To the Graduate Council:

I am submitting herewith a thesis written by Teresa Jean Ragain entitled "Factors Affecting the Development in Culture and the Pathogenicity of Septoria glycines Hemmi." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Agricultural Biology.

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FACTORS AFFECTING THE DEVELOPMENT IN CULTURE AND THE PATHOGENICITY OF *SEPTORIA GLYCINES* HEMMI

A Thesis

Presented for the

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ABSTRACT

Brown spot, caused by Septoria glycines Hemmi, is one of the most destructive diseases of soybeans in Tennessee. The objectives of this study were to (a) evaluate the effect of temperature, humidity, precipitation, and age of the host plant upon infection of soybean leaves by S. glycines and (b) determine the resistance of eight soybean cultivars to the pathogen.

The optimum temperature for linear growth and sporulation of S. glycines on potato-dextrose agar and V-8 juice agar was 24 to 28 C. Czapek-Dox agar was not a satisfactory medium for growth and sporulation.

Atomizing a suspension of S. glycines pycnidiospores upon the soybean leaves was the most effective method of inoculation in controlled-temperature chambers. Distributing a spore or mycelial suspension over the leaves with a glass rod did not result in brown spot symptoms. Disease incidence was low in all experiments with the effect of temperature on infection. There was no significant difference among the four temperatures. Disease incidence of soybeans inoculated at 29 C day - 25 C night was significantly higher in a growth chamber containing a cool-moisture humidifier than in a chamber with ambient humidity.

Brown spot symptoms of soybeans in the field were rated weekly during the summer of 1978. The two major increases in symptoms occurred in late June and in late August and September. Each increase occurred 10 to 18 days after a period with relative humidity of 90% or higher

for at least 18 hours and two consecutive days with one inch or more of precipitation. Monitoring spore release with a Kramer-Collins spore sampler was of little use in predicting an increase in symptoms, as only four S. glycines pycnidiospores were collected in 1741 hours of sampling.

There were no significant differences in disease severity in eight soybean cultivars inoculated in the greenhouse. However, significant differences occurred among disease severity in the same eight cultivars when detached leaves were inoculated. Brown spot symptoms developed on detached leaves of Essex, Lee 74, and York, but did not develop on Bedford, Centennial, Dare, Forrest, or Pickett 71.

Additional monitoring of brown spot symptoms in the field is necessary to confirm the effect of temperature, precipitation, and humidity on the development of brown spot. Results of detached leaf inoculations should be compared with field inoculations to assess the reliability of this method of determining resistance to brown spot.

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CHAPTER I

INTRODUCTION

The importance of soybeans as a crop in Tennessee has increased markedly in recent years, with the acreage almost doubling between 1969 (41) and 1977 (19). Soybean diseases are becoming more important for two reasons. Diseases are more destructive when the crop is grown repeatedly in the same field without rotation, and financial loss from disease increases as greater acreage is cultivated even if loss per acre is unchanged (5,11,27).

Brown spot, caused by Septoria glycines Hemmi, is one of the most destructive diseases of soybeans in Tennessee (36). In seasons when conditions are favorable for its development, brown spot can cause severe defoliation and yield reduction (12). Several workers have observed that the pathogen spreads most rapidly in warm, moist weather (5,21) and is absent or causes only slight damage in dry seasons (1,4). Hemmi observed that the development of symptoms on previously uninfected leaves ceased in mid-summer, and the same phenomenon has occurred in Tennessee (35). However, little information has been published on specific environmental conditions influencing the development of the disease.

There are several factors which may affect the occurrence of brown spot, such as humidity, precipitation, temperature, light quality, cyclic sporulation of the fungus, and susceptibility of the host at

different ages. The objective of this study was to evaluate the effect of some of these factors by the following methods:

1. Determination of optimum temperatures for growth and sporulation of the fungus in pure culture.

2. Controlled-temperature chamber studies of the effect of temperature and humidity on infection of soybeans by S. glycines.

3. Comparison of disease severity of naturally infected plants in the field with spore release, temperature, rainfall, and relative humidity.

This study also included determination of resistance to brown spot of eight commercial cultivars, seven of which are recommended for production in Tennessee.

CHAPTER II

LITERATURE REVIEW

I. SYMPTOMS

Brown spot of soybeans was first reported by Hemmi (21). He observed the symptoms in 1914 on the leaves of soybeans grown in several provinces of Japan and named the pathogen Septoria glycines Hemmi. Brown spot is one of the earliest diseases to appear on soybeans in the spring (13). The first symptoms are irregular dark-brown patches which appear on the cotyledons after the unifoliate leaves have opened. Angular reddish-brown spots 1 to 5 mm in diameter appear on the unifoliate leaves before the cotyledons fall (52). The spots appear on both surfaces of the leaves (21), with the reddish-brown color more noticeable on the underside of the leaf (13). The lesions are angular and distinct in outline, limited by small veins, and unbordered. Old spots, which frequently turn gray in the center, often contain scattered pycnidia. Small spots may coalesce into larger brown patches, and the infected leaves may turn yellow or brown and fall off. The symptoms occur progressively closer to the top of the plant as the season advances (21). The spots on the mature leaves are generally smaller than those on the lower leaves, often less than 1 mm in diameter (22). If conditions are favorable for the spread of the pathogen, the older leaves may become so spotted by late summer that individual lesions are indistinguishable (52). Brown spot sometimes causes severe defoliation and yield reduction (12).

Although it is most noticeable on the leaves, the disease also occurs on the stems and pods. The lesions on the stems are brown, surrounded by pale green areas, vary from tiny specks to areas several centimeters in length, and are indefinitely margined (52).

II. MORPHOLOGY OF THE PATHOGEN

S. glycines is a member of the order Sphaeropsidales in the Fungi Imperfecti (9). The hyphae are 2 to 4 µm in width (21), and the pycnidia are 44 to 125 µm, commonly 60 to 70 µm, in diameter (22). The pycnidia form in mature lesions on leaves, stems, and pods (52) and are globose or subglobose, with thin membranaceous walls which are brown or dark brown in color (21). The short round papilla is at first covered, then erumpent with a comparatively large ostiole. Although the pycnidia are found under the epidermis on both sides of the leaf, they are usually on the upper side. Hemmi (21) describes the spores as "hyaline, filiform, more or less curved, or sometimes straight, smooth, guttulate, obscurely septated, one to four-celled, 21.0 to 52.5 µm long, 1.4 to 2.1 µm broad." He later reported that spores produced on the leaves were 16.6 to 52.5 x 1.3 to 2.1 µm, and those on potato decoction agar were 22.4 to 48.0 x 1.3 to 2.1 µm. The spores germinate within 24 to 50 hours at room temperature when placed in a drop of water or nutrient media (22). An increase in size occurs first, with the septa becoming clearly visible because of the constrictions, followed by the growth of one or two germ tubes from the ends of the conidia and from each cell at or near the septa (22,52).

The germ tubes are about 2 to 3 μm wide. Secondary conidia may be produced by budding from the germinating pycnidiospores or by their separation at the septa (22).

III. HISTOLOGY OF THE HOST-PARASITE RELATIONSHIP

Wolf and Lehman (52) placed S. glycines conidia in drops of water on soybean leaves and observed that the germ tubes entered the stomata 16 to 20 hours later. MacNeill and Zalasky (29) also observed stomatal penetration with no evidence of an appressorium in their histological study of the host-parasite relationship between S. glycines and soybeans. Penetration occurred exclusively on the lower side of the leaf. The penetrating hypha branched in the substomatal chamber and produced a thickened runner-hypha which branched freely and ramified intercellularly. Hyphae frequently invaded the phloem in the leaf veins, but were not found in the xylem (29).

In the central portion of the invaded area of the leaf, the contents of the cells disintegrated and the walls collapsed, producing a holonecrotic area. Cells near the necrotic area which were not contacted by progressing hyphae appeared unaffected until the initiation of pycnidial rudiments in the holonecrotic area. At this time, the margined cells beyond the advancing hyphae became yellow and granular, forming a plesionecrotic zone which never became colonized. MacNeill and Zalasky suggested that a diffusable, phytotoxic substance affecting cells some distance away from the focus of infection was produced upon formation of fruiting bodies. The phytotoxic substance may produce a

zone which acts as a barrier to lateral spread of the mycelium, limiting it to a discrete lesion (29).

Stomatal penetration and intercellular colonization were also found in the pods. Pycnidia formed in a pseudostroma in the mesocarp often broke through into the cavity of the pod. Occasionally the fungus entered the seed coat directly and formed sclerotial bodies in the seed. Seeds were also invaded via the placenta and funiculus. As a result of colonization of the funiculus and formation of sclerotial bodies in the hilum area, the seed was sometimes separated from its attachment tissue. Hyphae occasionally continued to spread from the hilum and cause macroscopic lesions on the cotyledons. This study established that soybean seeds can be invaded by S. glycines (29).

IV. EPIDEMIOLOGY

Since soybean pods are subject to infection by S. glycines, Wolf and Lehman (52) suggested that the pathogen may be seed-borne. They were unable to isolate the fungus from the seed, but noted that the diseased cotyledons could indicate seed-borne infection. Hemmi (22) soaked soybean seeds in a spore suspension for 24 hours before planting in sterilized soil. He concluded that seed-borne infection could occur since the cotyledons and lower leaves of the plants developed brown spot symptoms. MacNeill and Zalasky's study (29) also indicated S. glycines was seed-borne.

Wolf and Lehman (52) found that S. glycines conidia in diseased soybean leaves and stems were still viable in March after being stored outdoors all winter. Manns and Adams (30) found that abundant fruiting

of S. glycines occurred when over-wintered stubble was placed in moist chambers. Therefore, debris from the previous year's infected crop could serve as a source of inoculum when the field is planted in soybeans again. Hemmi tested this idea by growing S. glycines on cooked stems and pods of soybeans for 97 days, adding this inoculum to sterilized soil, and planting soybeans. Since brown spot occurred on cotyledons and lower leaves of some of the plants, he concluded that debris could be the inoculum source (22).

In the genus Septoria, spores are produced in pycnidia from which they exude in slimy masses forming cirrhi (38). These spores are usually spread by splash-liberation or insect dispersal. In splash dispersal, the smaller droplets may contribute spores to the air spora, although the larger droplets fall back rapidly onto the substratum (26). Sackston (38) found that spores are not dislodged from dry cirrhi or from exuding pycnidia by a current of air from a fan, but when water is sprayed from an atomizer into the air current, spores are dislodged in large numbers.

Brown spot has generally been most severe in damp, warm weather, or in poorly drained areas (1,5,13,16,21). It has been noted that the upper leaves are not invaded in mid-summer or in a dry region, although the lower leaves may be severely infected. Later in the season, the upper leaves may develop symptoms if weather conditions are available (22).

Wolf and Lehman (52) mentioned that brown spot was epiphytotic in 1923, presumably as a result of two protracted rainy periods, one in June and the other in late August and early September, with a lot of

damp, warm weather between. Other records concerning weather and development of brown spot epiphytotics are not available, but Shaner and Finney (42) have compared weather and disease incidence of Septoria leaf blotch of wheat epiphytotics (caused by Septoria tritici Rob. ex Desm.) in Indiana over a 20 year period. Although they stated that their conclusions must be regarded as tentative, their model predicts epiphytotics on the basis of rainfall frequency and the number of periods of two consecutive days of minimum temperature $\leq$ 7 C. It is likely that rainfall frequency and temperature would also be important in prediction of brown spot epiphytotics.

V. GEOGRAPHICAL DISTRIBUTION

Brown spot occurs in all major soybean-growing areas of the world. It was first reported from Japan (21). The disease has also been reported from other Asian countries, including Korea, China, and India (43). In the Soviet Far East, brown spot is listed as one of the three most important diseases of soybeans. The disease is widely distributed in Europe (16). In 1922, brown spot was observed in North Carolina, and has since been reported in a number of other states and in Canada (1,3,10,11,29,34,48,50,51).

VI. ISOLATION OF THE PATHOGEN AND CULTURAL CHARACTERISTICS

Wolf and Lehman (52) isolated S. glycines from diseased leaves, stems, and pods by macerating pycnidia-bearing tissues in sterile water and spreading drops of the suspension over the surface of hardened agar

plates. Hemmi (22) used 1/1000 mercuric chloride solution to surface-sterilize diseased portions of leaves before rinsing them in sterile water and placing them on agar plates. A technique for obtaining single-spore isolates of S. glycines from contaminated material was described by Schmitthenner and Hilty (40).

When incubated at 12 to 17 C, the colonies were olive brown and produced pycnidiospores exuded through the ostioles in sticky masses. Pycnidiospores germinated within 24 hours at temperatures between 16 and 36 C, with the germ tubes reaching the greatest length at 28 C. On potato decoction agar, the most vigorous growth occurred at 24, 28, and 32 C, with no marked differences among the three temperatures. The colonies were olivish black or grayish black stroma-like mycelial masses with velvety surfaces. However, the mycelial masses sometimes turned grayish white at unfavorable temperatures. Since pycnidiospores were exuded from the matured dark-colored pycnidia as white or grayish white sticky masses, their formation was easily detected. Light conditions seemed to have no effect on the formation of pycnidiospores, but fluctuation of daily temperatures (such as occur in the case of room temperature) was apt to accelerate their formation (22).

Benedict (7) reported production of pycnidiospores of S. glycines after streaking pycnidiospores from stock cultures on petri dishes of Sabouraud dextrose agar and incubating at 24 to 28 C for six days. More rapid sporulation of other Septoria species occurred with transfer of spore suspensions than with point inoculation (25,28). Near ultraviolet light has also been found to increase sporulation of some Septoria species (15).

VII. CONTROLLED ENVIRONMENTAL CONDITIONS

MacNeill and Zalasky (29) used controlled environmental conditions in their histological study of the host-parasite relationship between S. glycines and soybeans. Plants were cultured in four-inch pots and following inoculation were incubated at 22 C in a saturated atmosphere until it was established that infection had taken place.

In a study on the effect of Pseudomonas glycinea (Coerper) Stapp on the development of S. glycines, Benedict (7) cultured the soybeans in growth chambers which provided day and night temperatures of 80 and 72 F, respectively, 100% relative humidity, day length of 14 hours, and illumination of 1100 foot candles 16 inches above the plants. The lights in the chamber were turned off after each inoculation and switched on again the following morning to start the 14 hour photophase. The temperature was raised 10 F in the chamber from the eighteenth to the twenty-first day after seeding. Benedict did not specify whether the temperature was gradually raised 10 F over a three day period and left at that temperature or whether the temperature was raised 10 F for three days and then returned to the original temperature.

VIII. METHODS OF INOCULATION

Several methods have been used for inoculating soybeans with S. glycines. The most commonly used method is spraying spore suspensions on the leaves. Wolf and Lehman (52) sprayed suspensions of conidia from pure cultures on the leaves of healthy plants which had just begun to set seed. Infections developed promptly in plants growing in beds in

the greenhouse and in the field, with no attempt to conserve high degrees of humidity around inoculated plants. Small, brown discolorations appeared by the fifth day, and characteristic brown-spot lesions developed within the following week. Hemmi (22) also sprayed a spore suspension on healthy leaves, but he found it necessary to press the leaves between two fingers to flatten the numerous hairs before inoculation and to place the plants in a moist chamber at 24 to 25 C for 24 hours after inoculation. MacNeill and Zalasky (29) used a small hand sprayer to apply a spore suspension to the plants, following which the plants were incubated at 22 C in a saturated atmosphere until infection was established. Benedict (7) rubbed the upper surface of the leaves with a glass rod that had been dipped in the spore suspension.

Inoculations with other Septoria species have usually involved applying a spore suspension to the leaves with a hand atomizer and placing the plants in a saturated atmosphere (14,24,25,39). Spore concentrations of 1 - 30 x 10^6 spores/ml were used. Renfro and Young (37) and Beach (6) rubbed the leaves with fingers that had been dipped in the spore suspension.

IX. DISEASE RATING

Symptoms resulting from artificial inoculation of soybeans with S. glycines were rated by Benedict (7) on a five-point scale. This rating system, which is the only one available for brown spot of soybeans, is intended for rating damage on the individual leaf, not the entire plant.

X. RESISTANCE OF SOYBEAN CULTIVARS TO BROWN SPOT

Differences in susceptibility to brown spot have been observed among soybean cultivars (2,7,10,23,29,52).

CHAPTER III

MATERIALS AND METHODS

I. ISOLATION OF THE PATHOGEN

S. glycines was isolated from angular, reddish lesions which were 1-1/2 to 2 mm in diameter and distinct on both the upper and lower surfaces of unifoliate soybean leaves. Leaves were surface-sterilized in 0.525% sodium hypochlorite, rinsed twice in sterile water, and blotted on sterile filter paper to remove excess liquid. Each lesion and some adjacent healthy tissue were plunged into PDA in a petri dish. Cultures were incubated at room temperature.

II. OPTIMUM TEMPERATURES FOR LINEAR GROWTH AND SPORULATION IN CULTURE

Optimum temperatures for growth and sporulation of S. glycines in culture were determined on Czapek-Dox agar #58, V-8 juice agar #248, and modified potato-dextrose agar #167 (46). The potato-dextrose agar was modified to 17 g agar and 10 g dextrose per liter. Five-mm disks were cut with a cork borer from the margins of 11-day-old cultures on modified PDA. The disks were placed in the center of standard 9 mm petri dishes (approximately 20 ml medium/plate), and five dish-replicates of each medium were incubated at 16, 20, 24, 28, and 32 C.

After 14 days incubation, radial growth was determined by measuring the largest diameter and the one perpendicular to it of each colony. Sporulation was determined by flooding each colony with 10 ml of water

and gently rubbing the surface with a glass rod. The resulting spore suspension was transferred to a test tube, one drop of Tween® 20 was added, and the suspension was agitated. The spore concentration was determined with an AO Spencer Bright-Line hemacytometer. Four 5-mm^2 grids (0.5 mm^3 each) were counted and the counts were averaged to determine the spore production of each dish (46).

III. EFFECTS OF TEMPERATURE AND HUMIDITY ON INFECTION BY S. GLYCINES

The effect of temperature and humidity on infection of soybean leaves by S. glycines was studied in controlled-environment chambers. The cultivar Essex was used in all experiments. The plants were grown in the greenhouse in an artificial soil mix of the following composition: 0.5 bushel peat and 0.5 bushel perlite, with 151 g 6-12-12 fertilizer, 76 g superphosphate, 303 g lime, 9 g Sequestrene®, and 303 g vermiculite per bushel (45). Vermiculite was used instead of the gypsum in Toler's soil mix. Seeds were surface-sterilized in 0.9% sodium hypochlorite (44). Eight seeds were planted in each four-inch pot, and plants were thinned to three uniform plants per pot before inoculation.

Plants were inoculated when the unifoliate or trifoliate leaves were fully expanded. Immediately after inoculation, they were placed in temperature-controlled chambers. A 14 hour photophase was used in all experiments (7).

Temperature Experiments

Four growth chambers were used in this study. The temperatures 33, 29, 25, and 21 C were used during the 14 hour photophase and temperatures 4 C lower were used during the 10 hour scotophase. Inoculum was produced by streaking pycnidiospores across the surface of modified PDA (5 g dextrose/liter) in petri dishes. The dishes were incubated at room temperature. Spore suspensions were prepared by flooding each dish with sterile water and rubbing the colony surface with a glass rod. The spore suspensions were combined, an aliquot was diluted 1:19, and the spore concentration was determined with a hemacytometer (46). Tween® 20 was used as a surfactant. The inoculum was sprayed on the unifoliate leaves or lateral leaflets of the first trifoliate leaf with an atomizer to the point of run-off. Control plants were sprayed with sterile water. High humidity was maintained by covering the pots with plastic bags, which were removed 48 hours after inoculation. Fifteen days after inoculation, the plants were examined for brown spot symptoms, and isolations were attempted. Disease incidence of plants in each pot was rated one, two, three or four if there were zero, one, two, or three infected plants, respectively, per pot. This rating system was used in all inoculation experiments. Exceptions to the procedure given above are listed under each experiment. Information about age of cultures and plants at time of inoculation and number of plants inoculated is presented in Table 1.

TABLE 1. Experiments in Controlled-Temperature Chambers on the Effect of Temperature on Infection of Soybean Leaves by *Septoria glycines*

Experiment	Unifoliate leaves: Age of Plants (Days)	Unifoliate leaves: Number Pots Inoculated per Chamber	Trifoliate leaves: Age of Plants (Days)	Trifoliate leaves: Number Pots Inoculated per Chamber	Number Pots Controls per Chamber	Method of Inoculation	Age of Cultures (days)	Spore Concentration (millions per ml)	Disease Incidence: Number days after Inoculation
I	16	10	22	10	5	Spore suspension atomized on leaves	10	14.7	15
II	17	15	24	15	6	Spore suspension spread on leaves with glass rod	12	14.1	15
III	--	--	17	20	5	Mycelial mixture spread on leaves with glass rod	18	None	16
IV	--	--	17	15	4	Spore suspension atomized on leaves	11	11.3	21

Temperature experiment I. Three temperatures (33 C day--29 C night, 27 C day--23 C night, and 21 C day--17 C night) were used because one of the controlled-temperature chambers malfunctioned.

Temperature experiment II. The spore suspension was distributed over the leaves with a glass rod. Sterile water was applied to the controls.

Temperature experiment III. A mycelial suspension was spread on the leaves with a glass rod. The inoculum was prepared by blending 10 cultures from modified PDA with 200 ml sterile water for 20 seconds on low speed in a Waring blender which had been sterilized with 70% alcohol. A mixture of modified PDA and sterile water was spread on the controls. Plants were returned to the greenhouse bench after four days in the growth chamber.

Temperature experiment IV. Plants were not covered with plastic bags. After four days in the growth chambers, plants were returned to the greenhouse bench.

Humidity Experiment

The effect of humidity on infection of soybean leaves by S. glycines was studied in controlled-temperature chambers. Humidity remained at ambient level in one chamber, and humidity in the other was increased with a cool-moisture humidifier. Temperature in both chambers was 29 C during the photophase and 25 C during the scotophase. The

lateral leaflets of the first trifoliate leaf of 14-to 16-day-old plants were sprayed with a spore suspension containing 1.2 to 1.3 x 10^7 spores/ml. Tween ^{R} 20 was used as a surfactant. Control plants were sprayed with sterile water. Immediately after inoculation, 20 pots of inoculated plants and five pots of control plants were placed in each chamber, and the 10 hour scotophase was started. The plants were returned to the greenhouse bench after six days in the growth chambers. Seventeen days after inoculation, each leaflet was examined for symptoms. The experiment was repeated once.

IV. SPREAD OF S. GLYCINES IN FIELD CONDITIONS

Development of symptoms on field-grown soybeans was compared with spore release of S. glycines, temperature, rainfall, and relative humidity to explain why brown spot symptoms do not develop until late in the season on leaves produced in mid-summer even when the lower leaves are heavily infected. Spore release in the field was monitored with a Kramer-Collins spore sampler, which collected airborne particles in bands on a sillicone-coated microscope slide (49). One band was collected per hour, and the sampler was operated for a 24 hour period. The spore sampler was placed at ground level among soybean cultivar Coker 136 planted adjacent to the weather station at the University of Tennessee Plant Science Farm on 19 May 1978. The spore sampler was operated at least four days a week from 31 May 1978 to 29 September 1978, with the exception of the first week of September, when the spore sampler malfunctioned.

Brown spot symptoms were rated from 22 June to 28 September 1978. A two part rating system was used. The rating classes are presented

in Table 2. The first number indicated the location of the symptoms in three arbitrary divisions. The second number indicated the severity of the symptoms (number and size of lesions). Symptoms on 100 randomly chosen plants were rated each week, and ratings were averaged. Temperature and rainfall records were obtained from the U.T. Plant Science Farm weather station.

TABLE 2. Rating Classes for Symptoms of Brown Spot of Soybeans

Location Rating	Severity Rating
1 symptoms on lowest third of plant	0 no symptoms
2 symptoms on lower two-thirds of plant	1 light infection
3 symptoms on all three sections of plant	2 moderate infection
	3 heavy infection

V. DETERMINING RESISTANCE TO BROWN SPOT IN SOYBEAN CULTIVARS

Resistance of eight soybean cultivars to brown spot was tested with detached leaves in petri dishes and greenhouse plants. The following cultivars, seven of which are recommended for soybean production in Tennessee (19), were used in these experiments: Bedford, Centennial, Dare, Essex, Forrest, Lee 74, Pickett 71, and York.

Seeds were surface-sterilized in 0.9% sodium hypochlorite (44) and planted in a sterilized mixture of equal parts of sand, soil, and peat. Six seeds were planted in each four-inch pot, and plants were

thinned to three per pot before inoculation. The lateral leaflets of the first trifoliate leaf of 16-day-old plants were inoculated by atomizing a spore suspension with the concentrations presented in Table 3. Control plants were sprayed with sterile water.

TABLE 3. Spore Suspensions Used as Inocula in Determining the Resistance of Eight Soybean Cultivars to Septoria glycines

Trial	Spore Concentration (Spores/ml) Greenhouse Plants	Detached Leaves
I	1.2×10^6	1.2×10^6
II	2.2×10^7	1.0×10^7
III	1.0×10^7	

Twenty-four plants of each cultivar were inoculated and six plants were sprayed with sterile water as controls in greenhouse experiments. The pots were covered with plastic bags for 36 hours after inoculation. Five days after inoculation the plants were fertilized with a Rapid-Plant-Gro® solution. The leaves were rated for brown spot symptoms 15 days after inoculation. The rating system described in the temperature experiments was used.

In the detached leaf experiments, the leaves were floated in 20 ml of sterile water in sterile petri dishes and then inoculated. Six

leaves of each cultivar were inoculated and two sprayed with sterile water as controls in each of two trials. Detached leaves were rated for symptoms 19 days after inoculation. Leaves were given a rating of one if not infected, two if infected.

CHAPTER IV

RESULTS AND DISCUSSION

I. ISOLATION OF THE PATHOGEN AND CULTURAL CHARACTERISTICS

The pathogen isolated from lesions on soybean leaves produced colonies that were dark green, whitish on the upper surface, and slow-growing. Spores were found in faintly yellow cirrhi 10 days after transfer on modified potato-dextrose agar. The lengths of 100 spores from a 60-day-old culture were from 24.5 µm to 47.3 µm with a mean of 36.1 µm. The lengths of 100 spores from a 14-day-old culture were from 17.5 µm to 45.5 µm, with a mean of 31.2 µm. The measurements correspond well with those reported by Hemmi (22).

Spreading pycnidiospores on modified PDA in petri dishes usually resulted in visible whitish cirrhi within six to seven days. However, sporulation sometimes ceased for no obvious reason. In these cases, chilling, heating, and exposing the cultures to ultraviolet light all failed to induce sporulation.

II. OPTIMUM TEMPERATURES FOR LINEAR GROWTH AND SPORULATION IN CULTURE

The range and mean values for linear growth of S. glycines in culture are presented in Table 4. Linear growth was greatest at 24 and 28 C on all three media. In trial I, linear growth on Czapek-Dox agar (CDA) was significantly greater at 24 and 28 C. Growth at 20, 24, 28, and 32 C was significantly greater than that at 16 C in trial II. In

TABLE 4. Linear Growth of Septoria glycines on Czapek-Dox Agar (CDA), Modified Potato-Dextrose Agar (PDA), and V-8 Juice Agar (VJA) After 14 Days Incubation at Five Different Temperatures

		Linear growth (mm)			
		Mean[1]		Range	
Medium	Temperature	Trial I	Trial II	Trial I	Trial II
CDA					
	16	11.8 c[2]	11.8 b	8 - 14	10 - 13
	20	15.8 b	15.0 a	12 - 18	12 - 18
	24	22.2 a	18.0 a	20 - 24	14 - 21
	28	22.4 a	16.8 a	22 - 24	16 - 18
	32	13.2 c	15.4 a	11 - 16	13 - 17
PDA					
	16	20.4 c	24.0 c	20 - 22	21 - 26
	20	28.0 b	26.6 c	27 - 30	23 - 28
	24	29.0 b	31.4 b	27 - 30	30 - 34
	28	33.2 a	34.4 a	32 - 34	33 - 35
	32	20.0 c	15.8 d	14 - 24	13 - 21
VJA					
	16	20.6 c	21.2 c	20 - 22	19 - 24
	20	26.8 b	29.6 b	25 - 28	25 - 32
	24	29.0 ab	33.2 a	24 - 31	32 - 34
	28	30.2 a	32.8 a	30 - 31	31 - 35
	32	15.8 d	10.8 d	12 - 20	8 - 14

[1]Values are means for five replicates.

[2]Means within a medium in each trial which are followed by the same letter are not significantly different according to Duncan's multiple range test (P=0.05).

both trials, growth on modified potato-dextrose agar (PDA) was significantly greater at 28 C. Linear growth was significantly greater on V-8 juice agar (VJA) at 24 and 28 C, with no significant difference between these two temperatures. Growth of the fungus on CDA was sparse, while dense, stroma-like mycelia developed on PDA and VJA.

The range and mean values for sporulation of S. glycines are presented in Table 5. The sporulation values were much lower on CDA than on PDA or VJA. There was no significant difference in sporulation on CDA among the five temperatures. The greatest sporulation on CDA occurred at 28 C in trial I and 32 C in trial II. Sporulation values on PDA were significantly higher at 24 and 28 C in the first trial and at 28 C in the second. Less sporulation occurred on PDA at 32 C than at 20 C. Sporulation was significantly greater on VJA at 20, 24, and 28 C in trial I and at 28 C in trial II. The highest sporulation values for both VJA and CDA occurred at 24 and 28 C. These results indicate that the optimum temperature range for sporulation of S. glycines on VJA and PDA is 24 to 28 C. Sporulation was more variable than linear growth. Sporulation at 28 C may have been stimulated by the greater fluctuation in temperature ($\pm$ 3 C) which occurred in that incubator compared to the $\pm$ 1.5 C fluctuation which occurred in the others (22).

The results of these experiments are similar to those of Hemmi (22), who found the optimum temperature for growth and sporulation of S. glycines in culture was approximately 24 to 28 C.

TABLE 5. Sporulation of Septoria glycines on Czapek-Dox Agar (CDA), Modified Potato-Dextrose Agar (PDA), and V-8 Juice Agar (VJA) After 14 Days Incubation at Five Different Temperatures

		Sporulation (10^5 spores/dish)			
		Mean[1]		Range	
Medium	Temperature	Trial I	Trial II	Trial I	Trial II
CDA					
	16	0.00 a[2]	0.04 a	0.00 - 0.00	0.00 - 0.10
	20	0.00 a	0.07 a	0.00 - 0.00	0.00 - 0.15
	24	0.05 a	0.14 a	0.00 - 0.10	0.00 - 0.25
	28	0.15 a	0.48 a	0.05 - 0.35	0.00 - 0.90
	32	0.09 a	1.67 a	0.05 - 0.15	0.75 - 2.25
PDA					
	16	0.00 c	0.04 d	0.00 - 0.00	0.00 - 0.10
	20	3.00 b	11.20 c	0.10 - 11.75	0.20 - 36.30
	24	11.32 a	46.25 b	0.65 - 23.55	6.60 - 81.90
	28	8.78 a	76.50 a	4.20 - 21.55	57.00 -104.45
	32	0.64 bc	8.39 c	0.15 - 1.85	0.75 - 19.35
VJA					
	16	0.06 c	0.25 c	0.00 - 0.20	0.05 - 0.45
	20	4.19 ab	1.99 bc	0.15 - 13.40	0.00 - 8.80
	24	14.75 a	2.43 bc	1.00 - 41.25	0.35 - 4.05
	28	6.30 a	40.41 a	2.05 - 14.35	8.25 - 65.10
	32	1.12 bc	9.14 b	0.20 - 3.15	0.65 - 20.85

[1]Values are means for five replications.

[2]Means within a medium in each trial which are followed by the same letter are not significantly different according to Duncan's multiple range test (P=0.05). The analysis variance and Duncan's multiple range test were performed on $\sqrt{x + 1}$ transformations of the sporulation values.

III. EFFECT OF TEMPERATURE AND HUMIDITY ON INFECTION BY *S. GLYCINES*

Temperature Experiments

Plastic bags placed over the pots after inoculation were unsatisfactory for maintaining high humidity when determining optimum temperatures for infection of soybeans by *S. glycines*. No typical brown spot symptoms were produced by distributing spore or mycelial suspensions over the leaves with a glass rod. Low infection rates were produced when spore suspensions were atomized on the foliage, with or without plastic bags covering the plants after inoculation.

Temperature experiment I. There was a low rate of infection in this experiment, with typical brown spot symptoms occurring on only 12 of the 180 plants inoculated. Number of plants infected and mean disease ratings are presented in Table 6. According to an *F* test, there was no significant difference between disease ratings at the three temperatures, at the two different stages of development, or between the treatments and the controls. It was difficult to distinguish brown spot symptoms on unifoliate leaves at 27 and 33 C because of yellowing, mottling, and generally poor condition of the leaves.

Temperature experiment II. The inoculation procedure in this experiment consisted of distributing a spore suspension with a glass rod over the leaf surfaces. This was necessitated by a low infection rate resulting from the procedure used in experiment I and low spore production in culture. Since early abscission of many of the leaves occurred, and no typical brown spot symptoms appeared, this inoculation technique was unsatisfactory.

TABLE 6. Incidence of Brown Spot on Soybean Plants Inoculated with Septoria glycines in Temperature Experiment I

Temperature	Inoculated Unifoliate Leaves		Inoculated Trifoliate Leaves	
	Number Plants Infected	Mean Disease Rating[1]	Number Plants Infected	Mean Disease Rating[1]
33 - 29 C	2	1.2	0	1.0
27 - 23 C	3	1.3	0	1.0
21 - 17 C	3	1.3	4	1.4

[1]Disease rating for individual pots

1 = 0 plant infected/pot
2 = 1 plant infected/pot
3 = 2 plants infected pot
4 = 3 plants infected pot

Temperature experiment III. The inoculation technique used in this experiment, which consisted of spreading a mycelial mixture on the leaves with a glass rod, was attempted because of the lack of sporulation in inoculum cultures. No typical brown spot symptoms occurred on any of the plants. Blending the mycelium may have rendered it incapable of causing infection. This method was unsatisfactory for inoculation with S. glycines.

Temperature experiment IV. Number of plants infected and mean disease ratings are presented in Table 7. Since there was a possibility that the poor condition of the leaves and premature abscission in the previsou experiments were caused by the plastic bags used to maintain high humidity, they were omitted in this experiment. Although the leaves were in better condition at the time of disease rating than in the other experiments, which made it easier to distinguish brown spot symptoms, only two plants became infected of the 180 inoculated. The humidity was probably too low to permit spore germination and invasion of the leaf. According to an F test, there were no significant differences between disease ratings at the four temperatures.

Humidity Tests

In previous experiments, use of plastic bags to maintain high humidity resulted in poor condition of the leaves and a low rate of

TABLE 7. Incidence of Brown Spot on Soybean Plants Inoculated with Septoria glycines in Temperature Experiment IV

Temperature (C)	Number Plants Infected	Mean Disease Rating[1]
33 - 29	1	1.07
29 - 25	0	1.00
25 - 21	1	1.07
21 - 17	0	1.00

[1]Disease rating for individual pots:

1 = 0 plant infected/pot
2 = 1 plant infected/pot
3 = 2 plants infected/pot
4 = 3 plants infected/pot

infection, while inoculation with no attempt to maintain high humidity also resulted in a low rate of infection. Placing a cool-moisture humidifier in the controlled-temperature chamber was tested as an alternate method of maintaining high humidity. In trial I there was a highly significant difference between the mean disease ratings of plants in the ambient and increased humidity chambers. The mean disease ratings are given in Table 8. In the growth chamber with increased humidity, 62% of the inoculated plants became infected. In the chamber with ambient humidity, only 7% of the inoculated plants became infected. In trial II, there was a significant difference between the mean disease ratings of plants in ambient and increased humidity. The mean disease ratings

TABLE 8. Incidence of Brown Spot on Soybean Plants Inoculated with Septoria glycines in Ambient and Increased Humidity Trial I

Humidity	Plants	Mean Disease Rating[1]
Increased	Inoculated[2]	2.85 a[3]
Increased	Control[4]	1.00 b
Ambient	Inoculated	1.20 b
Ambient	Control	1.00 b

[1]Disease rating for individual pots:

1 = 0 plant infected/pot
2 = 1 plant infected/pot
3 = 2 plants infected/pot
4 = 3 plants infected/pot

[2]Values are means for 20 replications.

[3]Means followed by the same letter are not significantly different (P = 0.01).

[4]Values are means for five replications.

are presented in Table 9. No plants became infected in the chamber with ambient humidity, and 12% of the inoculated plants in the increased humidity chamber became infected.

The results of this experiment emphasize the importance of humidity in infection of soybeans by S. glycines. Controlled-environment chambers with humidity controls would be extremely useful in finding the optimum temperature for infection of soybean leaves by S. glycines.

TABLE 9. Incidence of Brown Spot on Soybean Plants Inoculated with Septoria glycines in Ambient and Increased Humidity Trial II

Humidity	Plants	Mean Disease Rating[1]
Increased	Inoculated[2]	1.35 a[3]
Increased	Control[4]	1.00 b
Ambient	Inoculated	1.00 b
Ambient	Control	1.00 b

[1]Disease rating for individual pots:

1 = 0 plant infected/pot
2 = 1 plant infected/pot
3 = 2 plants infected/pot
4 = 3 plants infected/pot

[2]Values are means for 20 replications.

[3]Means followed by the same letter are not significantly different (P = 0.05).

[4]Values are means for five replications.

IV. SPREAD OF S. GLYCINES IN FIELD CONDITIONS

On 19 June 1978 brown spot symptoms were first noted in the plot of soybean cultivar Coker 136 on the U.T. Plant Science Farm. The lesions were up to 3 mm in size, distinct on both sides of the leaf, and reddish-brown with the red tint more noticeable on the lower surface of the leaf. The mean disease ratings for each week are presented in Table 10.

TABLE 10. Incidence and Severity of Brown Spot on Soybean Cultivar Coker 136 in Field Conditions in 1978

Date	Location[1]	Severity[1]
June 22	1.58[2]	1.26
29	1.14	2.17
July 6	0.91	1.36
13	0.85	1.02
20	0.92	1.06
27	0.79	0.73
August 3	0.77	0.75
11	0.77	0.76
17	0.97	0.93
24	0.56	0.56
31	1.56	0.97
September 7	1.91	0.98
14	2.42	1.05
22	2.59	2.03
28	2.98	2.67

[1]Disease rating for individual plants:

Location	Severity
1 symptoms on lowest third of plant	0 no symptoms
2 symptoms on lower two-thirds of plant	1 light infection
3 symptoms on all three sections of plant	2 moderate infection
	3 heavy infection

[2]Values are means for 100 plants.

On 22 June 1978 the severity rating was 1.26 and the location rating was 1.58, indicating that there were slight symptoms on the lower one-third to two-thirds of the plants. The location ratings decreased in late June and early July. This decrease reflected the rapid growth of the plants without a corresponding increase in symptoms on new growth. The severity rating increased in late June, as lesions increased in size and additional lesions appeared on leaves which already had some symptoms. From late June to mid-July, severity ratings decreased. Both severity and location ratings were between 0.5 and 1.1 from the middle of July to the end of August. At the end of August, there was a definite increase in the location rating, indicating that symptoms were beginning to occur higher on the plants. About three weeks later, in mid-September, the severity rating also increased sharply. Some lodging occurred from late July to the end of the season, but only sturdy, upright plants were included in the disease rating.

Spore release in the field was monitored for 1741 hours during the summer of 1978. Four S. glycines pycnidiospores were collected on spore sampler slides. The small number of spores collected is puzzling in view of the high disease ratings in late August and September. Splash dispersal by rain was probably the major method by which the spores were disseminated, but it seems that some of the small droplets of spore suspension should contribute to the air spora (26) and be collected by the spore sampler. A rotary intake adaptor to keep the intake orifice facing into the wind or methods to collect droplets with suspended spores might have increased the efficiency of the sampling (17).

Between 9:00 P.M. 12 June and 9:00 P.M. 13 June 1978, large numbers of structures similar to S. glycines pycnidiospores in length and width were found on the spore sampler slides. Smaller numbers were collected on several other days. These structures had an appendage similar to a germ tube at each end. However, they had no septa, were either straight or evenly curved, and were uniform in width instead of being slightly larger at one end as S. glycines pycnidiospores usually are. For these reasons, the structures were not included in the count of S. glycines spores.

Downy mildew, a foliar disease of soybeans caused by Peronospora manshurica (Naoum.) Syd. ex Gaum. (Syn. P. sojae Lehman and Wolf), was very common in the soybean plot for several weeks in the last half of July and part of August. P. manshurica sporangia were collected on the spore sampler slides in large numbers. The hours of the day when P. manshurica sporangia were collected were noted from 16 July to 24 July 1978. Marked diurnal periodicity was exhibited. Maximum numbers of sporangia were collected between 10:00 A.M. and 2:00 P.M. EDST. Diurnal periodicity of release of Peronospora tabacina Adams spores was reported in 1958 (47). Maximum release in that species generally occurred between 5:00 and 10:30 A.M.

Precipitation, relative humidity, and temperature were evaluated for their effect on the development of brown spot. Precipitation data are presented in Table 11. Rainfall exceeded one inch on eight days: 4 May, 8 June, 9 June, 13 June, 16 July, 26 July, 1 August, 12 August, and 13 August 1978 (32).

TABLE 11. Daily Precipitation at University of Tennessee Plant Science Farm from 1 May to 30 September 1978

Date	Precipitation (Inches)	Date	Precipitation (Inches)
May 1	0.10	July 10	0.02
2	0.17	16	2.00
4	1.03	25	0.31
5	0.09	26	1.08
8	0.18	28	0.06
9	0.57	29	0.03
13	0.49	Aug. 1	1.03
14	0.25	5	0.29
15	0.10	6	0.55
16	0.03	7	0.03
23	0.03	9	0.03
24	0.95	10	0.15
29	0.02	12	2.10
June 3	0.21	13	1.03
4	T	15	0.33
8	1.40	17	0.16
9	2.10	18	0.10
13	1.31	26	0.32
20	0.45	30	0.03
21	0.34	31	0.05
23	0.62	Sept. 11	0.67
July 2	0.49	15	0.27
3	0.22	23	0.03
9	0.45		

Source: National Oceanic and Space Administration, Vol. 83, Numbers 5-9. "Climatological Data, Tennessee." 1978.

Two major increases in disease rating occurred in late June and late August to mid-September. Each followed two consecutive days with daily precipitation greater than one inch. Disease ratings and daily precipitation are presented in Figure 1. The first appearance of symptoms on 19 June 1978 occurred 10 days after the heavy rains on 8 and 9 June, and the increase in disease ratings in late August occurred 11-18 days after the rains on 12 and 13 August. Wolf and Lehman (52) reported that characteristic brown spot lesions occurred on soybeans 12 days after inoculation with S. glycines.

Possibly a long period of rainfall and high humidity is necessary for dissemination of S. glycines spores and satisfactory conditions for infection. The pycnidia may exude pycnidiospores after being wetted, and the pycnidiospores may be splashed by subsequent rain. Gough (20) found that pycnidiospores were exuded from pycnidia in wheat leaves infected with S. tritici within 24 to 26 hours after the tissues had been submerged in water for 15 seconds. If S. glycines also exuded pycnidiospores under similar conditions, additional rainfall could spread the spores to other leaves of the same plant or nearby plants.

There were 12 times between 1 May and 30 September 1978 when the relative humidity was greater than or equal to 90% for at least 18 hours (33). These dates are given in Table 12. Rainfall occurred within 24 hours of each of these periods of high relative humidity.

From 19 May 1978, when the soybeans were planted, to 28 September 1978, when the last disease rating was done, daily maximum temperatures were between 23 and 36 C and minimum temperatures were between 9 and 22 C.

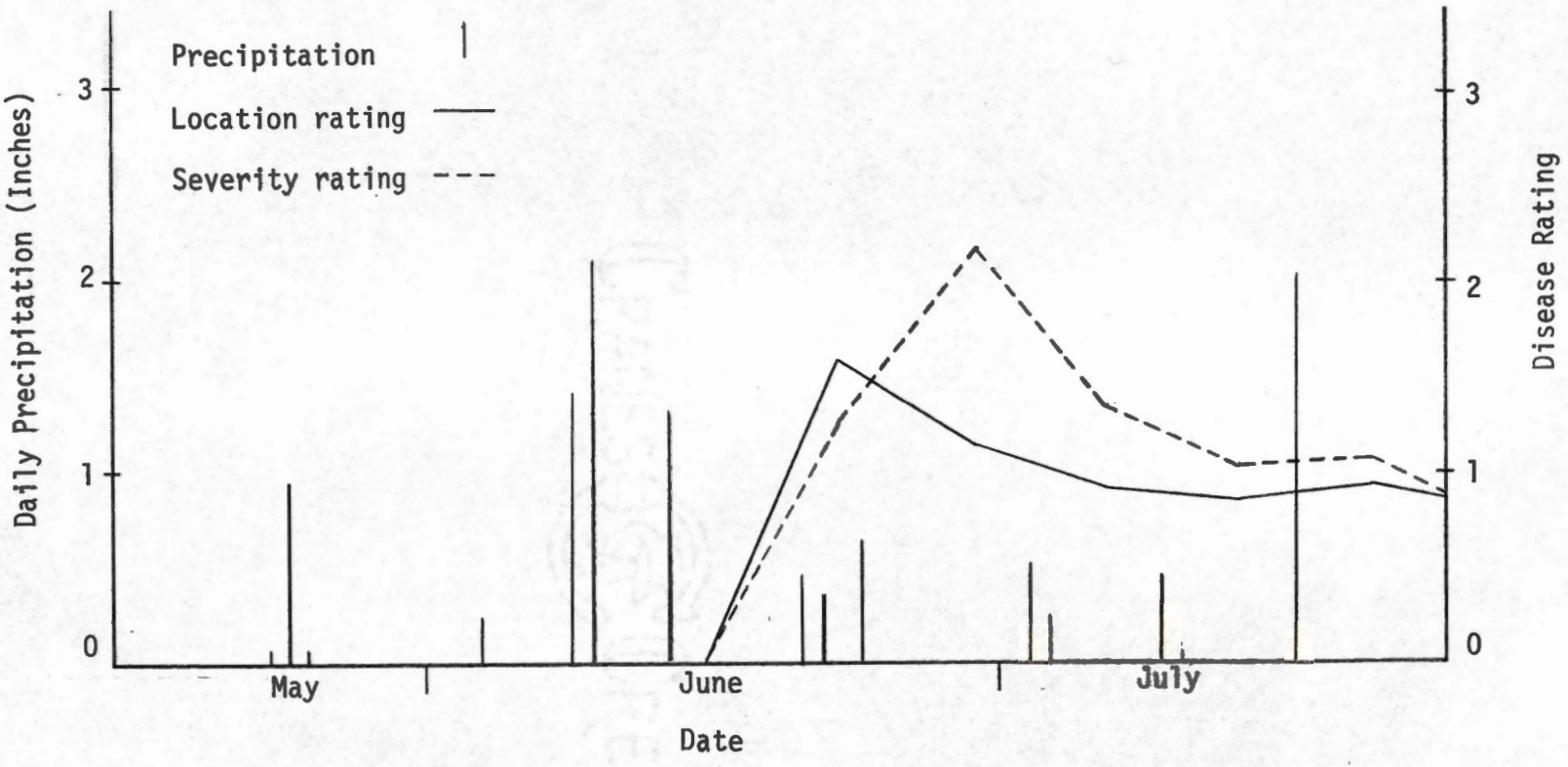

Figure 1. Daily precipitation at the University of Tennessee Plant Science Farm and brown spot disease rating of soybean cultivar Coker 136 during the summer of 1978.

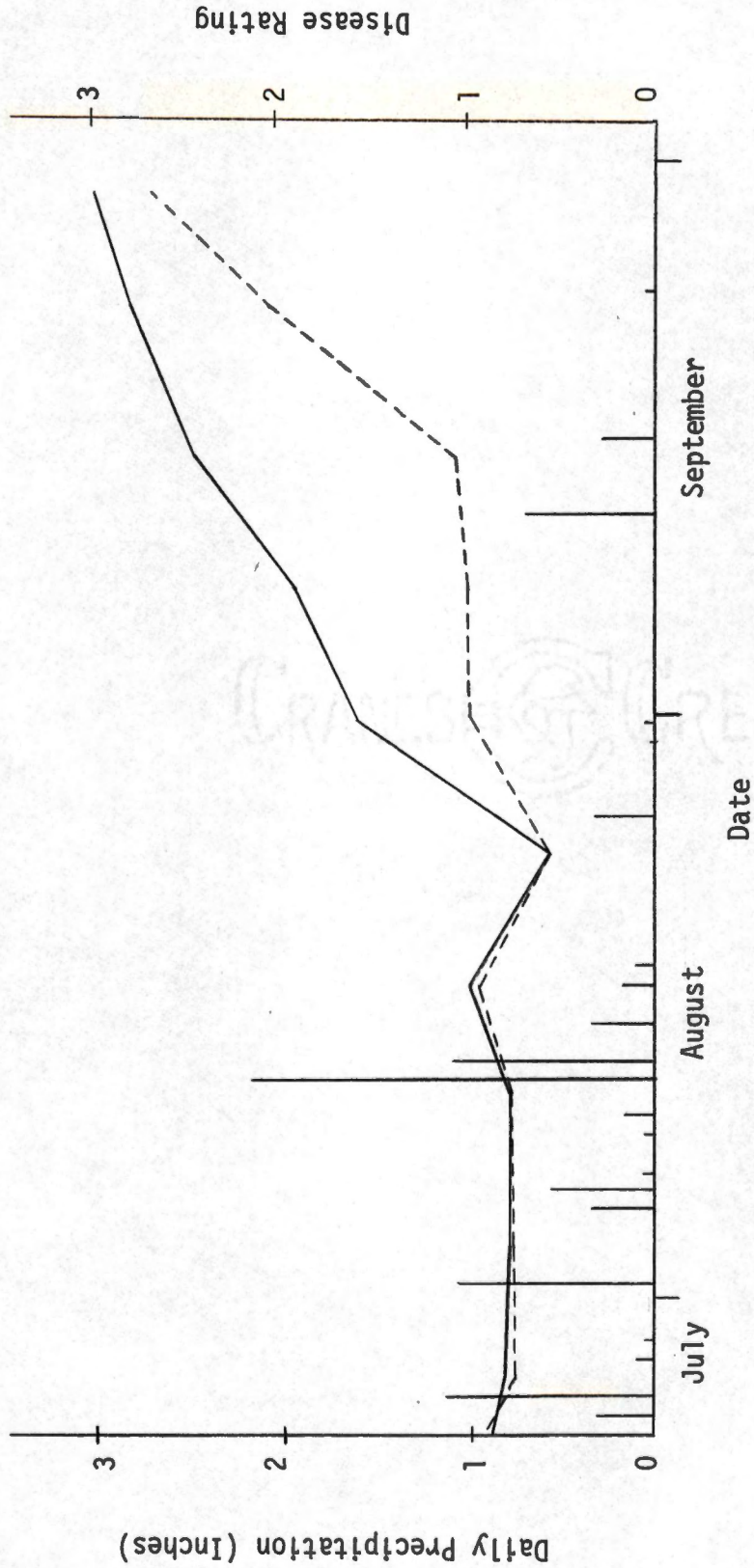

Figure 1. (Continued)

TABLE 12. Periods Between 1 May and 30 September 1978 with Relative Humidity ≥ 90% for at Least 18 Hours

Beginning of high humidity		Continuous Hours of R.H. ≥ 90%
Date	Time	
May 7	1:00 P.M.	42
June 2	7:00 P.M.	18
June 7	4:00 P.M.	39
June 19	4:00 P.M.	18
July 15	7:00 A.M.	27
July 24	10:00 P.M.	36
August 4	1:00 P.M.	24
August 8	7:00 P.M.	24
August 12	7:00 P.M.	18
August 29	7:00 P.M.	18
September 10	7:00 P.M.	18
September 14	1:00 P.M.	24

Source: National Oceanic and Space Administration,"Monthly Summary, Local Climatological Data," 1978.

There appears to be no relationship between temperature and the two major increases in brown spot symptoms in the field. The air temperature was close to the optimum temperature for S. glycines in culture (24 to 28 C) much of the time between 1 June and 30 September 1978. Although the optimum temperatures in culture and in the host plant are not necessarily the same, it seems unlikely that temperature alone was the cause of the apparent inactivity of S. glycines from mid-July to late August.

While increases in symptoms seemed to follow two-day periods of rainfall with 18 hours or more hours of high humidity, no definite conclusions can be drawn from one year's data. In years when temperature is less favorable for S. glycines, temperature may be a limiting factor.

Rainfall and humidity are undoubtedly important in the development of S. glycines, but other factors such as light intensity (8,18), temperature, and age of the host plant may also influence sporulation and infection.

V. DETERMINING RESISTANCE TO BROWN SPOT IN EIGHT SOYBEAN CULTIVARS

Greenhouse Trials

There were no significant differences among disease ratings of the eight cultivars in any of the three greenhouse trials. Mean disease ratingsfor each cultivar in each trial are presented in Table 13.

Detached Leaf Trials

Only three varieties, Essex, Lee 74, and York, became infected in the detached leaf trials. The mean disease ratings are presented in Table 14. In both trials there were significant differences among mean

Table 13. Incidence of Brown Spot on Soybean Plants of Eight Cultivars Inoculated with *Septoria glycines* in the Greenhouse

	Mean Disease Ratings[1]		
Cultivar	Trial I	Trial II	Trial III
Bedford	1.00[2]	1.25	1.12
Centennial	1.00	1.12	1.12
Dare	1.38	1.00	1.00
Essex	1.25	1.38	1.00
Forrest	1.50	1.12	1.25
Lee 74	1.25	1.38	1.00
Pickett 71	1.12	1.12	1.12
York	1.25	1.12	1.00

[1]Disease rating for individual pots:

1 = 0 plant infected/pot
2 = 1 plant infected/pot
3 = 2 plants infected/pot
4 = 3 plants infected/pot

[2]Values are means for eight replications (three plants each).

TABLE 14. Incidence of Brown Spot on Detached Leaves of Eight Soybean Cultivars Inoculated with *Septoria glycines*

Cultivar	Mean Disease Ratings[1] Trial I	Trial II
Bedford	1.00[2] d[3]	1.00 b
Centennial	1.00 d	1.00 b
Dare	1.00 d	1.00 b
Essex	1.33 c	1.33 ab
Forrest	1.00 d	1.00 b
Lee 74	1.67 b	1.33 ab
Pickett 71	1.00 d	1.00 b
York	2.00 a	1.67 a

[1]Disease rating for individual leaves

1 = not infected
2 = infected

[2]Values are means of six replications.

[3]Means within a trial followed by the same letter are not significantly different (P = 0.05).

disease ratings of the eight cultivars. In trial I the mean disease rating of York was significantly higher than Lee 74, which was significantly higher than Essex. The mean disease ratings of these three cultivars were significantly higher than those of the other five cultivars. In trial II the mean disease rating of York was significantly higher than Bedford, Centennial, Dare, Forrest, or Pickett 71. The ratings of Essex and Lee 74 were not significantly different from those of York or the other five cultivars.

Although the detached leaf trials indicate that York, Lee 74, and Essex are more susceptible than the other cultivars tested, these results should be compared with field cultivar trials to check the reliability of the detached leaf method of determining resistance. If the same differences in susceptibility occur in field trials as in detached leaf trials, this method could be a simple, useful tool for determining resistance of soybean cultivars to the brown spot pathogen.

Adventitious roots grew at the cut end of the petiole of all the detached leaves used in this study. Leaves which were transplanted to a peat, soil, and sand mixture or vermiculite in pots in the greenhouse stayed alive for over two months. None of the leaves developed a shoot from the callus tissue, but this does not eliminate the possibility that shoots might develop under other conditions. The development of adventitious roots from detached soybean leaves holds possibilities for research in nematology and plant breeding.

CHAPTER V

SUMMARY AND CONCLUSIONS

A temperature range of 24 to 28 C was most favorable for linear growth and sporulation of S. glycines. Modified potato-dextrose agar and V-8 juice agar were more satisfactory for growth and sporulation than Czapek-Dox agar. Sporulation was more variable than linear growth.

The most successful method of inoculation used during growth chamber studies on the effect of temperature on infection of soybeans by S. glycines consisted of atomizing a spore suspension upon the leaves. Distributing a mycelial or spore suspension over the leaves with a glass rod did not result in brown spot symptoms. No significant differences were observed in disease ratings of plants inoculated at 33, 29, 25, or 21 C. Significantly higher disease ratings occurred in a chamber with increased humidity than in a chamber with ambient humidity at temperatures of 29 C day and 25 C night. Experiments should be conducted in chambers with humidity control to determine the optimum temperature and number of hours of high humidity necessary for infection of soybean leaves by S. glycines.

Only four S. glycines pycnidiospores were collected in 1741 hours of monitoring spore release in the field with a Kramer-Collins spore sampler. These results were unsatisfactory considering the high level of infection in the field. Brown spot symptoms on naturally inoculated Coker 136 soybeans in the field increased in late June and again in late

August through mid-September. The two major increases in disease rating occurred 10 to 18 days after periods with relative humidity of 90% or greater for at least 18 hours and two consecutive days with daily precipitation greater than one inch. Several years of disease rating compared with weather data would be necessary to determine if this occurrence was more than coincidental. Development of brown spot in the field during mid-summer is probably limited by lack of rainfall and high humidity. Other factors, such as light intensity and possible cyclic sporulation of the fungus, should be investigated to determine their effect on infection of soybeans by S. glycines.

There were no significant differences among disease incidence on greenhouse plants of eight cultivars inoculated with S. glycines. Disease ratings of all cultivars were low. Significant differences in disease incidence occurred among cultivars when detached leaves were inoculated. In both trials, York, Lee 74, and Essex had higher disease ratings than Pickett 71, Forrest, Dare, Centennial, or Bedford. Comparisons with field trials should be made to evaluate the reliability of detached leaf inoculation as a method of determining resistance of soybeans to S. glycines.

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