicola and C. personata as Mycosphaerella arachidicola W. A. Jenkins and Mycosphaerella berkeleyii W. A. Jenkins, respectively. He also reported that C. arachidicola is generally abundant in August and early September, while C. personata (Cercosporidium personatum) is most prevalent from

September to harvest.

Deighton redefined the genera Passalora and Cercosporidium in 1967. He changed Cercospora personata (Berk. and Curt.) Ellis and Everhart to Cercosporidium personatum (Berk. and Curt.) Deighton (20). He also renamed the perfect stage of the C. arachidicola organism, Mycosphaerella arachidis Deighton due to the previous use of M. arachidicola Chochrjakov for a different leafspot of peanut in the Caucasus region of the U.S.S.R. This fungus has Ascochyta adzamethica Schoschiaschvii as a condial stage (21).

The reaction of a peanut plant to a Cercospora infection varies with the host variety, pathogen biotype or species, environmental conditions, and growth stage of host. The usual reaction of a susceptible plant to infection is the production of brown to black circular lesions 2-3 mm in diameter, which are frequently surrounded by a halo of yellow tissue. Abscission of the leaflets frequently occur in 2-3 weeks if several lesions are present (1, 27, 31, 32, 45).

The host range of the peanut Cercospora spp. has generally been considered to include only members of the genus Arachis (17, 31). A report of Cercospora arachidicola causing leafspot and severe defoliation of Stylosanthes sp. occurred in 1966 (1). The host ranges of a large number of Cercospora species have been inadequately determined due to the difficulty in producing conidia for cross inoculation. Therefore, the potential of other hosts for peanut Cercospora and Cercosporidium does exist.

Detached leaves have been used in numerous studies of pathogen disease cycles and host parasite interactions. The use of detached leaves is an attractive technique since it requires less space and test

material than other methods. With this technique it is possible to use a large number of isolates with little risk of contamination. Therefore, detached leaf techniques were emphasized in this study.

The purpose of this investigation was: 1) to determine if different isolates of Cercospora arachidicola and Cercosporidium personatum produce consistent differential leafspot reactions on different cultivars or species of peanuts; 2) to determine if additional hosts of Cercospora arachidicola and Cercosporidium personatum occur; 3) to establish a set of differentials for determining pathogenic species and races of Cercospora and Cercosporidium leafspots of peanuts if consistent differential leafspot reactions were discovered.

CHAPTER II

LITERATURE REVIEW

Detached Leaf

Detached leaves cannot be kept alive indefinitely in research studies. However, under the best conditions most detached leaves can be kept in good condition for about three weeks. Individual leaves have been kept alive for periods of up to six years (75).

Hooker and Yarwood (29) floated detached leaves of corn and Oxalis corniculata L. in glass petri dishes. Corn leaf sections floated in 5% sucrose solution with 20 ppm kinetin remained green and alive 14 to 21 days, sometimes for 34 days. Some corn genotypes were observed to live longer than others. Corn sections in distilled water deteriorated in 5-7 days. Oxalis leaves floated in distilled water lived 21-28 days. Addition of sucrose or kinetin did not increase survival time. Clinton and McCormick (18) found that the excised leaves of clover were too tender, and that death of leaves often occurred too early to secure definite results from rust inoculations. However, Waters (69) secured satisfactory rust infections on excised leaves of Alsike clover.

Borges et al. (12, 13) found that detached leaves of the Medicago sativa-falcata complex floated on water containing 5% sucrose was a suitable tool for studying biotypes of Stemphylium botryosum. Some workers had no contamination problems with clean but non-sterile procedures in their

work (12, 29). Other workers found that addition of carbohydrates to the suspension solution caused contamination problems (46, 54).

Verma and Petrie (66) used 0.25 ppm of benzyladenine and 0.8% agar in water as his suspension medium. The lower surfaces of detached turnip leaves were pressed into the medium. The addition of agar prevented the leaves from moving around in the petri dishes. Morrison (46) placed leaf disks of Helianthus annuus and Zinnia elegans on glass rod triangles in 100 mm petri dishes with porous filter paper and 25-30 ml of distilled water. He could maintain leaves of Helianthus 5-6 weeks and 7-8 weeks for Zinnia. The addition of 0.4 ppm of kinetin could increase the leaf usefulness to 7 and 10 weeks respectively.

Waggoner and Wallin (68) found that washing potato and tomato leaves in tap water and placing them on moist filter paper in petri dishes was a suitable technique for studying Phytophthora infestans. Lapwood and McKee (38) used a different technique to study infection of detached potato leaves by Phytophthora infestans. He placed detached leaves on terylene netting suspended in trays. The stacked trays were wrapped in polyethylene film after inoculation.

Mignucci (44) rooted excised unifoliolate leaves and trifoliate leaflets of soybeans on moistened absorbent paper in sterile trays covered with plastic. Leaves and leaflets produced adventitious roots, but no shoots. The rooted leaves were maintained for 2-3 months floating on sterile deionized water in petri dishes. The rooted leaves could be maintained up to nine months if placed in autoclaved sand and soil.

Melouk and Banks (42) developed a detached leaf method for screening peanut genotypes for resistance to leafspot caused by

Cercospora arachidicola. Individual leaf petioles, each supported by a foam rubber plug, were immersed in Hoagland's solution in test tubes. The inoculated leaves were placed in a polyethylene chamber on a greenhouse bench and maintained at 80 to 90% relative humidity. Cercospora lesions appeared in 8 to 10 days and leaflet defoliation began in 18 to 21 days after inoculation.

Scientists have been able to identify many race-host interactions with the various detached leaf methods. Their findings often corresponded to attached leaves. Waggoner and Wallin (68) found the same race-host interaction of potato isolates and tomato isolates of P. infestans on detached leaves of potato and tomato as on intact greenhouse plants. Since the detached leaves in the test were as nearly alike as possible, the detached leaf method did not pick up the greenhouse observation that a race from potato killed lower tomato leaves but not upper leaves. Detached leaf ratings of susceptible and resistant strains of turnip rape to white rust corresponded well with those of attached leaves and field grown plants (66).

A number of studies indicate that detached leaves of small grains may not react to a pathogen in the same way as an attached leaf (6, 14, 15, 54). Samborski et al. (54) found that the wheat cultivar Khapli, which is normally resistant to race 15B of stem rust became susceptible when detached and floated on water. The addition of 40 ppm benzimidazole caused the floated leaves to remain resistant to the rust. However, the addition of 1% glucose with the benzimidazole caused the cultivar to become susceptible again. A variety normally susceptible to race 15B stayed susceptible when floated in water or with benzimidazole added.

Browder (14) and Atif and Wilcoxson (6) observed that the

detached leaf study technique resulted in greater susceptibility of wheat to stem rust. This phenomenon was also found with crown rust and stem rust resistance in oats. Breakdown of rust resistance in oats was sometimes more rapid and complete in darkness than in light (15). Atif and Wilcoxson (6) also found that stem rust severity on wheat was significantly greater on leaves detached before inoculation, than after inoculation. The incubation period was significantly longer on leaves detached after inoculation.

Person et al. (49) discovered that benzimidazole solution prevented destruction of leaf chlorophyll. Carbohydrates supplied to excised leaves were found to be important for development of some rusts and powdery mildews (41, 64, 69). Yarwood (74) found that detached leaves collected in mid to late afternoon were more viable due to the increased carbohydrate present in the leaves at that time.

Culture Requirements

Miller (45) tested 40 different animal and vegetable products for a suitable culture medium for Cercospora. The best natural nutrient sources were sweet potato, beerwort, yeast extract, immature peanut seed, and cherry juice. High carbohydrate nutrients generally produce better vegetative growth while higher protein media usually supported more sporulation. Miller developed a peanut hull, sprouted-barley, sweet potato, basal-liquid medium that produced a more infective inoculum than his semi-synthetic basal agar cultures.

Sulaiman and Hande (62) found that nonsynthetic media like potato-dextrose agar, Czapeck's agar with yeast extract, radish-dextrose agar, and carrot-dextrose agar were favorable to growth and sporulation of

C. arachidicola and C. personata (Cercosporidium personatum). Synthetic media were unfavorable to growth and sporulation. Shantas' (55) work with C. personata (Cercosporidium personatum) agreed with Sulaiman's. He found that synthetic media were unfavorable to growth and sporulation unless yeast extract was added.

Abdou (1) found that good sporulation of C. arachidicola occurred on peanut leaflet extract, oatmeal, lima bean and mycopil agar media at room temperature. Peanut leaflet extract and oatmeal agar yielded the greatest sporulation. Abdou also found that light was not necessary for normal sporulation.

Smith (57) produced abundant conidia of C. arachidicola on peanut oatmeal agar with incubation under continuous cool-white fluorescent light at 28 C.

Abdou (1) found that good sporulation of C. personata occurs on peanut leaf extract, oatmeal and cornmeal agars at room temperature. It was also found that light was necessary for sporulation on peanut leaf extract agar, although light was not required for sporulation on naturally infected peanut leaves. Neutral light was best. Yellow, green and blue light also gave good conidial production. Orange light completely inhibited sporulation.

Miller (45) determined the temperature growth range for three cultural races of C. arachidicola on basal agar (semi-synthetic). The cardinal temperatures were: Race 1A = 4 C, 32 C, 35 C; Race 2A = 5 C, 25 C, 34 C; Race 3A = 2 C, 25 C, 35 C.

Sulaiman's (62) examination of an isolate of C. arachidicola from the Poons district of India on potato-dextrose agar yielded similar findings. The isolate could grow at temperature below 10 C to 32 C,

however, sporulation was good only from 24 C to 28 C.

Miller (45) determined the temperature growth ranges for three cultural races of C. personata on basal agar. The cardinal temperature for the C. personata races were: Race 1P = 4 C, 30 C, 34 C; Race 2P = 4 C, 25 C, 34 C; Race 3P = 4 C, 30 C, 34 C.

Sulaiman's and Hande's (62) examination of two isolates of C. personata (Cercosporidium personatum) on potato-dextrose agar yielded similar results. Isolate one could not grow at 10 C and there was no sporulation at 32 C, although the isolate sporulation was 24 C to 26 C. Isolate two would grow below 10 C and at 32 C. The best growth and sporulation was between 24 C and 28 C.

Shanta (55) found that only young cultures of C. personata (Cercosporidium personatum) sporulate and that sub-culturing had to be made by transferring spores at intervals of 2-3 weeks. Colonies resulting from mycelial transfer did not sporulate. When mixtures of mycelium and conidia were used in making transfers, the sporulating character was gradually lost in 8-9 months. Nagel (48) also found that transfer of conidia was preferred over transfer of mycelium.

Inoculation and Infection

Cercospora arachidicola and C. personata (Cercosporidium personatum) inocula have been prepared in several ways. A common method of obtaining inoculum was to flood inoculated culture plates with 20-30 ml of distilled water and brush the conidia free with a camel hair brush (1, 26, 57). Melouk (43) also flooded his culture plates, however, he did not use a camel hair brush to loosen the conidia. Abdou (1) added 3-4 drops of Tween 80 per 100 ml of inoculum. The

resultant suspension was filtered through one to four layers of cheesecloth to remove large mycelial fragments. Ramakrishna and Apparao (52) and Chahal and Sandhu (16) obtained Cercospora inoculum by washing infected peanut leaves in sterile water, incubating the washed leaves for 72 hours and then washing the spores from the leaves. Others used a more natural technique for obtaining their inoculum. They placed pots of peanuts outside during the rainy season and spread peanut leaves infected with C. arachidicola over and around each pot (16, 24).

Hassan and Beute (26) found that inoculum suspensions of 5,000, 10,000 and 15,000 conidia per ml made little difference in the relative position of the peanut cultivars in number of C. arachidicola lesions produced, although the total number of lesions per cultivar decreased with the lower inoculum concentrations. The relative position of the cultivars remained approximately the same. Abdou (1) found that approximately 15,000 conidia of C. arachidicola per ml gave adequate lesions for suitable disease evaluation. Melouk and Banks (42) preferred to use 20,000 conidia per ml in their disease evaluation of detached leaves.

The inoculum was applied to peanuts in several ways. The inoculum was frequently applied by an atomizer (1, 26, 42, 52). Other ways include application with a camel hair brush to the leaf surface (27); spashing the inoculum on the plants (45); or simply allowing rains to spash the spores on the test plants (16).

The handling of inoculated plants in greenhouse studies during the incubation period varied slightly on several points. The inoculated plants were generally placed in an incubation chamber with humidity levels varying from 85-100% and for periods of 2-8 days at temperatures

from 24-30 C (1, 3, 45, 52, 60, 63). Miller (45) found that inoculated plants needed light during incubation to obtain good infections.

Tests on Standard Jumbo Runner cultivar showed that race 1A of C. arachidicola caused infection at temperatures from 10 C to 32 C with optimum about 29 C. The optimum temperature for lesion development on leaves infected with race 1A was 18 C. This was for size of lesion not rate of development. Leaf shedding of race 1A infected leaflets was most rapid at 35 C, but occurred at 18-40 C. Lesion diameter increased in size, but no shedding occurred below 18 C (45).

Miller (45) found that race 1P of C. personata (Cercosporidium personatum) caused infections at temperatures from 16 C to 32 C with optimum at 24 C. The optimum temperature for large leaf lesion development for race 1P was 24 C. Leaf shedding of race 1P infected leaflets occurred from 21 C to 40 C with optimum temperature of about 30 C. Temperature below 21 C enabled the lesions to slowly enlarge without leaf shedding.

Rathaiah (53) reported that Cercospora beticola developed more penetrations of sugarbeet stomata under nightime wetting and daytime drying compared to continuous wetting or with nightime drying and daytime wetting.

Host Range

It has generally been held that the Cercospora species are fairly strict in their parasitism, each species being limited to one host species or to a few very closely related host species (59). Lieneman (40) listed 516 Cercospora species in North America in 1929. Most of these species have a single host. Many of the species differ from each

other only slightly morphologically, however, due to their presence on different hosts, they have been made valid species (47, 70).

The host range of Cercosporidium personatum appears to be very limited. It is believed to only infect species of Arachis (17, 31). Tharp (63) reported a collection of Cercospora personata (C. personatum) on living leaves of Cassia occidentalis L. at Palestine, Texas, on October 30, 1915. He believed that the collection was not C. personata var cassia occidentalis because it did not have slender articulated threads and one septate spores which are typical of C. personata var cassiae occidentalis. Chupp (17) states that reports of C. personata on Cassia are erroneous and that the fungus only occurs on peanut, Arachis hypogaea. He also states that C. personata var cassiae occidentalis is not related to C. personata (Cercosporidium personatum), but is a synonym of Cercospora occidentalis. Abdou (1) mentions an unpublished report from W. C. Cooper of North Carolina State University which states that C. personata (C. personatum) infected Stylosanthes sp. Abdou was unable to verify this report in greenhouse studies.

The host range of Cercospora arachidicola also appears to be limited. Chupp (17) reports that the fungus only occurs on the genus Arachis. However, Abdou (1) in 1966 found an isolate of C. arachidicola that could cause distinct lesions with severe defoliation of Stylosanthes sp. in greenhouse studies.

Conway and Freeman (19) found that Cercospora rodmanii has a limited host range. In a study of 58 species representing 22 families, C. rodmanii infected green leaves only on waterhyacinth, Eichhornia crasspies. The study also indicated that the fungus could infect older or dying leaves from four additional species representing three plant

families.

A similar finding with Cercospora brassiciola is reported. Of 11 genera and 12 species representing 8 families, only the genus Brassica was highly susceptible. However, four genera and species representing two families showed slight susceptibility. The study also showed that C. brassiciola became progressively more severe as the age of inoculated hosts increased. Disease on 45 day old plants was very severe while symptoms on 25 and 35 day old plants were considerably milder (37).

This finding also is supported in studies on sweet clover by Jones (35). He found that Cercospora infections were far more successful on older leaves of plants and on stems of second year growth after blossoming.

Many researchers have had difficulty in cross infection among species of Cercospora from legumes (9, 35, 47). However, other workers have been able to obtain cross infections easily with the same Cercospora spp. from legumes (11, 39). Part of the difference may be in the inoculum. Many workers used macerated mycelium plus conidia as inoculum (11, 19, 25, 33, 39). Other workers use only conidia as inoculum (9, 37, 47).

Berger (10) found that cross infections of Cercospora isolates from Trifolium, Medicago and Melilotus were generally more pathogenic on species within their own genera, however, exceptions did occur. This phenomenon was also reported by Latch (39). Their studies indicated that less host specificity may exist for Cercospora on the legumes than previously thought.

The diversity of hosts infected by some Cercospora spp. in host range studies indicate that these Cercospora spp. have a very wide host

range. There may also be considerable synonymy in some common species of Cercospora (11, 25, 33, 39, 65, 67). These reports show that in determining host range for Cercospora, the use of only related plants could miss a large number of host species, since many of the species of Cercospora will infect a nonrelated host species but not infect a closely related species (25, 34).

Cercospora Leafspot Resistance

The criteria for resistance to Cercospora leafspot on peanut is very important. If the size of lesions was used, one variety would be more susceptible. If the number of lesions was used, another variety might be more susceptible (23, 27). The percentage of abscised leaves is another criterion that is sometimes used (60).

Aulakh et al. (7) developed two types of disease ratings for Cercospora leafspot. One type was rating plants as very susceptible to highly resistant based on the percentage of leaf area diseased. The second type of disease rating was based on the incidence of disease. The disease rating ranged from a trace (highly resistant) to very heavy (highly susceptible).

Hemingway (27) noted that varieties with light-green foliage were without exception susceptible or very susceptible to Cercospora leafspot. All dark-green varieties showed some degree of resistance. He also observed that the darker leaves had much thicker palisade tissue. He thought that this characteristic might be a partial reason for the observed resistance.

Peanuts in a vegetative state of growth usually show more resistance to Cercospora leafspot than mature peanuts. Miller (45) found that he

could develop a more resistant peanut plant by clipping the gynophores throughout the growing season. It was also reported that spreading type peanuts were generally more resistant to Cercospora arachidicola and C. personata (Cercosporidium personatum) than bunch type peanuts (45).

Gibbons and Bailey (24) correlated resistance of field-grown peanuts to size of their stomatal apertures. Species with stomatal aperture of less than 12 mm had no lesions and species with larger mean stomatal apertures developed more lesions corresponding to the increase in the size of stomatal aperture. However, Hassan and Beute (26) did not believe that changes in stomatal size was the mechanism for increases resistance.

Races and Biotypes

The existance of races and biotypes of Cercospora from peanut is suggested in cultural studies by Miller (45). He identified 122 different cultural types of Cercospora arachidicola from 697 isolates. He was also able to identify five cultural types of C. personata (Cercosporidium personatum) from the five isolates examined.

Cultural races of both Cercospora remained phenotypically constant under similar environmental conditions for six years. The stable races might look different in different cultural conditions, but revert to original phenotype when environmental stimuli were removed. The original cultural identities of the races were retained on inoculation and reisolation from leaf lesions of peanuts.

Miller described differences in symptoms between his race 1A and race 2A of C. arachidicola on the peanut cultivar Spanish 146. Race 1A produced blackish leaf lesions with sharp margins, while race 2A

produced light brown lesions with a small yellow border. Race 1A was considered more pathogenic since it produced more large lesions and caused more rapid defoliation than race 2A.

Sulaiman and Hande (62) reported differences in pathogenicity of two isolates of C. personata (C. personatum) on the peanut variety, Spanish Improved. Isolate number one required an incubation period of 15-16 days and produced lesions 5-8 mm or less in size, mostly irregular, confluent, brown in color, without sharp margins and with a pale yellow faded halo on the upper leaf surface. Isolate number two produced lesions in 8-10 days. The mature spots were 2-5 mm or less, mostly circular and nonconfluent, dark brown to black in color with sharp margins and with a clear brown-yellow halo. Isolate number one caused no defoliation with 34% of leaf area infected; whereas isolate number two defoliated 70% leaflets with 30% of leaf area infected.

Field observations where a mixture of races were present revealed no striking differences in lesion expression on the same cultivar with either C. arachidicola or C. personata (Cercosporidium personatum). In fact there appeared to be a constancy of expression of varieties (45).

The identification of races in other species of Cercospora has been reported. Solel and Wahl (58) identified three races of Cercospora beticola on sugarbeet. The lesion type and intensity of sporulation were similar for the three races. However, the density of lesions on sugarbeet leaves was a consistent differential for the three races. No significant variation in relative lesion density occurred in up to five successive reisolation and reinoculation cycles of seven monospore subcultures.

The use of the number of lesions for identification of Cercospora

races has also been used for Cercospora sonjina on soybeans. The difference between the highly susceptible and intermediate cultivars was in number of lesions per plant rather than size of lesion or abundance of sporulation. Resistant cultivars had only occasional spots, and these were usually small and nonsporulating (5, 50).

CHAPTER III

MATERIALS AND METHODS

Inoculum Preparation

Peanut leaves infected with Cercospora arachidicola and Cercosporidium personatum were collected from various locations (Tables I and II). Single spore isolates were obtained from these leaves by picking off single spores from Cercospora lesions with a spore pick. The spore pick is a bristle from a camel hair brush glued to the tip of a dissecting needle. The conidia were picked from lesions under a dissecting microscope at a magnification of 40X with a spore pick sterilized by dipping in 95% ethanol. The spores were placed in a petri dish of peanut leaf extract agar (PLX). On fresh leaves the conidia were picked directly from the leaf without any sterilization of the leaf tissue. Contamination was seldom a problem.

Extra tissue preparation was made prior to picking conidia from dried leaves. The Cercospora spot was first cut out of the leaf and washed in running tap water for 15 minutes. The spot was then surface sterilized in 10% clorox for 90 seconds and placed in a petri dish containing water agar. Cercospora arachidicola lesions were placed with the upper leaf surface up and Cercosporidium personatum lesions were placed with the lower leaf surface up. The plates were placed under continuous fluorescent light at room temperature for 3-4 days and then

TABLE I

CERCOSPORA ARACHIDICOLA ISOLATES STUDIED

Isolate	Location Collected	Date Collected	Host Cultivar	Spot Description
A42A1	Stillwater, Oklahoma	October 9, 1977	Comet	Dark brown lesion, distinct yellow halo
A42B1	Stillwater, Oklahoma	October 9, 1977	Comet	Dark brown lesion, no halo
A75A4	Yoakum, Texas	September 12, 1978	Starr	Unknown
A78A1	Perkins, Oklahoma	October 21, 1978	Florunner	Small lesion, very faint narrow halo
A82B1	Ft. Cobb, Oklahoma	October 24, 1978	Tamnut 74	Unknown
A84A1	Stillwater, Oklahoma	November 1, 1978	Florunner	Very small lesion, faint very narrow yellow-green halo

TABLE II

CERCOSPORIDIUM PERSONATUM ISOLATES STUDIED

Isolate	Location Collected	Date Collected	Host Cultivar	Spot Description
P44B1	Perkins, Oklahoma	October 9, 1977	Comet	Unknown
P75B1	Yoakum, Texas	September 12, 1978	Starr	Unknown
P75C1	Yoakum, Texas	September 12, 1978	Starr	Unknown
P81A1	Stillwater, Oklahoma	October 21, 1978	Tamnut 74	Dark round lesion, no halo
P82A1	Ft. Cobb, Oklahoma	October 24, 1978	Tamnut 74	Unknown
P82C1	Ft. Cobb, Oklahoma	October 24, 1978	Tamnut 74	Unknown

single spores were picked off with the spore pick with the same procedure as with fresh leaves. Six single spore isolates of C. arachidicola and six single spore isolates of C. personatum were used in the test (Tables I and II).

Conidial and colony measurements were made of Cercospora arachidicola and Cercosporidium personatum isolates. Two isolates of Cercospora sp. collected from pencil-flower, Stylosanthes biflora, were also examined. Fifteen single conidia from each isolate were grown for nine days on peanut leaf extract (PLX) agar. The inoculated PLX agar plates were sealed with tape and maintained at 22 C under continuous fluorescent light of 2,530 lux.

Measurements were made with a micrometer eyepiece on an Olympus microscope. Conidial production was estimated by placing three single spore colonies into a drop of water on a slide (1). The spores were released by crushing the mycelium. A cover slip was then added. Examination of ten microscopic fields at 125X magnification was used to determine sporulation. Coloration of Cercospora colonies and media around the colonies was also noted.

The Cercospora and Cercosporidium isolates were grown on PLX agar. Preparation of PLX agar was slightly modified from PLX agar used by Abdou (1). The medium was made with 200 ml of peanut leaf extract, 800 ml of distilled water, and 12 gms of agar flakes. The peanut extract was prepared by boiling 100 gms of washed peanut leaves in 1000 ml of distilled water for 25 minutes. Distilled water was added to return the liquid to original level and then brought back to a boil. The extract was filtered through four layers of cheesecloth. No noticeable differences in Cercospora growth was observed between extract

prepared from fresh or frozen peanut leaves. Storage of the extract in frozen form did not appear to harm the Cercospora growth properties.

Cercospora and Cercosporidium isolates were grown on PLX agar in nine cm plastic petri dishes. The inoculated petri dishes were sealed with tape and placed under continuous fluorescent lights at 2530 lux and temperature of 21 C to 30 C.

The petri dishes were inoculated by using a flamed transfer needle to cut out blocks of agar covered with sporulating Cercospora or Cercosporidium isolates and streaking them on the new agar plates. The inoculated petri plates were used as an inoculum source after approximately two weeks growth.

The inoculum was prepared by washing the plates with 20 ml of sterilized distilled water containing one drop of Tween 20 per 100 ml of water. The plates were washed for 15 minutes and then decanted into a beaker. Spore counts of the spore suspension were made with an American Optical Bright-line Hemacytometer. The mean of three counts was used as the spore density unless counts of less than 5,000 conidia per ml were obtained, at which time five conidial counts were made. Inoculum with a spore density of 20,000 spores per ml was normally used. High concentrations of spores were diluted to the proper concentration with distilled water containing one drop Tween 20 per 100 ml of water.

Host Range

Host range studies with detached leaves were made in a Sherer Model CEL 512-37 growth chamber, a Sherer-Gillette Model CEL 255-6 growth chamber, and a climate control room. Host range studies were performed in the climate control room and the Sherer-Gillette Model CEL 244-6

growth chamber from 1978 through July 1979. All host range tests from June 1979 through February 1980 were made in the Sherer Model CEL 512-37 growth chamber.

The climate control room was constantly illuminated with fluorescent light at 2330 lux. Equipment difficulties prevented precise regulation of temperature. The temperature in the room fluctuated from 21 C to 30 C. Temperature measurements inside the petri plates were approximately 3 C above room temperature. Humidity in the room was not controlled, however, the moisture present in the petri plates should have produced a humid condition inside the dishes.

The Sherer-Gillette Model CEL 255-6 growth chamber was maintained at a temperature setting of 20 C night and 24 C day. The chamber was set for a 16-hour light period with a fluorescent light intensity of 3400 lux.

The Sherer Model CEL 512-37 growth chamber was set at a temperature of 22 C from 6 p.m. to 12 a.m. and at 27 C from 12 a.m. to 6 p.m. Temperatures taken inside the closed petri dishes showed a maximum temperature of 30 C at 5-6 p.m. and a minimum temperature of 22 C at 4-6 a.m. Only half of the fluorescent lights in the chamber were used. These lights were set to be on from 6 a.m. to 10 p.m. The fluorescent light intensity was 20,500 lux. The incandescent lights in the chamber were used from 10 a.m. to 8 p.m. Their intensity was 1,050 lux. A Hankscraft Model 240 humidifier was used to increase the humidity in the growth chamber. The humidity was 80% during light periods and 100% during dark periods.

Leaves and leaflets of host range test plants were collected from field and greenhouse grown plants. The leaves were normally collected

mid to late afternoon, since Yarwood (74) stated that leaves detached in late afternoon showed more vitality. The specimens were washed in running tap water for five minutes. Large leaves were cut into sections. The leaflets of compound leaves were normally detached from the leaf except in species where the leaves were very small. In Krameria a short section of stem with small leaves attached was used. The specimens were washed in 10% clorox for 90 seconds and then rinsed in tap water. Most leaves had a waxy cuticle which prevented wetting of the leaves except where injuries to the leaf were present. The leaf material was then placed in nine cm glass petri dishes containing three sheets of S-32915-D filter paper and five ml of tap water. The test plates were randomized and inoculated from all directions by applying a spore suspension to the leaf surfaces with a hand atomizer. The inoculum was applied until the leaves were thoroughly covered. Fifteen ml of inoculum was sufficient for inoculating up to 20 plates. All plates receiving the same inoculation were randomized to prevent biased inoculation of a test species. The Cercospora arachidicola inoculum was composed of 1-6 single spore isolates (Table I). The Cercosporidium personatum inoculum was also composed of 1-6 isolates (Table II).

A spore suspension was made as described in the inoculum preparation section. A spore density of 20,000 conidia per ml was desired for the test, however, this density could not always be used. In C. arachidicola a spore density as low as 11,000 spores per ml was used once and a couple of spore densities near 50,000 spores per ml were used. Spore densities were generally lower for C. personatum. Spore densities ranged from 2,000 conidia per ml to 17,000 per ml. The

average spore density for C. personatum was approximately 9,000 conidia per ml.

Peanut leaflets were also inoculated in all host range tests. If the peanut leaflets did not average at least three lesions per leaflet with at least 3/4 of the leaflets infected, the test was not considered valid.

Control plates were also prepared by spraying test leaves of host range plants as well as peanut leaves with a solution of one drop Tween 20 in 100 ml distilled water and other control plates with distilled water only. Three to six replicates of each treatment were made, depending on quantity of leaf material and space available. All test plates were placed in the climate control room or one of the growth chambers in a completely randomized design. Plates were lightly sprayed once each evening for 14 days with distilled water. After 14 days, distilled water was added only to prevent the plates from drying out. Periodic observations were made with a final observation made 30 days following inoculation.

A leaf was not considered infected with Cercospora or Cercosporidium until sporulation was observed. Representative single spore isolates were collected from these infections and cultured on PLX agar for two weeks. Representative isolates were then used to inoculate peanut leaflets. Since testing all Cercospora and Cercosporidium infections for pathogenicity would be prohibitive, 1-5 single spore isolates from each infected host range species was inoculated back to peanuts.

A further study for possible hosts of Cercospora arachidicola and Cercosporidium personatum was made by examining Cercospora lesions on

weeds and plants in and near peanut fields at Perkins and Stillwater, Oklahoma. Single spore isolates were made from these Cercospora. Cercospora isolates producing spores on PLX agar were used to inoculate peanut leaves by using standard inoculation procedures.

A final procedure of the host range study was to plant legumes and other selected plants into the rows of a peanut field severely infected with C. arachidicola (Table III). The plants were planted during August and September of 1979. These plants were lightly sprinkled with water at dusk each day. The plants were examined for Cercospora lesions until frost. Conidia from suspected Cercospora lesions were plated on PLX agar. Non-sporulating lesions were surface sterilized in 10% clorox and placed in petri dishes of water agar and incubated under continuous fluorescent light. The plates were examined for production of Cercospora spores at frequent intervals.

Single and Multispore Infections

Florunner peanut leaves were excised from plants late in afternoon. The excised leaves were third through fifth leaf from the terminal on 45 day old plants. The detached leaves were washed in running tap water for five minutes. The leaflets were then removed and washed in 10% clorox for 90 seconds and then washed in tap water for two minutes. Four washed leaflets were placed on three sheets of filter paper, S-32915-D, moistened with five ml of tap water in nine cm shallow glass petri dishes.

The peanut leaves were inoculated at different conidial levels from a C. arachidicola isolate, A75A4, grown for two weeks on PLX agar. A drop of distilled water was placed on each leaflet and then conidia were

TABLE III

PLANTS PLANTED IN PEANUT FIELD SEVERELY INFECTED WITH *CERCOSPORA ARACHIDICOLA*

Species Genus Common Name	Form Planted	
	As Plants	As Seed
Acacia angustissima Prairie Acacia	X	
Beta vulgaris Beet, Detroit Dark Red [b/]		X
Canavalia ensiformis Jack Bean		X
Cassia marilandica Maryland Sennea	X	
Cassia obtusifolia Sicklepod		X
Desmanthus illinoensis Illinois Bundleflower	X	
Glottidium vesicarium Bladder Pod	X	X
Glymnocladus dioica Kentucky Coffee-tree	X	
Medicago sativa Alfalfa [b/]	X	
Neptunia lutea Gold Plume	X	
Phaseolus vulgaris Bush Bean, Top Crop		X
Blackeyed Bean		X
Pinto Bean		X
Pisum arvense Austrian Winter Pea	X	
Rhamnus caroliniana Indian Cherry	X	
Robinia pseudo-acacia Black Locust [b/]	X	

TABLE III (Continued)

Species Genus Common Name	Form Planted As Plants	 As Seed
Sesbania exaltata Sesbania		X
Stylosanthes biflora Pencil-flower a/	X	

a/ Naturally infected by Cercospora arachidicola

b/ Naturally infected by other Cercospora spp.

hand picked from the inoculum plate with a camel-hair-spore pick and placed in the droplet of water. This operation took place under a dissecting microscope at 50X magnification. Treatment one contained one spore per droplet. Treatment two contained ten spores per droplet. Treatment three contained 20 spores per droplet. Treatment four was inoculated with a spore suspension applied with a hand atomizer. Treatment five was a control containing peanut leaflets lightly sprayed with distilled water. Five replications of each treatment were prepared. The plates were placed in a Sherer Model CEL 512-37 growth chamber.

Fifteen days after inoculation observations and measurements were made as to number of lesions, diameter of lesions, width of halo, halo color, halo characteristics and sporulation. Diameter of lesions and halo were made with a millimeter ruler. Sporulation was estimated by counting the number of spore producing stroma per lesion.

Observations were again made twenty-two days following inoculation. If a lesion had heavy sporulation, a change was made in sporulation measurement. The lesion was washed with five ml of 10% solution of Tween 20 in distilled water and then washed with five ml of distilled water. Five counts of the resultant conidial suspension were made with an American Optical Bright-line Hemacytometer. This would give an estimate of the total number of conidia washed from the lesion. Final observations were made 30 days after inoculation.

Cultivar Isolate Interaction

Leaflets of Florunner, Valencia and Tamnut 74 peanut cultivars and three wild peanut species were used in this experiment. The three wild

peanut species were P. I. number 276233 (Arachis sp., section RHIZOMATOSAE), P. I. number 262141 (A. cardenasii Krap. & Greg., (nomen nudum) section ARACHIS), and P. I. number 276234 (A. chacoense Krap. & Greg., (nomen nudum) section ARACHIS). The leaflets were collected from greenhouse plants, then washed and placed in shallow glass petri dishes as described in the host range study. The six single spore isolates of Cercospora arachidicola (Table I) were grown on PLX agar for two weeks. The inoculum was collected and prepared as described in section on inoculum preparation.

The conidial density was adjusted to 20,000 conidia per ml for each fungal isolate by diluting the spore suspension with distilled water containing one drop of Tween 20 per 100 ml of water. Four plates of each peanut variety or species were inoculated with each C. arachidicola isolate. Four petri plates were used as control for each peanut variety and species used in the study. The control plates were lightly sprayed with distilled water containing one drop of Tween 20 per 100 ml of water. All petri dishes receiving the same inoculation treatment were randomized on a table to prevent biased inoculation of cultivars. The inoculum was applied to the petri dishes of each isolate from all sides with a hand atomizer containing 30 ml of inoculum.

The inoculated plates were placed in a Sherer Model CEL 512-37 growth chamber with light and temperature settings as given in the host range study. The plates were lightly sprayed each evening with distilled water for ten days. After ten days, distilled water was added to plates only to prevent drying, and it was not applied directly on the leaflets.

Counts of the number of lesions per leaflet were made ten days following inoculation. The following measurements and observations were made on each plate 15 days and 30 days following inoculation: 1) leaflet color, 2) uniformity of leaflet color, 3) leaflet texture, 4) percentage of leaflet contaminated, 5) size and color of lesion halos, 6) percentage of leaflet covered with lesions, 7) amount of sporulation, 8) diameter of largest single lesion on leaflet.

The same study was made with six isolates of Cercosporidium personatum (Table II). Two modifications to the study were made. The conidial density of the inoculum was 3,000 per ml for isolates P75B1, P81A1 and P82A1 and 1,000 conidia per ml for isolates P44B1, P82C1 and P75C1. The second modification was the use of the number of lesions instead of the percentage of leaf coverage with lesions in observation number six.

Electron Microscope

Florunner peanut leaflets and pencil-flower, Stylosanthes biflora, leaflets were washed and in running tap water for five minutes and then surfaced sterilized in 10% clorox solution for 90 seconds. They were then washed for two minutes in tap water. Four peanut leaflets or five Stylosanthes leaflets were placed in glass petri dishes containing three sheets of filter paper moistened with five ml of tap water.

Treatment one was inoculation of peanut and Stylosanthes leaflets with Cercospora arachidicola isolate A75A4 at a spore density of 126,000 per ml. Treatment two was inoculation of peanut and Stylosanthes leaflets with Cercosporidium personatum isolate P44B1 at a spore density of 13,000 per ml. Treatment three was a control sprayed with distilled

water. Three replicates of each treatment for peanut and Stylosanthes were made.

The plates were placed in a Sherer Model CEL 512-37 growth chamber for 60 hours and 80 hours with the temperature and light settings as indicated in the host range study.

Sections about two mm square were cut from the inoculated peanut and Stylosanthes leaflets at the two different times and fixed in buffered glutaraldehyde for 2 hours. The specimens were washed three times in ten minute buffered washes. The tissue was then post fixed in 2% OsO_4, a mixture of 1:1 osmium to cacodylate buffer, for two hours. The tissue was dehydrated in the following steps:

a. ½ osmium to ½ 50% OH as wash.

b. 50% ETOH as wash.

c. 50% ETOH as wash.

d. 70% ETOH for ten minutes.

e. 90% ETOH for ten minutes.

f. 95% ETOH for ten minutes.

g. 100% ETOH for ten minutes.

The specimens were then critical point dried in a Polaron Critical Point Drier with CO_2 as transition phase. The leaf tissue was attached to metal studs with double sided tape. The specimens were coated with gold-palladium for two minutes in a Hummer II sputter-coater device. At this point the specimens were examined with a JEOL JSM 35 scanning electron microscope at magnifications from 600X to 6000X.

The observations were directed toward discovering the method of penetration of pencil-flower by C. arachidicola and C. personatum.

Observations of the inoculated peanut leaflets were used for a comparison of fungal activity.

CHAPTER IV

RESULTS

Host Range

A wide range of reactions to detached leaf treatment occurred among the host-range-test species. Many detached peanut leaflets could be maintained for considerable time in moist petri dishes with little adverse effect. In one test detached peanut leaflets were kept for two months with little loss of color or texture. However, certain host-range-test species deteriorated rapidly. Scurf-pea would turn dark and produce a yellow color on the moist filter paper in a couple of days. This made it difficult to perform a good Cercospora pathogenicity evaluation with this species on moist filter paper in petri dishes.

None of the other species were as sensitive as scurf-pea, however, several species sometimes started leaf deterioration within a week (Table IV). Pencil-flower, bladder pod, and wild peanut P.I. 276235 were extremely sensitive to excess moisture. The leaflets could be kept in good form for a considerable time if they were maintained with little free moisture. If considerable free moisture was present the leaves tended to deteriorate rapidly.

Collecting field grown plant material for detached leaf studies sometimes brought in fungal contaminates even when no visible symptoms

TABLE IV

PLANTS INCLUDED IN DETACHED LEAF STUDY OF HOST SPECIFICITY OF *CERCOSPORA ARACHIDICOLA* AND *CERCOSPORIDIUM PERSONATUM*

Family Genus and Species Common and Cultivar name	*C. arachidicola*	*C. personatum*
Chenopodiaceae		
Beta vulgaris		
Beet, Detroit Dark Red	13/38	0/16 [d]
Swiss Chard [c]	7/21	0/16 [d]
Chenopodium sp.		
Goosefoot	36/89	0/64
Fagaceae		
Quercus Virginiana		
Live Oak	6/28	0/16 [d]
Krameriaceae		
Krameria secundiflora		
Sandspur	0/30	0/18 [d]
Leguminosae		
Acacia angustissima		
Prairie Acacia [c]	0/18 [d]	0/18 [d]
Amorpha canescens		
Lead Plant	0/30 [d]	0/30 [d]
Arachis cardenasii		
Wild Peanut, P.I. 262141	53/120	2/120
Arachis chacoense		
Wild Peanut, P.I. 276235 [c]	45/153	0/186
Arachis sp.		
Wild Peanut, P.I. 276233	14/144	0/144
Baptisia leucophaea		
False Indigo	0/12 [d]	0/12 [d]
Canavalia ensiformis		
Jack Bean	0/27	-
Cassia marilandica		
Maryland Sennea	0/12 [d]	-
Cassia obtusifolia		
Sicklepod	10/80	-

TABLE IV (Continued)

Family Genus and Species Common and Cultivar name	C. arachidicola	C. personatum
Leguminosae (continued)		
Coronilla varia Crown Vetch	0/40 d/	0/40 d/
Desmanthus illinoensis Illinois Bundleflower c/	0/35	0/21 d/
Desmodium sp. Large Leaf Tick-clover	0/24 d/	0/24 d/
Desmodium sp. Large Leaf Tick-clover	0/30 d/	0/30 d/
Gleditsia triacanthos Honey Locust	0/21 d/	0/21 d/
Glottidium vesicarium Bladder Pod c/	40/131	-
Lathyrus odoratus Sweetpea	3/52	0/52
Melilotus alfa White Sweet Clover	3/32 d/	0/32 d/
Melilotus officinalis Yellow Sweet Clover	2/24 d/	0/24 d/
Medicago sativa Alfalfa, Cody	45/78	0/76
Pisum arvense Austrian Winter Pea	15/56	0/79
Psoralea sp. Scurf-Pea c/	0/64	0/64
Stylosanthes biflora Pencil-flower	10/102	6/102
Vicia villosa Winter Vetch	10/64	0/64
Vicia sp. Vetch	2/32 d/	0/32 d/

TABLE IV (Continued)

Family Genus and Species Common and Cultivar name	C. arachidicola	C. personatum
Onagraceae		
Oenothera sp. Evening Primrose	6/28	0/16 [d]
Oxalidaceae		
Oxalis corniculata Creeping Wood-sorrel	8/78	1/64

a/ Numerator - number of leaves infected

b/ Denominator - number of leaves or leaflets inoculated

c/ Species with tendency to rapidly deteriorate as detached leaves

d/ Results from a single test

e/ No C. arachidicola or C. personatum infections occurred on any non-inoculated control leaves of any of the host-range-test species.

were observed on the leaves. The test leaves were surface sterilized in 10% clorox. However, symptomless infections were not eliminated. Greenhouse grown plants also contained fungal contaminates.

Sweet clover and pencil-flower both frequently produced a contaminate Cercospora during the study. The presence of this contaminate required careful observations to prevent incorrect identification as Cercospora arachidicola or Cercosporidium personatum infections. The Cercospora contaminate developed an indistinct stroma instead of a distinct stroma. Also the Cercospora contaminates produced a loose spreading mycelial growth on PLX agar rather than the compact growth typical of C. arachidicola or C. personatum. The Cercospora contaminates would not infect peanut.

Alternaria spp. and Colletotrichum spp. were the two most common contaminates. Colletotrichum was especially serious on vetch and wild peanut, P.I. 276235. It could degrade test leaves in a very short time. Alternaria was the most common contaminate with most test species. This contaminate was especially troublesome due to its production of a black stroma that closely resembles a nonsporulating Cercospora stroma. Consequently, only sporulating Cercospora could be used for reliable infection readings.

Cercospora arachidicola spores were collected from 19 of 31 plant species tested as detached leaves (Table IV). C. arachidicola was capable of producing sporulating lesions on healthy tissue only in pencil-flower and wild peanut, P.I. 262141 and the commercial peanut cultivars, Florunner, Tamnut 74, and Valencia. C. arachidicola could invade healthy tissue of alfalfa and the rhizomatous peanut P.I. 276233 (Figures 1 and 2), but lesions did not develop beyond a black fleck

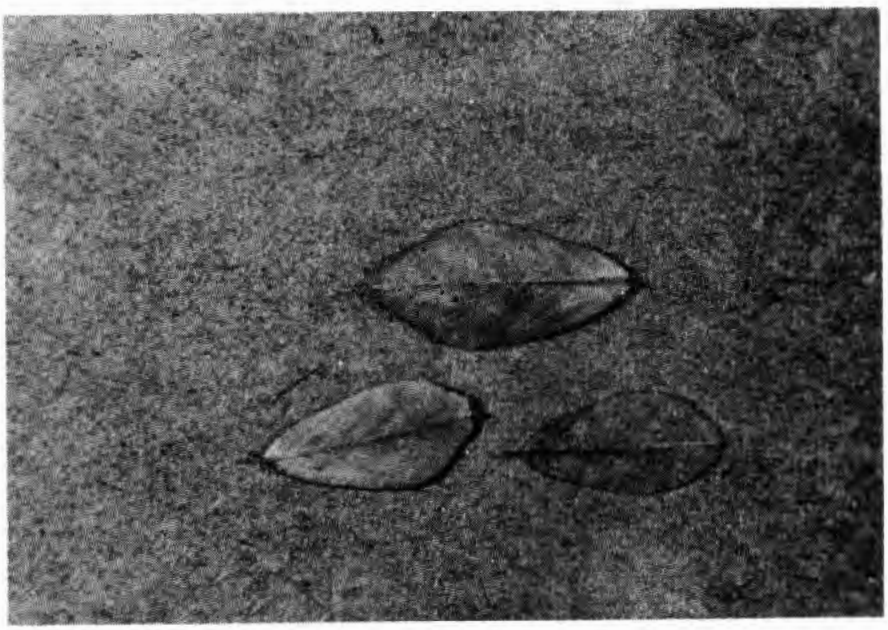

Figure 1. Small Cercospora arachidicola Lesions on Alfalfa, Medicago sativa, Leaflets 13 Days Following Inoculation. Distinct Light Green Halos Present Around Lesions.

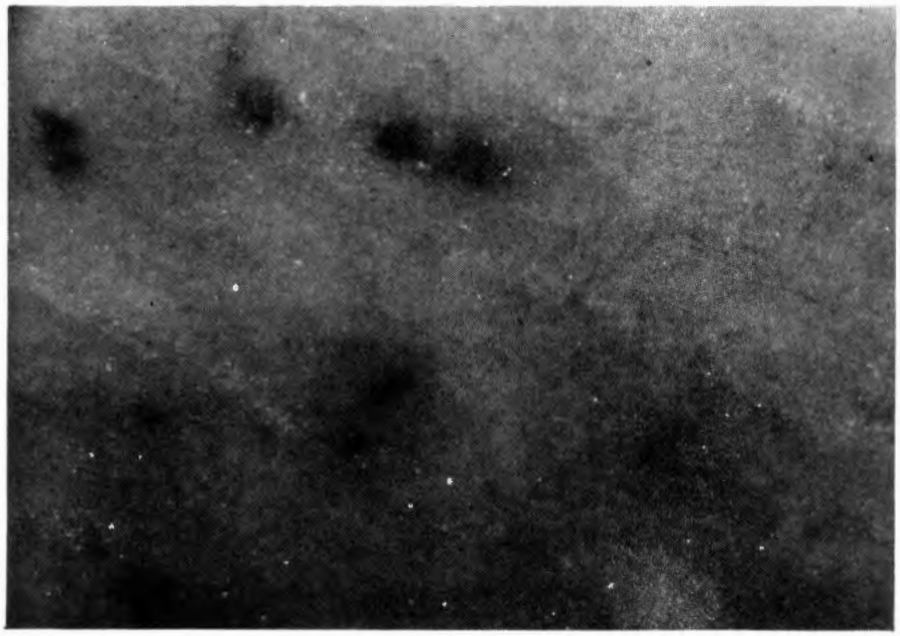

Figure 2. *Cercospora arachidicola* Lesions on Green Leaflet of Wild Peanut, P.I. 276233. No Sporulation Present 30 Days Following Inoculation. No Stromata Present. 32X.

until the leaflet tissue started to deteriorate. At that time a distinct stroma developed and sporulation occurred (Figure 3).

Green areas of leaflets containing black flecks were washed, surfaced sterilized and incubated on PLX agar. Abundant C. arachidicola conidia developed in the flecks in 5-10 days.

C. arachidicola appeared to act as a very weak parasite on many host species. In these hosts Cercospora infected tissue that had been injured through mechanical injury, by heat and drying, or oedema. Figure four shows a cut edge of beet leaf with a row of sporulating C. arachidicola stromata. Heavy sporulation of C. arachidicola was common along cut edges of beet, Chenopodium sp., live oak, sicklepod, and Swiss chard. The infected leaves often remain green except for a row or two of cells along the cut edges. C. arachidicola infections often occurred on petiole tips of Chenopodium sp. (Figure 5). Abundant sporulation of C. arachidicola sometimes occurred in wilted or dead leaf areas of susceptible host-range-test species. However, sporulation was generally not as heavy as infections along cut or injured edges. C. arachidicola very seldom occurred on a wet portion of the leaf. The raised portions of the leaves were normally infected.

The host range study of Cercosporidium personatum revealed very few hosts. Significant sporulation occurred only on pencil-flower and wild peanut, Arachis cardenasii P.I. 262141 and the commercial peanut cultivars. A very weak infection occurred on creeping wood-sorrel, Oxalis corniculata. This infection produced only a few conidia in a necrotic fleck on the upper surface of the leaf (Figure 6). The infections on pencil-flower and Arachis cardenasii were distinct brown to black lesions. Sporulation occurred on the lower leaflet surface in

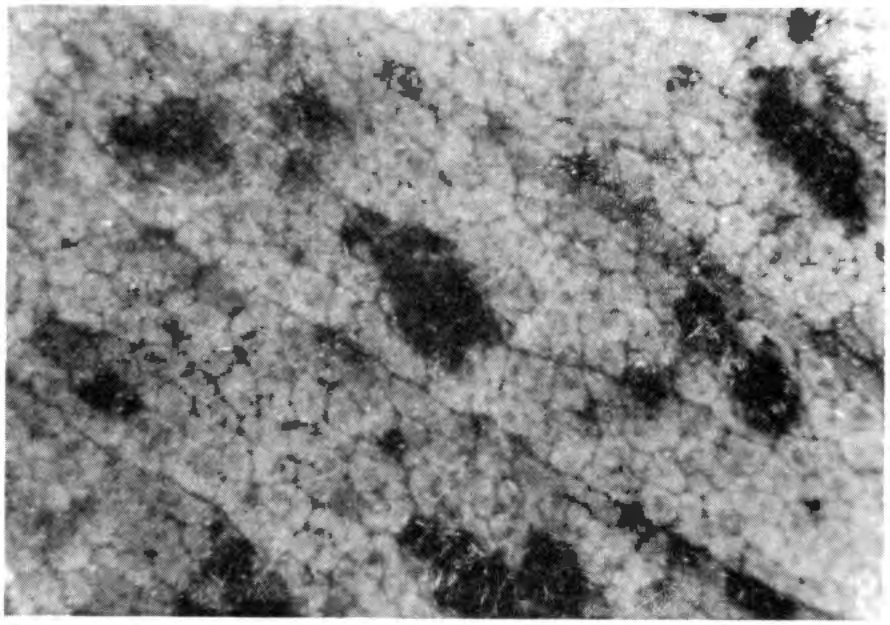

Figure 3. Cercospora arachidicola Lesions on Deteriorated Leaflet of Wild Peanut, P.I. 276233. Abundant Sporulation Present 30 Days Following Inoculation. Distinct Stromata Present. 32X.

Figure 4. Cercospora arachidicola Sporulating on Cut Edge of Beet, Beta vulgaris, Leaf 18 Days Following Inoculation. 32X.

Figure 5. *Cercospora arachidicola* Sporulating on Cut Petiole Tip of *Chenopodium* sp., 18 Days Following Inoculation. 32X.

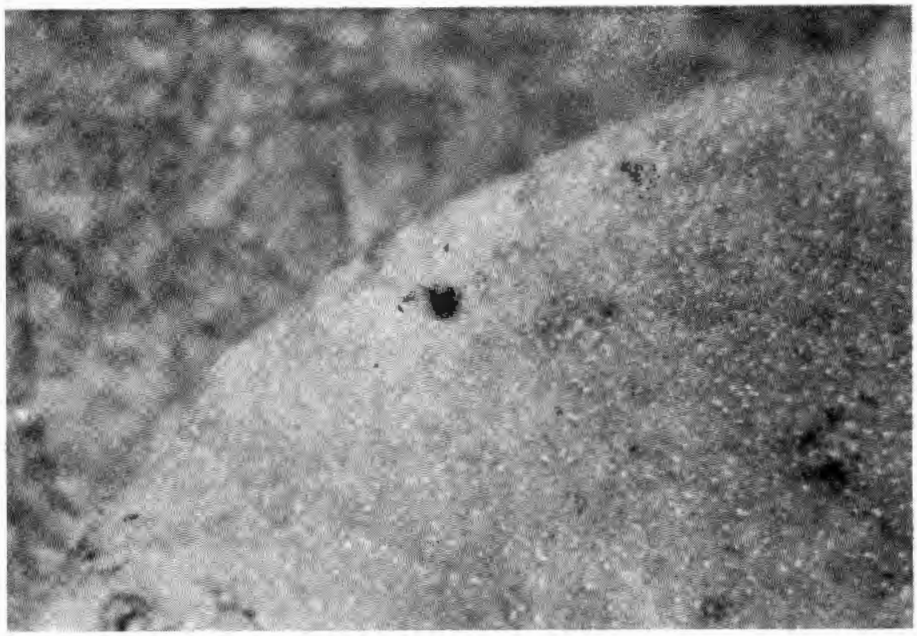

Figure 6. *Cercosporidium personatum* Lesion on Creeping Wood-sorrel, *Oxalis corniculata*, 30 Days Following Inoculation. Very Few Conidia Present. 40X.

both species.

Small necrotic flecks occurred on the wild peanut, P.I. 276233. These flecks may have been C. personata infection points. No sporulation or stromata developed from these flecks. Deterioration of the leaf tissue did not trigger stromata or conidial production in the flecks with C. personatum as it did with C. arachidicola. This could indicate that these flecks may be due to other factors.

Examination of plants growing in and adjacent to peanut fields severely infected with C. arachidicola were made in late summer and fall of 1978 and 1979. Cercospora spp. were recovered from ten different species (Table V). Cercospora lesions collected from pencil-flower (Figure 7) were very distinct and appeared similar to Cercospora lesions on peanuts. The pencil-flower lesions did not become as large as peanut lesions due to pencil-flower's smaller leaflets and early abscission of infected leaflets. The Cercospora isolates collected from alfalfa, pencil-flower, sweet clover, honey locust and unknown herbaceous vine produced abundant conidia on PLX agar. Their pathogenicity was tested on detached peanut leaflets. The pencil-flower isolates were the only isolates that were capable of infecting detached peanut leaflets. The pencil-flower Cercospora isolates produced symptoms identical to C. arachidicola symptoms on inoculated peanut leaflets (Figure 8). The Cercospora isolates from pencil-flower appeared identical to the original isolates when they were back inoculated to pencil-flower.

Scanning electron microscope observations of pencil-flower, Stylosanthes biflora, and Florunner peanut were made following inoculation with Cercospora arachidicola isolate A75A4 and Cercosporidium personatum isolate P44B1. The small number of conidia and the presence of germ

TABLE V

HOSTS FROM WHICH *CERCOSPORA* SPECIES WERE COLLECTED

Family Genus and Species Common Name	Date Collected	*Cercospora* Species Collected
Leguminosae		
Cercis canadensis		
Redbud	August 3, 1979	*C. cercidicola*
Desmanthus illinoensis		
Illinois Bundleflower	September 2, 1979	*C. desmanthi*
Gleditsia triacanthos		
Honey Locust	October 11, 1979	*C. condensata*
Medicago sativa		
Alfalfa	September 2, 1979	*C. medicaginis*
	September 25, 1979	*C. medicaginis*
Melilotus officinalis		
Yellow Sweet Clover	June 18, 1979	*C. davsii*
	September 2, 1979	*C. davsii*
Stylosanthes biflora		
Pencil-flower	August 3, 1978	*C. commonsii*
	June 18, 1979	*C. commonsii*
	September 30, 1979 a/	*C. arachidicola*
	October 17, 1979 a/	*C. arachidicola*
Chenopodiaceae		
Chenopodium sp.		
Goosefoot (Lambsquarter)	September 2, 1979	*C. dubia*
Liliaceae		
Smilax sp.		
Greenbrier	September 2, 1977	*C.* sp.
Rubiaceae		
Cephalanthus occidentalis		
Button Bush	September 2, 1977	*C. cephalanthii*
Unknown		
Unknown Herbaceous Vine	October 23, 1979	*C.* sp.

a/ Isolates that produced lesions on peanuts.

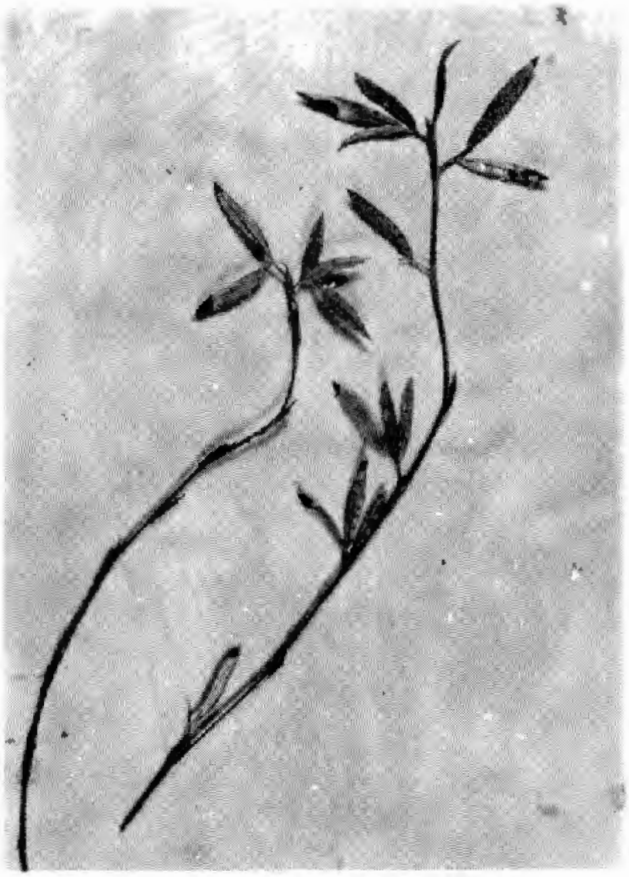

Figure 7. Pencil-flower, Stylosanthes biflora, Stems Collected from Peanut Field. Numerous Cercospora arachidicola Lesions Present.

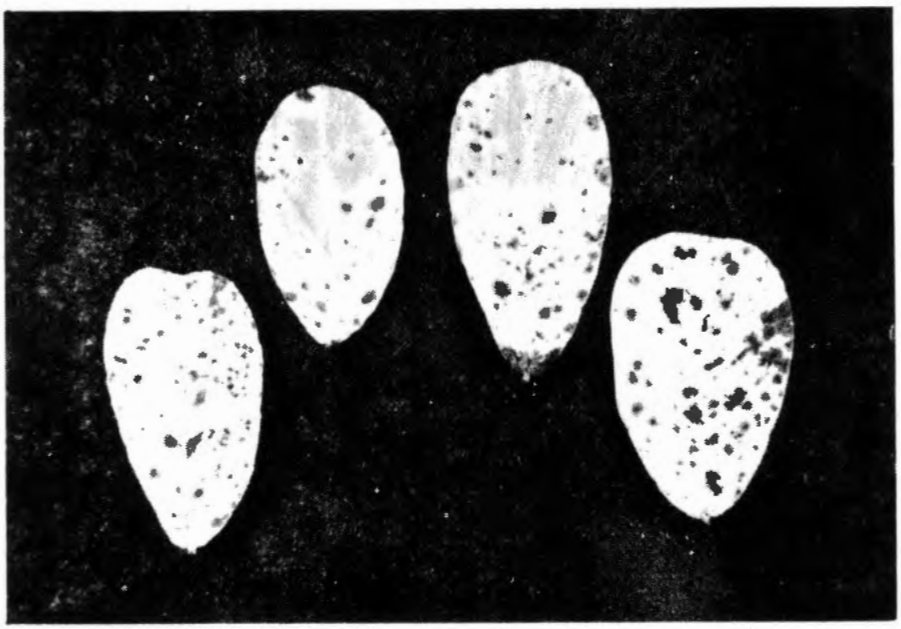

Figure 8. Cercospora Isolate 103B1 Lesions Produced on Peanut 20 Days Following Inoculation. Isolate 103B1 Was Collected from Pencil-flower, Stylosanthes biflora.

tubes without connecting conidia on the leaf surface suggest that most of the Cercospora conidia were removed during preparation of the leaf tissue for examination.

Little or no directional movement of the germ tubes of C. arachidicola and C. personatum toward the stomata of pencil-flower or peanut were detected. The germ tubes of both fungal species would often go around or across stomata of both plant species without any attempts to affect penetration. The fungus sometimes appeared to make a special effort to avoid entering a stoma. It was noted that stomata of pencil-flower were usually open (Figures 9 and 10) while stomata of peanut were usually closed or had very narrow open slits (Figure 11).

A germ tube of C. arachidicola can be seen forming an appressorium at the corner of a pencil-flower stoma in Figure 9. A second germ tube can be seen missing the stomatal opening. A bulge on this germ tube over the stomatal opening may be the start of a second appresorium. A small structure can be seen between the two germ tubes. A comparison with infection pegs produced by C. arachidicola on peanut (Figure 11) suggests that the small structure in Figure 8 is an infection peg penetrating the stomatal opening of the pencil-flower.

Observations of C. personatum on pencil-flower show that direct penetration through the epidermis is a means of infecting pencil-flower (Figure 10). In this picture a germ tube can be seen developing a small appressorium next to a protrusion on the leaf surface. The appressorium is adhering so tightly to the leaf surface that it is difficult to discern the boundary between the appressorium and the leaf. The photograph (Figure 10) is not conclusive, however, it does suggest that a direct penetration of the epidermis may be occurring.

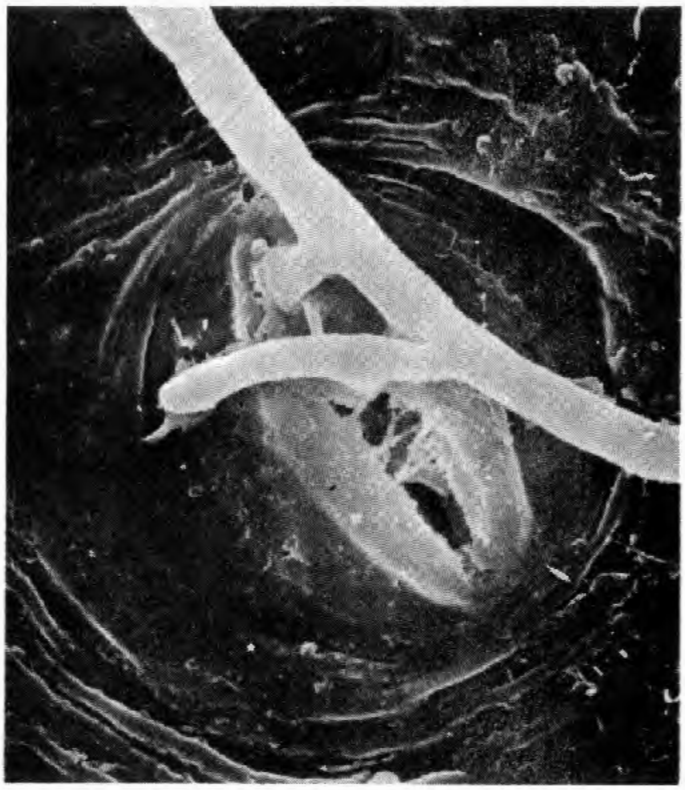

Figure 9. Two Germ Tubes of Cercospora arachidicola Developing from Cells of Conidium. The Short Germ Tube Has Produced an Appressorium at the Corner of a Stoma of Pencil-flower, Stylosanthes biflora. An Infection Peg Has Developed from the Appressorium and Penetrated the Open Stoma. 3200X.

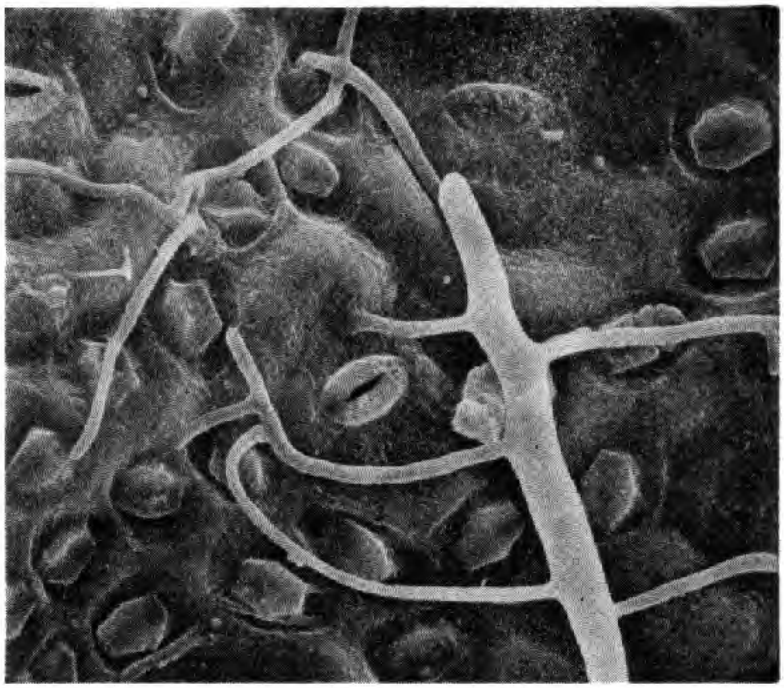

Figure 10. *Cercosporidium personatum* Conidium with Six Germ Tubes on Leaf of Pencil-flower, *Stylosanthes biflora*. The Short Germ Tube Has Formed an Appressorium at the Base of a Ridge on the Leaf. 1200X.

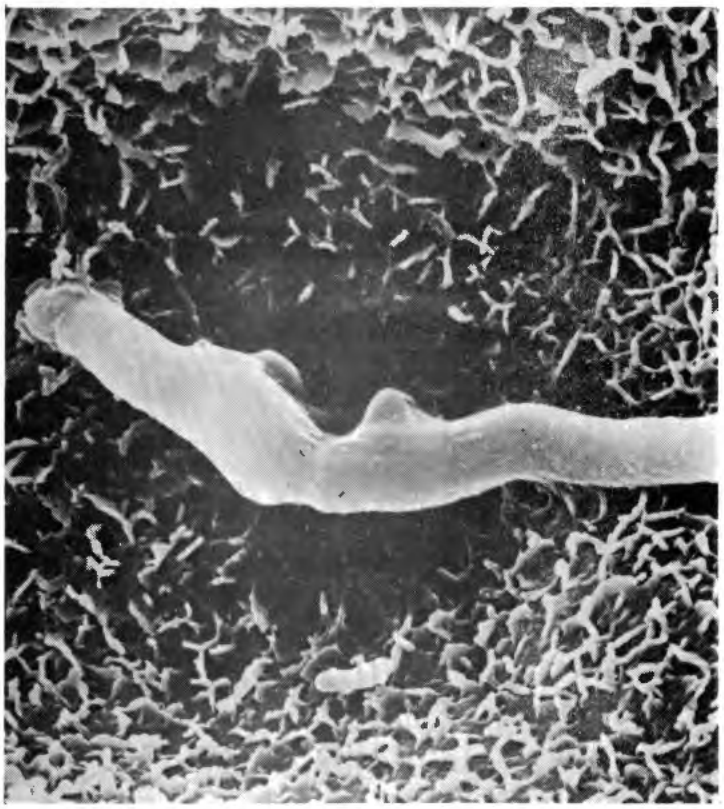

Figure 11. Multicellular Appressorium of Cercospora arachidicola Adjacent to Peanut Stoma. Two Infection Pegs from Appressorium Penetrating Open Stoma. 5400X.

Very few C. arachidicola or C. personatum infection sites were discovered. Only two probable infection sites per pathogen were observed. No direct penetrations by C. arachidicola through the epidermis were found and no penetrations by C. personatum through stomatal openings were discovered.

Single and Multispore Infections

The leaflets inoculated with a single conidium from Cercospora arachidicola isolate A75A4 developed three sporulating lesions and one nonsporulating lesion out of 20 possible infection points (Table VI) (Figure 12). The leaflets inoculated with ten conidia developed five sporulating lesions and one necrotic fleck out of 20 possible infection sites. The leaflets inoculated with 20 conidia per site produced nine sporulating lesions and one non sporulating lesion. Several satellite or double lesions occurred at some of the ten and 20 conidia infection sites. The infection rate with the low number of conidia appears to be higher than normally reported for Cercospora infections.

The average size of necrotic lesions from ten conidial inoculations were larger at all three test measurement periods (Table VII). The average size of the 20 conidia lesions was slightly smaller than the ten conidia and single conidium lesions until the third measurement period at which time the diameter increased rapidly. The individual lesions varied considerably in their diameter. Single spore lesions were often larger than the smaller ten spore and 20 spore lesions. The reverse was also true.

The halos around the lesions were well developed at 15 days. The average halo width ranged from 0.42 mm to 0.75 mm (Table VIII). The

TABLE VI

NUMBER OF SPORULATING CERCOSPORA ARACHIDICOLA, ISOLATE A75A4, LESIONS PRODUCED BY FLORUNNER PEANUT WITH INOCULUM LEVELS OF ONE, TEN, AND TWENTY CONIDIA PER LEAFLET

Inoculum Level	Days Following Inoculation		
	15	22	30
Single conidium	3/20	3/20	3/20
Ten conidia	3/20	5/20	5/20
Twenty conidia	5/20	8/20	9/20

a/ Numerator - number of sporulating lesions

b/ Denominator - number of inoculated sites

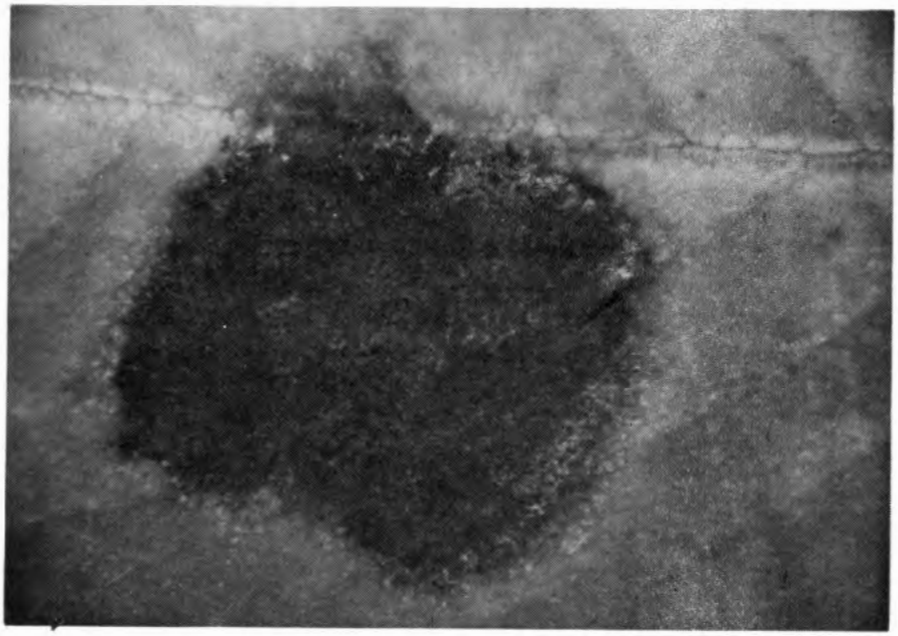

Figure 12. *Cercospora arachidicola* Lesion on Florunner Peanut 30 Days Following Inoculation. Lesion Produced by a Single Conidium from Isolate A75A4. 10X.

TABLE VII

DIAMETERS OF *CERCOSPORA ARACHIDICOLA*, ISOLATE A75A4, LESIONS ON FLORUNNER PEANUT WITH INOCULUM LEVELS OF ONE, TEN, AND TWENTY CONIDIA PER LEAFLET a/

Inoculum Level	Days Following Inoculation		
	15	22	30
Single conidium	1.31 b/	2.38 b/	2.88 b/
Ten conidia	1.38 b/	2.60 c/	3.50 c/
Twenty conidia	1.61 d/	2.41 e/	3.20 e/

a/ Measurements given in millimeters

b/ Mean of 4 lesions

c/ Mean of 5 lesions

d/ Mean of 9 lesions

e/ Mean of 10 lesions

TABLE VIII

WIDTHS OF HALOS SURROUNDING *CERCOSPORA ARACHIDICOLA*, ISOLATE A75A4, LESIONS ON FLORUNNER PEANUT WITH INOCULUM LEVELS OF ONE, TEN, AND TWENTY CONIDIA PER LEAFLET a/

Inoculum Level	Days Following Inoculation		
	15	22	30
Single conidium	0.75 b/	1.00 b/	1.19 b/
Ten conidia	0.42 b/	1.25 c/	0.55 c/
Twenty conidia	0.59 d/	1.00 e/	0.50 e/

a/ Measurements given in millimeters

b/ Mean of 4 lesions

c/ Mean of 5 lesions

d/ Mean of 9 lesions

e/ Mean of 10 lesions

color of the halos were generally a light green to yellow green. By the 22nd day the average measurement had increased to a range of 1.00 to 1.25 mm, and the color was a clear yellow. Exceptions were the lesions that had measured less than one mm in diameter in the preceding week and a single spore lesion that did not increase in diameter from the 1.5 mm diameter of the previous week. The halos around these four lesions were light green to yellow green.

A general decline in lesion halos was observed 30 days following inoculation. Most of the halos had turned a yellow green. In some cases portions of yellow halos from previous measurements had turned green. This caused the combined lesion halo diameter to be smaller than it was the previous week.

C. arachidicola conidia were washed from lesions with abundant sporulation at 22 and 30 days following inoculation (Table IX). Great extremes in the number of conidia recovered from the different lesions occurred. Only two lesions were counted in the 22-day old infections from single spores. The count in one lesion was 38,000 conidia per lesion and the count in the other lesion was 2,000 conidia per lesion. The extremes of conidia recovered and the limited number of lesions counted makes these measurements of limited value.

There was also great fluctuation between 22 day and 30 day conidial counts. The counts were sometimes considerably larger, considerably smaller or near the same. It was noted that after a lesion was washed of conidia, no new conidia developed in the center of the lesion. The new sporulation occurred only along the edge of the lesion. This observation was also supported by the fact that only lesions that had a major increase in diameter between the first and second conidial

TABLE IX

NUMBER OF *CERCOSPORA ARACHIDICOLA*, ISOLATE A75A4, CONIDIA RECOVERED PER LESION FROM FLORUNNER PEANUT WITH INOCULUM LEVELS OF ONE, TEN, AND TWENTY CONIDIA PER LEAFLET

Inoculum Level	Days Following Inoculation	
	22	30
Single conidium	20,000 a/	5,333 b/
Ten conidia	23,333 b/	44,000 b/
Twenty conidia	31,500 c/	16,500 d/

a/ Mean of 2 lesions

b/ Mean of 3 lesions

c/ Mean of 4 lesions

d/ Mean of 8 lesions

counts produced major sporulation gains. However, a comparison among the spore counts per lesion in Table IX with the lesion diameter increases in Table VII does not support this observation.

Cultivar Isolate Interaction

A general comparison of the reaction of the different peanut cultivars and species showed some distinct differences. The Florunner cultivar showed the greatest adaptibility to the moist filter paper in petri dish experiment. The leaflets usually maintained a normal color with little tendency toward chlorosis except under heat and moisture stress or Cercospora arachidicola and Cercosporidium personatum infections. Tamnut 74 and Valencia leaflets had a strong tendency for veinal chlorosis with some tendency for general chlorosis.

Large yellow halos and blotches were present around most Cercospora arachidicola and Cercosporidium personatum lesions on Florunner peanuts. The amount of yellow around the Cercospora lesions on Florunner was considerably greater than for Valencia and Tamnut 74 cultivars (Tables X and XI). This phenomenon was observed in all experiments and all isolates of C. arachidicola and C. personatum.

The yellow halos and blotches had distinct borders. The halo appeared as a light green ring soon after the Cercospora lesions could be seen. The halo usually took on a bright yellow color 2-3 weeks after inoculation. At this time irregular yellow blotches also began to appear as extensions of the halo. The yellow blotches often connected Cercospora lesions that were sometimes up to two cm apart. The lesion halos had a tendency to degrade to a tan color if the leaflets were placed under severe moisture stress with plentiful moisture added

TABLE X

HALO DEVELOPMENT ON PEANUT LEAFLETS 30 DAYS FOLLOWING INOCULATION BY *CERCOSPORA ARACHIDICOLA*

C. arachidicola Isolate	Peanut Cultivar			Mean
	Florunner	Valencia	Tamnut 74	
A42A1	5.1	2.9	2.6	3.6
A42B1	4.3	2.6	2.7	3.2
A75A4	5.4	2.7	2.8	3.6
A78A1	4.8	2.8	2.8	3.4
A82B1	5.1	3.1	2.3	3.4
A84A1	5.1	2.6	1.9	3.2
Mean	5.0	2.8	2.5	

LSD 0.01 cultivar means = 0.7

LSD 0.05 isolates means = nonsignificant

a/ Ratings 0-9 as follows: 0 = no halos; 1-3 = most lesions with small indistinct halos; 4-6 = most lesions with moderate halo development; 7-9 = most lesions with large distinct halos.

TABLE XI

HALO DEVELOPMENT ON PEANUT LEAFLETS 30 DAYS FOLLOWING INOCULATION BY CERCOSPORIDIUM PERSONATUM a/

C. personatum Isolate	Peanut Cultivar			
	Florunner	Valencia	Tamnut 74	Mean
P44B1 b/	5.5	2.2	1.8	3.1
P75C1 b/	5.2	2.6	3.1	3.7
P82C1 b/	7.0	2.8	2.5	3.4
Mean	5.6	2.5	2.5	
P75B1 c/	5.5	2.5	1.9	3.3
P81A1 c/	4.9	2.8	2.9	3.5
P82A1 c/	5.5	2.6	2.3	3.4
Mean	5.3	2.6	2.4	

LSD 0.01 cultivar means 1000 conidia = 1.4

LSD 0.05 isolate means 1000 conidia = nonsignificant

LSD 0.01 cultivar means 3000 conidia = 0.9

LSD 0.05 isolate means 3000 conidia = nonsignificant

a/ Ratings 0-9 as follows: 0 = no halos; 1-3 = most lesions with small indistinct halos; 4-6 = most lesions with moderate halo development; 7-9 = most lesions with large distinct halos.

b/ Isolates used at inoculum density of 1000 conidia per ml

c/ Isolates used at inoculum density of 3000 conidia per ml

later. The green leaflet tissue would remain normal color and texture. This tendency was also observed when the leaflets were kept in low light with excess moisture.

C. arachidicola produced significantly fewer and smaller lesions on Florunner than on Tamnut 74 and Valencia (Tables XII and XIII). No significant differences were found in sporulation of C. arachidicola on Florunner and Tamnut 74 cultivars or among the different Cercospora isolates (Table XIV). However, sporulation was significantly reduced in Valencia at the five percent level of probability.

Care had to be used in the ten day observations to prevent the counting of small dark flecks as infection sites. Similar flecks also occurred on the noninoculated leaflets. The flecks remained small and did not develop into normal Cercospora lesions. The flecks could usually be distinguished from Cercospora infection when second measurements were made. These flecks were more common on Tamnut 74 and Valencia leaflets than Florunner leaflets.

The measurements 30 days following inoculation showed that C. arachidicola isolate A82B1 was less virulent than the other isolates. Isolate A84A1 appears to be the most virulent (Tables XII and XV). The other isolates were scattered between these two isolates in their virulence.

The interaction of Cercosporidium personatum with the peanut cultivars was very similar to the interaction observed with C. arachidicola. C. personatum produced very strong halos around lesions of Florunner peanuts and weak halos around lesions on Tamnut 74 and Valencia peanuts (Table XI). Florunner consistently produced fewer and smaller C. personatum lesions than Tamnut or Valencia cultivars. Tamnut lesions

TABLE XII

NUMBER OF LESIONS PER PEANUT LEAFLET 15 DAYS FOLLOWING INOCULATION BY *CERCOSPORA ARACHIDICOLA*

C. arachidicola Isolate	Peanut Cultivar			
	Florunner	Valencia	Tamnut 74	Mean
A42A1	9.06	17.18	14.19	13.48
A42B1	5.44	13.06	9.81	9.44
A75A4	6.25	12.94	10.19	9.79
A78A1	6.50	14.56	20.56	13.88
A82B1	4.38	13.00	7.94	8.44
A84A1	14.50	13.62	17.31	15.15
Mean	7.69	14.06	13.33	

LSD 0.01 cultivar means = 3.28

LSD 0.01 isolate means = 4.63

TABLE XIII

DIAMETER OF LARGEST SINGLE CERCOSPORA ARACHIDICOLA LESION ON PEANUT LEAFLET 15 DAYS FOLLOWING INCOCULATION a/

C. arachidicola	Peanut Cultivar			
Isolate	Florunner	Valencia	Tamnut 74	Mean
A42A1	1.69	1.86	2.21	1.93
A42B1	1.54	2.13	1.83	1.86
A75A4	1.93	2.06	2.14	2.04
A78A1	2.19	2.06	2.44	2.23
A82B1	1.45	1.61	1.65	1.58
A84A1	1.70	2.00	1.97	1.89
Mean	1.77	1.96	2.05	

LSD 0.05 cultivar means = 0.19

LSD 0.01 isolate means = 0.37

a/ Diameter given in millimeters.

TABLE XIV

SPORULATION OF CERCOSPORA ARACHIDICOLA ON ADAXIAL SURFACE OF PEANUT LEAFLETS 30 DAYS FOLLOWING INOCULATION a/

C. arachidicola	Peanut Cultivar			
Isolate	Florunner	Valencia	Tamnut 74	Mean
A42A1	7.5	5.2	7.6	6.8
A42B1	6.8	7.8	7.9	7.5
A75A4	7.6	6.9	7.1	7.2
A78A1	6.8	7.1	8.2	7.4
A82B1	6.6	6.4	7.2	6.7
A84A1	7.6	6.8	7.3	7.2
Mean	7.1	6.7	7.5	

LSD 0.05 cultivar means = 0.5

LSD 0.05 isolate means = nonsignificant

a/ Ratings 0-9 as follows: 0 = no sporulation; 1-3 = light sporulation; 4-6 = moderate sporulation; 7-9 = heavy sporulation.

TABLE XV

PERCENTAGE OF PEANUT LEAFLET COVERED WITH *CERCOSPORA ARACHIDICOLA* LESIONS 30 DAYS FOLLOWING INOCULATION

C. arachidicola	Peanut Cultivar			
Isolate	Florunner	Valencia	Tamnut 74	Mean
A42A1	30	26	51	36
A42B1	18	29	50	32
A75A4	24	29	47	33
A78A1	23	35	67	42
A82B1	20	23	41	28
A84A1	43	44	68	51
Mean	26	31	54	

LSD 0.01 cultivar means = 8

LSD 0.01 isolate means = 12

were significantly larger than Florunner lesions (Tables XVI and XVII).

Sporulation of C. personatum was the greatest on Florunner with Tamnut 74 close behind. Valencia was a distant third in sporulation with sporulation averaging 40% less than Florunner (Table XVIII). The sporulating stroma in the Florunner lesions were denser than in the lesions of the other two cultivars.

Identification of differences among isolates of C. personatum was hampered due to the use of two inoculum levels and prevented accurate comparisons. However, isolate P75C1 appears to be the most virulent isolate. This isolate produced more lesions, larger lesions and heavier sporulation on the peanut cultivars than any of the other Cercosporidium isolates (Tables XVI, XVII and XVIII). All six Cercosporidium isolates developed prominent halos.

Faint signs of C. personatum infections could usually be seen 9-10 days following inoculation. There did not appear to be any cultivar or isolate with a tendency to develop earlier or later than the other isolates or cultivars.

A direct correlation existed between the halo appearance of the parent lesion of a Cercospora arachidicola isolate and the characteristics of the halos on inoculated peanut leaflets in petri dishes. This can be seen by comparing the halo development of an isolate (Table X) with the appearance of the parent spot (Table I). Isolate A42A1 and isolate A42B1 were obtained from lesions on plants only a few feet apart. Isolate A42A1 which was obtained from a lesion with a distinct yellow halo had the strongest halo of any isolate in the study. Isolate A42B1 which was obtained from a lesion with no halo had the weakest halo of any isolate in the study. However, this

TABLE XVI

NUMBER OF LESIONS PER PEANUT LEAFLET 30 DAYS FOLLOWING INOCULATION BY CERCOSPORIDIUM PERSONATUM

C. personatum Isolate	Peanut Cultivar Florunner	Valencia	Tamnut 74	Mean
P44B1 a/	1.69	1.75	1.63	1.69
P75C1 a/	7.19	7.94	8.94	8.02
P82C1 a/	1.25	2.13	2.19	1.86
Mean	3.38	3.94	4.25	
P75B1 b/	3.88	5.94	5.81	5.21
P81A1 b/	6.13	6.44	9.25	7.27
P82A1 b/	4.50	7.38	5.69	5.85
Mean	4.83	6.58	6.92	

LSD 0.05 cultivar means 1000 conidia = nonsignificant

LSD 0.01 isolate means 1000 conidia = 2.39

LSD 0.05 cultivar means 3000 conidia = nonsignificant

LSD 0.05 isolate means 3000 conidia = nonsignificant

a/ Isolates used at inoculum density of 1000 conidia per ml

b/ Isolates used at inoculum density of 3000 conidia per ml

TABLE XVII

DIAMETER OF LARGEST SINGLE *CERCOSPORIDIUM PERSONATUM* LESION ON PEANUT LEAFLET 30 DAYS FOLLOWING INOCULATION a/

C. personatum Isolate	Peanut Cultivar			Mean
	Florunner	Valencia	Tamnut 74	
P44B1 b/	2.41	3.23	3.17	2.96
P75C1 b/	3.47	3.32	4.69	3.85
P82C1 b/	3.25	3.58	4.00	3.71
Mean	3.08	3.38	4.02	
P75B1 c/	2.78	3.03	4.50	3.42
P81A1 c/	3.16	3.25	4.19	3.53
P82A1 c/	3.19	3.97	4.59	3.92
Mean	3.04	3.42	4.43	

LSD 0.01 cultivar means 1000 conidia = 0.78

LSD 0.01 isolate means 1000 conidia = 0.76

LSD 0.01 cultivar means 3000 conidia = 0.44

LSD 0.05 isolate means 3000 conidia = 0.33

a/ Diameter given in millimeters

b/ Isolates used at inoculum density of 1000 conidia per ml

c/ Isolates used at inoculum density of 3000 conidia per ml

TABLE XVIII

SPORULATION OF CERCOSPORIDIUM PERSONATUM ON ADAXIAL SURFACE OF PEANUT LEAFLETS 30 DAYS FOLLOWING INOCULATION a/

C. personatum Isolate	Peanut Cultivar			
	Florunner	Valencia	Tamnut 74	Mean
P44B1 b/	2.1	2.1	2.1	2.1
P75C1 b/	6.5	3.1	4.9	4.8
P82C1 b/	2.1	2.4	3.7	2.7
Mean	3.5	2.5	3.5	
P75B1 c/	5.4	2.4	3.7	3.8
P81A1 c/	5.2	2.5	3.9	3.9
P82A1 c/	4.8	2.8	4.7	4.1
Mean	5.1	2.5	4.1	

LSD 0.05 cultivar means 1000 conidia = nonsignificant

LSD 0.01 isolate means 1000 conidia = 1.4

LSD 0.01 cultivar means 3000 conidia = 1.2

LSD 0.05 isolate means 3000 conidia = nonsignificant

a/ Ratings 0-9 as follows: 0 = no sporulation; 1-3 = light sporulation; 4-6 = moderate sporulation; 7-9 = heavy sporulation.

b/ Isolates used at inoculum density of 1000 conidia per ml

c/ Isolates used at inoculum density of 3000 conidia per ml

correlation was not significant at the five percent level of probability.

The three wild peanut species used in the cultivar isolate interaction study were of limited value. Factors reducing their value to the study were: Peanut P.I. 276235 deteriorated very quickly in the experiment; Peanut P.I. 262141 developed considerable number of lesions, however, a lot of contamination on the leaflets prevented accurate observations; Peanut P.I. 276233 sporulated only on deteriorated leaflets.

CHAPTER V

DISCUSSION

Cercospora arachidicola was capable of infecting healthy pencil-flower leaves in detached leaf studies. Examination of inoculated leaves with a scanning electron microscope demonstrated that C. arachidicola could penetrate stomata of pencil-flower. Cercospora collected from lesions on pencil-flower in the field showed typical growth characteristics of C. arachidicola on PLX agar. One isolate from pencil-flower (Table XIX) did have conidia longer than the length given for C. arachidicola (17). However, this is probably not a reason for keeping this isolate from being identified as C. arachidicola since many Cercospora authorities state that conidial length is a highly unreliable measurement and is highly influenced by the environment (17, 30, 59, 71, 70). Observations of C. arachidicola taken on PLX agar (Table XX) also showed some isolates with conidia outside the normal accepted length measurements of 35-110 μ (17). The shape and width are generally considered the most important taxonomic characteristic of Cercospora conidia (17, 59). Welles (70) stated that the Cercospora taxonomic system assumes that: 1) environmental effects such as moisture and temperature do not effect the size of fruiting structures, 2) hosts do not affect the size of conidia and 3) reaction of the host is a response to specific stimulus of the pathogen. The only important taxonomic criteria that he found in separating various Cercospora

TABLE XIX

CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF *CERCOSPORA ARACHIDICOLA* ISOLATES COLLECTED FROM PENCIL-FLOWER AND STUDIED ON PEANUT LEAF EXTRACT AGAR a/

Characteristic	Isolate	
	103B1	103A1
Diameter of Colony b/		
Range	520-920	630-900
Mean	744	795.6
Sporulation c/	+++	+++
Length of Conidia b/		
Range	82.5-144.0	3.0-3.5
Mean	110.6	87.6
Width of Conidia b/		
Range	3-3.8	3-4.5
Mean	3.3	4.0
Number of Septa per Conidium		
Range	2-8	0-8
Mean	4.8	3.7
Colony Color	Gray	Gray
Discoloration of Medium	Amber	Amber

a/ Ten measurements made 9 days following single conidium transfer

b/ Measurements in microns

c/ +++ = good sporulation

TABLE XX

CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF *CERCOSPORA ARACHIDICOLA* ISOLATES STUDIED ON PEANUT LEAF EXTRACT AGAR a/

Characteristics	Isolates					
	A42A1	A42B1	A75A4	A78A1	A82B1	A84A1
Diameter of Colony b/						
Range	230-800	550-780	560-960	620-850	700-1020	500-820
Mean	632	683	772	784	850	612
Colony Color	Gray	Gray	Gray	Gray	Gray	Gray
Discoloration of Medium	Amber	Amber	Amber	Amber	Amber	Amber
Sporulation c/	+++	+++	+++	++++	++	++
Length of Conidia b/						
Range	67.5-96	60-127.5	52.5-105	51-117	67.5-127.5	67.5-96
Mean	84.2	94.7	56.2	83.7	101.0	84.2
Width of Conidia b/						
Range	2.0-4.5	3.0-4.5	3.8-4.5	3.0-4.5	3.0-4.5	3.0-4.5
Mean	3.5	3.7	4.0	3.8	3.9	3.5
Number of Septa Per Conidium						
Range	2-7	1-8	1-9	0-10	2-8	2-7
Mean	5.0	4.4	4.8	3.9	4.8	5.0

a/ Ten measurements made 9 days following single conidium transfer

b/ Measurements in microns

c/ ++ = fair sporulation; +++ = good sporulation; ++++ = abundant sporulation

species were physiological behavior on artificial media and extent of parasitism. Cercospora isolates from pencil-flower showed typical C. arachidicola lesion development on inoculated detached peanut leaves.

Cercosporidium personatum was found capable of infecting healthy leaves of pencil-flower in detached leaf studies. This was verified by inoculations back to peanuts. Scanning electron microscope observations showed C. personatum germ tubes penetrating the epidermis of pencil-flower.

The observations of direct penetration by C. personatum through the epidermis and penetration by C. arachidicola through the stoma do not mean that these are the only methods these pathogens use to infect pencil-flower. Since the number of observed fungal penetrations of pencil-flower was very small, it is impossible to say that C. arachidicola infections of pencil-flower occur only through stomatal openings and the C. personatum infections take place only by direct penetration of the epidermis.

The verification of pencil-flower, Stylosanthes biflora, as a highly susceptible host for Cercospora arachidicola and a probable host for Cercosporidium personatum should be considered when control measures are established for Cercospora leafspot on peanuts. Stemen and Myers (61) stated in their book on Oklahoma flora that Stylosanthes biflora was quite common on dry soils in Oklahoma. Pencil-flower was found to be common in several native grass pastures near Stillwater, Oklahoma. This plant is not distinctive and could probably constitute 20-30% of the vegetation of a pasture without being noticed. The ramifications of pencil-flower as a very susceptible host of C. arachidicola could be of major importance in the near future. There is great interest in various

species of Stylosanthes as a forage crop in tropical and subtropical regions. Tests of the forage value of this genus are currently being made in South and Central America, East Africa, Southeast Asia, Australia, Phillippines and Florida (56, 22). Peanuts are grown commercially in many of the above named areas. The planting of peanuts adjacent to Stylosanthes pastures could lead to severe Cercospora leaf-spot problems.

The infection of deteriorating or stressed leaves of various plant species by C. arachidicola (Table IV) indicates that these plants may not be a common host for Cercospora arachidicola, however, they may be a source of inoculum under certain conditions. These weak hosts may also have no role in the life cycle of C. arachidicola. Chupp (17) states that Cercospora spp. are never wholly saprophytic, however, they can attack a host semi-saprophytically when the host tissue is saturated with water. He believed that host range examinations of Cercospora spp. should be made only with conidia and an absence of excess moisture. Observations in this study show that the Cercospora infections were more prevalent on the drier portions of the leaves of the weak host. These areas frequently contain tissue collapsed due to moisture stress.

The normal Oklahoma condition of summer drought with fall rains may make it possible for C. arachidicola to infect the stressed leaves of many plant species during the fall. Most leaves have many small necrotic areas during the fall. It is possible that C. arachidicola could be dormant or semi-dormant in leaves of various plants. With deterioration of the leaf Cercospora could begin active growth. The production of heavy sporulation on dead or deteriorating leaves could indicate that C. arachidicola is a much better saprophyte than is

generally believed.

The use of detached leaves on moist filter paper for host range studies has some serious problems. Plant species show considerable variation in their reaction as detached leaves in a moist environment. Leaves and leaflets of some species can be maintained for long periods without signs of stress. Other species start showing signs of stress almost immediately. Common reactions are change of color, loss of turgor and leaf bleeding. This results in a different level of leaf vitality for the different species. This could pose a problem of rating the relative susceptibility of different species of plants to a pathogen. Studies in the cereals have shown that some leaves that are resistant to a pathogen when attached to a plant may become susceptible to the pathogen when detached (6, 14, 15, 54).

A single conidium of each of Cercospora arachidicola and Cercosporidium personatum were found capable of infecting detached peanut leaves. The observed infection rate was higher than some reported rates. One percent was reported to be the standard infection rate for Cercospora omphakodes (36).

A lack of consistancy in the size of lesions and halos were noted in inoculations with the same number of conidia. The cause does not appear to be due to the conidia used in the experiments. Ten individual conidia from the inoculation plates were transferred with a camel-hair-spore pick under a steromicroscope onto PLX agar. Ten of ten conidia germinated and produced normal colonies. This suggests that environmental conditions in the individual petri dishes, the physiological condition of the peanut leaflets, and pesticide applications to greenhouse plants are potential causes of different lesion reactions.

Uneven moisture stress of the leaflets in the test plates were sometimes observed. This factor appeared to be the primary variation capable of affecting the development of Cercospora lesions.

The miticide, Plictran, was sometimes used for mite control. Observations suggest that this pesticide has a deleterious effect on Cercospora arachidicola. A waiting period with a minimum of two weeks was used after any pesticide application prior to initiation of experiments and the test leaves were thoroughly washed. This waiting period may not be sufficient for degradation of Plictran. Consequently, this factor could also contribute to variable lesion development.

Detached leaflets on moist filter paper in petri dishes were capable of detecting host pathogen interaction differences among Florunner, Valencia and Tamnut 74 cultivars of peanuts. This procedure was also capable of detecting different levels of pathogenicity among isolates of Cercospora arachidicola and Cercosporidium personatum.

The consistently greater development of yellow halos and blotches around C. arachidicola and C. personatum infections on Florunner peanuts was very apparent. This characteristic could indicate that Florunner peanuts are incompatible with C. arachidicola and C. personatum. With major physiological changes occurring in the peanut leaflet after infection. This results in the development of large yellow areas on the leaf. Tamnut 74 and Valencia cultivars did not react as severely to C. arachidicola and C. personatum infections as did Florunner. This could indicate that Tamnut 74 and Valencia cultivars have greater tolerance to Cercospora leafspot.

Characteristics of the halo of the original lesion of a C. arachidicola isolate could be identified by the prominance of the halos

produced by the isolate on detached leaves. This suggests that the pathogen has a major influence over the characteristics of the halo produced around a lesion. However, several researchers have stressed the importance of the host and environmental influence on halo formation (32, 52). The correlation between observations on detached leaves and the parent spot did not hold true with the P81A1 isolate of C. personatum in which the original characteristics of the halo was known.

Florunner consistently had the lowest or near the lowest number of lesions per leaflet in the cultivar isolate study. This number may be misleading. Although, an attempt was made to keep the relative size of the leaves among the cultivars near the same, Florunner leaves on the plants were generally slightly smaller and this may have carried over into leaf selection for the experiments. Observations where the percentage of leaflet covered with lesions instead of actual number of lesions present showed Florunner with lower percent lesion coverage than other cultivars. Therefore, the discovery that Florunner has fewer lesions per leaf appears to be valid.

Tamnut 74 had more lesions and a larger percentage of leaflet covered by lesions than Florunner or Valencia. Valencia generally supported the lowest sporulation rate. All of these cultural characteristics were the same for C. arachidicola and C. personatum. This suggests that the genes controlling these characters in peanuts are related for both Cercospora leafspot species. It might also indicate just similar responses by the leaflet to C. arachidicola and C. personatum infections. Higgins (28) reported that resistance to C. arachidicola was inherited independently of C. personatum and vice versa.

Certain isolates of Cercospora arachidicola were consistent in producing more and larger lesions with heavier sporulation. Sporulation and number and size of lesions appeared to be independent of the halo formation.

The relative number of lesions produced appears to be the key to detecting resistance. Cercospora studies on soybeans and beets have determined several physiological races with this character (5, 50, 51, 58, 72). In race 2 of Cercospora sojina the resistance was traced to a single dominant gene (51).

A major drawback of using detached leaves in petri dishes for screening for Cercospora resistance is that the defoliation parameter cannot be observed. Reports indicate that this could be a major resistance character (45, 62). Melouk's and Banks' (42) detached leaf method with leaf petioles in Hoaglands solution overcomes that drawback to detached leaf study for resistance, however, it may not be as easy to keep a large number of Cercospora isolates separated.

Great variation in the replications in a test and the leaflets in a petri dish show that great care has to be used in comparing findings from one study to another. Melouk and Banks (42) found that the leaf reactions to C. arachidicola of certain peanut introductions tested gave different results than those obtained by other researchers (2). Single spore isolates of Cercospora were normally used in screening for resistance in these studies. The use of a highly virulent or low virulent isolates could greatly alter the resistance reactions of a peanut plant. In this study certain C. arachidicola and C. personatum isolates consistently produced higher or lower relative infection reading on all plant cultivars tested, but the readings were not the highest or lowest

when compared with an isolate on a different variety. An exmaple of this can be seen in Table XV. Isolate A84A1 has the highest infection of any isolate on any cultivar, however, if isolate A84A1 on Florunner is compared with C. arachidicola isolates on Tamnut 74, it would have the second lowest percentage of leaflet coverage with Cercospora lesions. The observation that a need for consistancy or uniformity in testing procedures in screening for resistance is supported by Sowell (60).

The use of the two wild peanuts P.I. 262141 and P.I. 276235 to differentiate C. arachidicola and C. personatum was not effective in this study due to the deterioration of leaflets of test plants and the lack of uniform conidial concentration in the inoculum. Peanut P.I. 262141 was reported to have resistance to C. personatum and peanut P.I. 276235 was reported to have resistance to C. arachidicola (8).

Several isolates could be identified in this study as to number of lesions, size of lesions and sporulation. However, with the peanut varieties studied no differential host could be selected from reaction of isolates without comparison with other isolates in the study to obtain a relative infection level.

CHAPTER VI

SUMMARY

1. Pencil-flower, Stylosanthes biflora, was very susceptible to Cercospora arachidicola under field conditions. Pencil-flower was also susceptible to Cercospora arachidicola and Cercosporidium personatum in detached leaf studies.

2. Cercospora arachidicola infected pencil-flower, Stylosanthes biflora, by penetration of stomata with infection pegs.

3. Cercosporidium personatum infected pencil-flower, Stylosanthes biflora, by direct penetration of the epidermis from an appressorium.

4. Injured or stressed leaves of a large number of legume and non-legume plants were susceptible to C. arachidicola. It appeared to act as a saprophyte or semi-saprophyte on dead or dying leaves of a large number of plant species.

5. Cercospora arachidicola produced dark flecks in healthy leaves of alfalfa and wild peanut P.I. 276233 in detached leaf studies. These infections remained dormant until the infected leaves began to deteriorate. Stromata production and sporulation occurred in dark flecks with deterioration of infected leaves.

6. Cercosporidium personatum developed trace sporulation on creeping wood-sorrel, Oxalis corniculata. The infected area was minute.

7. Leaves from different plant species deteriorate on moist filter paper in petri dishes at different rates. This affected any host

parasite interaction comparisons made with detached leaves.

8. Cercospora arachidicola lesions from multiple conidial infections were not significantly different from single conidial infections.

9. Environmental conditions and physiology of the leaves were major contributing factors to spot characteristics of Cercospora arachidicola and Cercosporidium personatum infections.

10. The Florunner peanut cultivar developed significantly fewer Cercospora arachidicola and Cercosporidium personatum lesions than Tamnut 74 or Valencia peanut cultivars.

11. Florunner peanuts developed smaller lesions than Tamnut 74 or Valencia peanuts when infected with Cercospora arachidicola or Cercosporidium personatum.

12. Cercospora arachidicola or Cercosporidium personatum infections resulted in significantly larger more distinct halo development on Florunner peanut than on Tamnut 74 or Valencia peanuts.

13. Cercospora arachidicola and Cercosporidium personatum isolates differed in degree of development of lesions, sporulation and lesion size on different peanut cultivars but appeared to be related to prevalence of infection rather than genotype and could not be used to quantify resistant or susceptible reactions.

14. On basis of hosts tested none were suitable for race identification or species separation of Cercospora arachidicola and Cercosporidium personatum in detached leaf studies.

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