Influence of Air Temperature on Brown Stem Rot of Soybean

D. V. Phillips

Assistant Professor, University of Georgia, College of Agriculture Experiment Stations, Georgia Station, Experiment, Georgia 30212.

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ABSTRACT

The influence of air temperature on brown stem rot (BSR) of soybean was determined, using several soybean cultivars inoculated with different isolates of *Cephalosporium gregatum*. Maximum BSR symptom development occurred from 15 to 27 C. Symptom development, rated on a scale of 0-4, was 3.0 at 27 C, 2.0 at 30 C, and 1.0 at 32 C. Cultivars were about equally susceptible to BSR, but *C. gregatum* isolates differed in virulence. The influence of temperature was the same regardless of cultivar or isolate. Air temperatures in field microplots were high enough to seriously limit BSR development for only a minor part of the growing season, and BSR development in these plots was extensive. Thus, air temperatures may have little influence on BSR development in the field. Phytopathology 61:1205-1208.

Additional key words: Glycine max.

Brown stem rot (BSR) of soybean (*Glycine max* [L.] Merr.) caused by *Cephalosporium gregatum* Allington & Chamberlain has been a serious disease in midwestern USA for many years (1, 13). The incidence of this disease in Iowa increased rapidly with increasing soybean acreage (4), and extensive yield losses have been demonstrated (6). In recent years, BSR has been found in Mexico (9) and in several southern states (8, 14) where soybean acreage has increased rapidly.

Allington & Chamberlain (1) reported that low air temperature was necessary for rapid BSR development. They found that soybean plants inoculated with *C. gregatum* remained symptomless at air temperatures above 21 C, but rapidly developed symptoms below 21 C. Chamberlain & McAlister (3) reported that symptom development was equally inhibited at 18 C night-32 C day or at constant 32 C, and that symptoms developed faster in older than in young plants. They concluded that air temperature and physiological age of the host governed the progress of BSR in the field, but that air temperature was more influential.

If high air temperature inhibits disease development, this might prevent BSR from becoming a serious disease in southern USA as suggested by Allington & Chamberlain (1). Dunleavy (4), however, was unable to show a close correlation between air temperature in the field and incidence of BSR in Iowa. Since high incidences of BSR have been reported from North Carolina (12) and Georgia (10), the effect of air temperature on BSR development was re-examined.

MATERIALS AND METHODS.—Soybean seeds were planted in vermiculite in a greenhouse with a day-length of at least 16 hr. Seedlings were transferred to a complete mineral salt solution (11) in plastic trays 14 to 21 days after planting, and placed in a growth chamber at 21 C with a 12-hr day-length at ca. 2,300 ft-c. Seven days later, the plants were inoculated and the growth chambers adjusted to the desired temperature (± 2 C).

Plants were inoculated by making a hole through the hypocotyl with a hypodermic needle 2-3 cm below the cotyledonary node. An inoculum suspension was injected into the hole until it ran out both sides of the hypocotyl. A similar hole was made in the hypocotyl of control plants, but no inoculum was injected. Inoculum was prepared by adding a small amount of agar with mycelium and conidia to a flask containing 100 ml of a sterile medium composed of the mineral salt solution above plus 5 g/liter technical maltose. The flasks were put on a gyrotory shaker at 200 excursions/min at 20-24 C. After 7 days, the flasks contained at least 3×10^6 spores/ml. To break up large masses of mycelium, cultures were blended 1 min in a Waring Blendor. The entire culture containing spores, mycelial fragments, and fermented medium was used as inoculum. In experiments where more than one isolate was used, the inoculum of each isolate was adjusted to the same optical density (equal to ca. 2×10^7 spores/ml).

Disease ratings were made 28-30 days after inoculation by splitting the stem longitudinally and visually checking for discoloration in the pith or xylem. Each plant was rated according to the following scale: 0 = no discoloration through the 1st node above the inoculation point (cotyledonary node); 1 = discoloration through the 1st node; 2 = discoloration through the 2nd node; 3 = discoloration through the 3rd node; and 4 = discoloration through the 4th node or above. In several experiments, the mean length of discolored stem in plants rated 1 = 5 cm, 2 = 13 cm, 3 = 17 cm, and 4 = 28 cm.

Seed was planted 2 June 1969 in field microplots (0.13 m^2 bordered by polyethylene cylinders) which had been fumigated with methyl bromide (175 g/m^3). Seedlings were inoculated 23 June by the method described above. In October just prior to maturity of each cultivar, the stems were split longitudinally and length of the discolored portion of the stem measured. The air temperature was recorded on a hygrothermograph near the microplots from 1 June to 31 October.

RESULTS.—Brown stem rot symptoms developed in nearly all inoculated plants; therefore, mean symptom ratings reflect primarily the length of internal stem browning rather than the percentage of plants with symptoms. No BSR symptoms were detected in control plants in growth chamber experiments, but BSR symptoms were found in some controls in field plots.

Effect of temperature on symptom development.—Mean symptom ratings of inoculated plants at 15 or 21 C were lower than those at 27 C in one experiment (Table 1), but in other experiments (Table 2) there was no significant difference in the ratings of plants at 15 and 27 C. The mean rating of all inoculated plants from three experiments (174 determinations/temperature) at 15 C was 3.5 and at 27 C was 3.6. Individual *C. gregatum* isolates were equally pathogenic at 15 and 27 C (Table 1), and individual soybean cultivars were equally susceptible at 15 and 27 C (Table 2).

The mean symptom rating of inoculated plants at 32 C was significantly lower than the mean of plants at 15 or 27 C (Tables 2, 3). The mean for all inoculated plants from three experiments (180 determinations/temperature) was 3.3 at 27 C and 0.6 at 32 C. Symptom development was less at 32 C than at 27 C, regardless of the soybean cultivar or *C. gregatum* isolate (Tables 2, 3). The symptom rating at 30 C was less than at 27 C, but higher than at 32 C (Table 3), indicating that symptom development may decrease with increasing temperatures above 27 C.

Disease development and temperatures recorded in field microplots.—All plants inoculated with isolates 5 and 39 developed BSR symptoms, as did 94 and 95% of those inoculated with isolates 36 and 43, respectively. Brown stem rot symptoms were found in 3% of the noninoculated control plants. The internal stem browning was more extensive in plants inoculated with isolates 5 and 39 than in those inoculated with isolates 36 and 43 (Table 4).

The air temperature near the microplots was between 15 and 27 C more than 60% of the growing season, and 32 C or above less than 5% of the growing season (Fig. 1). Air temperature reached 32 C or higher on 36 days, but remained at 32 C or higher for more than 1 hr on 22 days only, and for more than 4 hr on 15 days.

TABLE 1. Effect of air temperature on brown stem rot symptom development in Lee soybeans inoculated with different isolates of *Cephalosporium gregatum*

Isolate no.	Symptom rating[a]		
	15 C	21 C	27 C
5	4.0 f[b]	3.7 ef	4.0 f
36[c]	3.0 def	2.9 cdef	3.6 ef
37	4.0 f	4.0 f	4.0 f
38	3.7 cf	3.4 def	3.7 ef
39	2.3 cd	1.8 bc	3.4 def
40	0.5 a	1.2 ab	1.1 ab
41	2.7 cde	2.7 cde	3.6 ef
Mean	2.9 A	2.8 A	3.3 B

[a] 0 = No discoloration through the 1st node above the inoculation point (cotyledonary node); 1 = discoloration through the 1st node; 2 = discoloration through the 2nd node; 3 = discoloration through the 3rd node; and 4 = discoloration through the 4th node or above. Each figure denotes mean of 10 determinations.

[b] Numbers followed by the same letter are not significantly different at the 1% level; capital letters used to compare the means at different temperatures.

[c] ATCC 11073.

TABLE 2. Effect of air temperature on brown stem rot symptom development in soybean cultivars inoculated with *Cephalosporium gregatum* isolate 5

Soybean cultivar	Symptom rating[a]		
	15 C	27 C	32 C
Experiment 1			
Lee	3.7 a[b]	3.0 a	
Semmes	3.7 a	3.7 a	
P.I. 84946-2	4.0 a	3.5 a	
9 other cultivars[c] (mean)	3.7 a	3.4 a	
Mean	3.7 A	3.4 A	
Experiment 2			
Lee	4.0 c	3.9 c	0.3 a
Semmes	4.0 c	4.0 c	2.0 b
4 other cultivars[d] (mean)	4.0 c	4.0 c	0.1 a
Mean	4.0 B	4.0 B	0.5 A

[a] 0 = No discoloration through the 1st node above the inoculation point (cotyledonary node); 1 = discoloration through the 1st node; 2 = discoloration through the 2nd node; 3 = discoloration through the 3rd node; and 4 = discoloration through the 4th node or above. Each figure denotes mean of 4 determinations (experiment 1) or 10 determinations (experiment 2).

[b] Numbers followed by the same letter are not significantly different at the 1% level; capital letters used to compare the means at different temperatures.

[c] Hill, Pickett, Davis, Dare, Bragg, Coker 207, Hood, Hardee, Jackson.

[d] Hampton 266, York, Delmar, Ga. 59-871.

TABLE 3. Effect of air temperature on brown stem rot symptom development in Lee and Semmes soybeans inoculated with different isolates of *Cephalosporium gregatum*

Soybean cultivar	Isolate no.	Symptom rating[a]		
		27 C	30 C	32 C
Experiment 1				
Lee	5-1[c]	3.3 d[b]		0.5 ab
Lee	36-1	1.9 c		0.1 a
Semmes	5-1	4.0 e		0.9 b
Semmes	36-1	2.3 c		0.8 b
Mean		2.9 B		0.6 A
Experiment 2				
Lee	5	3.8 f	2.5 cd	0.9 ab
Lee	36	2.8 de	2.0 bcd	1.5 ab
Semmes	5	3.5 ef	2.1 bcd	0.7 a
Semmes	36	1.7 bc	1.3 ab	0.7 a
Mean		3.0 C	2.0 B	1.0 A

[a] 0 = No discoloration through the 1st node above the inoculation point (cotyledonary node); 1 = discoloration through the 1st node; 2 = discoloration through the 2nd node; 3 = discoloration through the 3rd node; and 4 = discoloration through the 4th node or above. Each figure denotes mean of 18 determinations (experiment 1) or 12 determinations (experiment 2).

[b] Numbers followed by the same letter are not significantly different at the 1% level; capital letters used to compare the means at different temperatures.

[c] Isolates No. 5-1 and 36-1 are monoconidial isolates from No. 5 and 36 (ATCC 11073), respectively.

TABLE 4. Brown stem rot symptom development in soybean cultivars inoculated with different *Cephalosporium gregatum* isolates in field microplots at Experiment, Ga., in 1969

Soybean cultivar	Internal stem browning (% of stem length) Isolate no.			
	5	36[b]	39	43
Lee	81[a]	17	79	21
Bragg	77	13	88	14
Hampton 266	89	14	91	13
Coker 102	86	14	81	12
Mean	83	14	84	15

[a] Each figure denotes mean of 10 determinations.
[b] ATCC 11073.

Susceptibility of soybean cultivars.—There were no striking differences in the susceptibility of cultivars in growth chambers or in field microplots. There were slight differences in the susceptibility of Lee and Semmes in some experiments (Tables 2, 3). However, the mean symptom ratings of all inoculated plants from four experiments, 2.1 for Lee and 2.2 for Semmes, indicate no difference for those cultivars.

The field resistance reported for P.I. 84946-2 (2) was not apparent when this cultivar was inoculated (Table 2). In an experiment terminated early due to failure of the growth chambers, there was no apparent difference in the susceptibility of Lee and P.I. 84946-2 inoculated with eight isolates of *C. gregatum* at 15 or 27 C.

Virulence of C. gregatum isolates.—There was wide variation in virulence among isolates in both growth chamber and field tests (Tables 1, 4). There was a slight difference between the virulence of isolates 5 and 36 (ATCC 11073) in growth chamber experiments. The means (102 determinations/isolate) for all plants from two experiments inoculated with isolates 5 and 36 were 2.7 and 2.1, respectively. The difference in virulence of these isolates was greater in field microplots where symptom development occurred over a longer time (Table 4). Isolate 40 was less virulent than other isolates tested (Table 1).

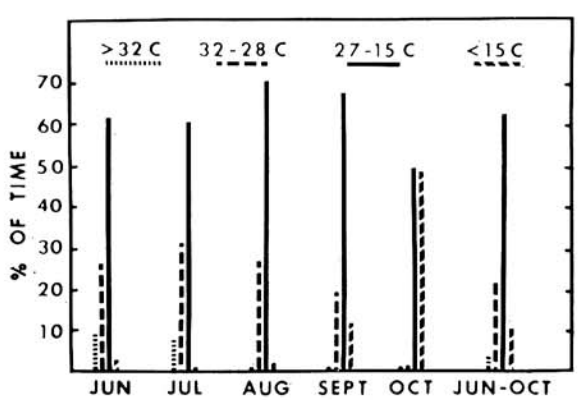

Fig. 1. Air temperatures near field microplots at Experiment, Ga., 1 June to 31 October 1969. Bars represent the percentage of time the temperature was in the indicated range for each month and for the total period.

DISCUSSION.—Brown stem rot development was extensive and unaffected by air temperatures from 15 to 27 C. At 30 C, symptom ratings were about 33% lower than at 27 C; and at 32 C, about 67% lower than at 27 C. These results differ considerably from those reported by Allington & Chamberlain (1), who reported extensive disease development at 15 C, very little at 21 C, and none at 27 C. Chamberlain & McAlister (3) reported more extensive disease development in plants held at 18 C night-24 C day temperatures than in those at 18 C night-32 C day temperatures or at constant 32 C, but their results indicate some disease at 32 C. In this study, symptoms developed as extensively at 27 C as at 15 C regardless of the soybean cultivar or *C. gregatum* isolate. Apparently, the report of suppression of BSR symptoms by air temperatures above 21 C (1) represents a special case and is not generally applicable to soybeans with BSR. Since disease development was less extensive at 30 C than at 27 C, it appears that the upper limit for maximum disease development lies somewhere between these two temperatures. The lower limit for maximum disease development was not determined, but is 15 C or below.

The mean of 50% of the stem with internal browning in the field microplots is extensive disease development compared to a mean of 18% reported for naturally infected plants in Iowa (5). This extensive disease development in a year when the mean temperature for the growing season was less than 1 C below normal indicates that normal air temperatures as far south as mid-Georgia are not highly inhibitory to disease development.

Chamberlain & McAlister (3) concluded that disease development was influenced by both age of plants and air temperature, with temperature being more influential. They concluded that high daytime temperature inhibited disease development, as disease development at constant 32 C was equal to that at 32 C day-18 C night. They did not specify the length of the day and night periods, but it is probable that the day period at 32 C in their experiments was several hours long.

Since days when the temperature is 32 C or above for more than a short time are infrequent during the growing season and the disease develops well at 30 C, it is unlikely that normal air temperature would greatly inhibit BSR in the field. The appearance of symptoms late in the growing season may be primarily a result of increased susceptibility of older plants (3) rather than disease inhibition by high temperatures early in the growing season. It is possible that extensive spread of the pathogen within the vascular tissues occurs before internal stem browning. Thus, this symptom may not be a reliable indication of disease progress early in the growing season.

Differences in susceptibility to BSR among soybean cultivars have been reported (7), but in these experiments differences were small and inconsistent. The possibility of a resistance factor in the roots of P.I. 84946-2 might explain the conflicting reports of resistance in the field (2) and susceptibility when inoculated in the hypocotyl in this study. Kunkel & Dunleavy

(7), however, concluded that a symptom-mitigating factor in less susceptible cultivars was located in the aerial portions of the plants rather than in the root system.

The variability among *C. gregatum* isolates is a factor that should be considered in research on BSR. A more thorough investigation of the differences in virulence among isolates is in progress.

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