Brown Spot Development and Yield Response of Soybean Inoculated with Septoria glycines at Various Growth Stages

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ABSTRACT

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Brown spot (which is incited by *Septoria glycines*) caused 17.1% yield loss on Essex soybeans in field tests in 1976; yield and seed size were negatively correlated with percent leaf area diseased. Yield loss was due to reduction in seed size. Symptoms appeared 2 wk later in 1977 than in 1976; disease levels were lower and loss was nil. The periods for maximum symptom development for Essex plants inoculated with *S. glycines* at the second trifoliolate stage, at flowering, and at full-pod stage were approximately 21, 40, and 21 days, respectively. The longer incubation period of infections initiated near flowering accounted for the marked reduction in brown spot of soybean during midsummer.

Additional key words: Glycine max.

Brown spot of soybean (*Glycine max* [L.] Merr.), which is caused by *Septoria glycines* Hemmi (1), was reported in North Carolina by Wolf and Lehman in 1928 (8). MacNeill and Zalosky (4) described the histopathology of the disease, and Ross (6) indicated that brown spot was the predominant leaf disease present in spray tests in North Carolina during a 1974 study of late season foliar diseases of soybeans. In the latter study, soybean yields were increased with benomyl sprays and irrigation during the final month of the growing season but brown spot levels were not reported.

When Hemmi (1) described brown spot of soybean, he reported that the disease incidence increased most rapidly in damp, warm weather; however, in midsummer the disease did not occur on the upper leaves. If conditions were favorable for the fungus, disease reappeared actively again in September. Factors responsible for the lack of disease on upper leaves in the midsummer might include unfavorably high temperatures, lack of sufficient rainfall, and/or a resistant physiological phase of the plants.

The objectives of this research were to determine: (i) potential yield loss due to brown spot, and (ii) the susceptibility of soybean leaves at different ages or developmental stages in an attempt to explain the lower incidence of the disease in midsummer.

MATERIALS AND METHODS

Yield loss tests.—In 1976, cultivar Essex soybeans were planted on 26 May in three-row plots, 6.1 m long, with 1.8 m of soybeans as a buffer between plots, and in an area of a field that was planted in corn in 1975; the remainder of the field was in soybeans in 1975 and 1976. Distance between rows was 0.96 m. Plots were inoculated with an aqueous spore suspension containing 1×10^5 *S. glycines* spores/ml and 0.5% gelatin. A CO_2-pressurized sprayer operated at 1.76 kg-force/cm^2 was used to thoroughly wet the upper and lower leaf surfaces with the suspension. Treatments were: (i) inoculations on 21 and 28 June; (ii) inoculations on 21 and 28 June, 29 July, and 5, 21 August; (iii) inoculations on 21, 28 June, 29 July, and 5, 21 August; and (iv) the control sprayed with 0.5% gelatin solution on all inoculation dates. Treatments were replicated eight times in a randomized complete block design.

Inoculum was produced by growing *S. glycines* on PDA, pH 4, for 2-3 wk, at 24 C. Plates were then flooded with distilled water, the agar surface was lightly rubbed to release the spores, and the suspension was strained through four layers of cheesecloth. The spore suspension was kept at 5 C or placed on ice when carried to the field and was adjusted to 100,000 spores/ml immediately before being applied. Percent defoliation was estimated visually and percent leaf area diseased was determined on the remaining leaves on 13 September according to the scale developed by Main et al (5) for tobacco leaves. Twenty leaflets, selected at random in each plot, were rated and averaged to obtain the percent leaf area diseased for each plot.

On 18 May 1977, three-row plots, 6.1 m long, of soybean cultivars, Essex, Forrest, and Centennial were planted in a field that had not been planted to soybeans for the previous 8 yr. A randomized complete block design was used and each plot was separated by two rows of Lee 74 soybeans on the sides and a 3 m alley at each end. Treatments, which were replicated six times, were: (i) Essex inoculated on 14 June (two trifoliolates); (ii) 18 July (flowering); (iii) 12 August (pods 2 cm long); (iv) Forrest and Centennial inoculated on 18 July (flowering); and (v) all cultivars sprayed with 0.5% aqueous gelatin on inoculation dates as the control.

On 28 September, foliage disease ratings were made as in 1976 except that 10 leaflets were rated in each plot. Percent defoliation was calculated by dividing the height of the lowest leaf from the soil line on the main stem by the

height of the plants. Pods were beginning to turn yellow in the Essex and Forrest plots.

After maturity, the center row of each plot was trimmed to 5.5 m, cut, and threshed with a stationary thresher. After drying, the total seed weight per plot and the weight of 100 seed was determined as measures of yield and seed size, respectively. Data were analyzed by analysis of variance and linear regression. In 1977, covariance and multiple regression analyses were used to correct for stand differences.

Influence of developmental stages on symptom development.—Essex soybeans were planted in 15-cm-diameter clay pots and thinned to one plant per pot soon after emergence. Plantings were made at monthly intervals from May through August 1976 and the plants were grown outdoors. Upper and lower leaf surfaces were sprayed in August with a suspension containing 1×10^5 *S. glycines* spores/ml and 1% gelatin until a continuous film formed. Plants were covered with plastic bags after inoculation, and the pots were placed in saucers of water on a bench in an air conditioned greenhouse (24 C), for 48 hr. Then the plants were placed outdoors again. Disease ratings were based on the number of defoliated leaves on the main stem and the percentage of the leaf area of the remaining leaves that was diseased.

In 1977, Essex soybeans were planted in 3-m, one-row plots in the same field used for the yield tests. Four plots were inoculated with *S. glycines* at 3-wk intervals beginning 14 June, as described for the yield loss test. Infection data were taken approximately 3 wk after each inoculation. Percent defoliation was calculated either by comparing total nodes to the number of nodes without leaves on 10 plants or by comparing the average height of the lowest leaf to total plant height. Foliage disease ratings were made as in the yield loss test. The disease index (DI) was calculated as [% defoliation + % remaining leaves] × [proportion of remaining leaf area diseased].

RESULTS

Field loss.—In 1976, the effect of the June inoculations could not be assessed because of the large amounts of defoliation from natural infection in noninoculated plots in May and June. Chlorotic halos around lesions as a result of the 29 July inoculation were first observed on 23 August. Inoculations in July and August significantly increased disease ratings and reduced yields compared to those of the noninoculated control (Table 1). Yield was significantly, negatively correlated with percent leaf area diseased ($r = -0.69$, Fig. 1-A), defoliation ($r = -0.68$), a combination of the percent leaf area diseased and defoliation with multiple regression ($r = -0.70$) and was positively correlated with seed size ($r = 0.80$). Seed size was highly significantly, negatively correlated with percent leaf area diseased ($r = -0.75$, Fig. 1-C), defoliation ($r = -0.68$), and combination of both ($r = -0.70$).

In 1977, the various inoculations of Essex produced significantly different amounts of disease but no differences in yield or seed size (Table 1). Seed size was not correlated with percent leaf area diseased (Fig. 1-D), defoliation, or the combination of both. Yield was significantly correlated with percent diseased leaf area ($r = 0.57$, Fig. 1-B), but a cross-products analysis of yield and percent leaf area diseased showed no significant correlation of these variables among the treatments. Yield was not correlated with defoliation.

More brown spot occurred in 1976 than in 1977 (Fig. 1-A,B). In 1976, half the plots had plants with leaf areas diseased greater than 40%, and only 9% of the plots had less than 20% leaf area diseased; in 1977, only 20% of the plots had greater than 40% leaf area diseased, whereas 40% of the plots had less than 20% leaf area diseased.

Inoculations had nonsignificant effects on the amount of disease, yield, and seed size of Forrest, and on yield and

TABLE 1. Effects on disease severity and yield[a] of inoculating field-grown soybeans at different dates with *Septoria glycines*

Inoculation dates, cultivars	Leaf area diseased[b] (%)	Defoliation[c] (%)	Yield (kg/ha)	Weight of 100 seed (g)
1976, Essex				
Control	26	56	3,009	12.2
21, 28 Jun	24	53	3,151	12.4
21, 28 Jun; 29 Jul; 5 Aug	58	88	2,605	11.0
21, 28 Jun; 29 Jul; 5,21 Aug	57	90	2,495	10.8
LSD ($P = 0.01$)	7	8	399	0.2
1977, Essex				
Control	11	47	2,576	14.1
14 Jun	20	50	2,347	14.6
18 Jul	45	64	2,686	14.4
12 Aug	36	64	2,661	15.4
LSD ($P = 0.05$)	11	10	399 NS[d]	1.0 NS
1977, Forrest				
Control	11	45	3,203	13.8
18 Jul	21	47	3,197	13.9
LSD ($P = 0.05$)	12 NS	9 NS	477 NS	1.8 NS
1977, Centennial				
Control	1	34	3,056	15.4
18 Jul	4	40	3,338	15.0
LSD ($P = 0.05$)	3	5	555 NS	0.5 NS

[a]Disease assessments were made on 13 September 1976 and 28 September 1977.
[b]Percentage of remaining leaf area affected.
[c]Height of lowest leaf from soil ÷ total plant height.
[d]The F-test showed nonsignificant differences between means.

seed size of Centennial (Table 1). The differences in the percentages of defoliation and leaf area diseased between inoculated and uninoculated Centennial treatments were small, but significant (Table 1).

Disease development.—Plants inoculated at flowering stage and at the beginning of pod-filling manifested less disease 18 days after inoculation than did plants inoculated when the second trifoliolate was expanded or when pods were full (Table 2). Plants inoculated when pods were 1 cm long and at initiation of pod-filling had less area diseased 12 days after inoculation than did plants inoculated when the second trifoliolate was expanded or when the pods were full. Plants inoculated when pods were 1 cm long did not develop disease equal to that of the other aged plants until 40 days after inoculation (Table 3). Symptoms developed more rapidly on older leaves than on younger leaves; for example, the oldest inoculated leaf of plants seeded on 1 July 1977 had 50% leaf area diseased compared to less than 5% leaf area diseased on the youngest inoculated leaf. The results from these tests are supported by those from field inoculations (Table 4). Lesions on young (14 July) and old (8 September) field-grown plants had chlorotic halos 30 days after inoculation, but plants inoculated at flowering required approximately 60 days for similar symptom development.

TABLE 2. Disease indices on Essex soybeans grown in pots outside 18 days after inoculating simultaneously with *Septoria glycines* on 31 August 1976

Date of planting	Developmental stage at inoculation	DI[a]
13 Aug	Two trifoliolates	91
16 Jul	Flowering	5
14 Jun	Beginning pod-fill	17
15 May	Full-pod	68
LSD ($P = 0.01$)		28

[a]Disease index (DI) = [% defoliation + % leaves remaining × [proportion of remaining leaf area diseased], and was calculated using only inoculated leaf area.

DISCUSSION

In 1976, there was a yield loss of 17.1% in plots inoculated five times with *S. glycines* compared to the uninoculated control. More severe disease in some seasons may lead to even greater loss. Yield loss occurs primarily through the reduction in seed weight (seed size) as one would expect with a late-season foliage disease. Although the analysis of variance of 1977 data showed no significant yield differences among treatments caused by the disease, there was a positive correlation of yield with percent of leaf area diseased. The nonsignificant differences in yield and seed size among plants that received different inoculation treatments indicate that the above correlation should be nonsignificant. A cross-product analysis of yield by percent leaf area diseased was

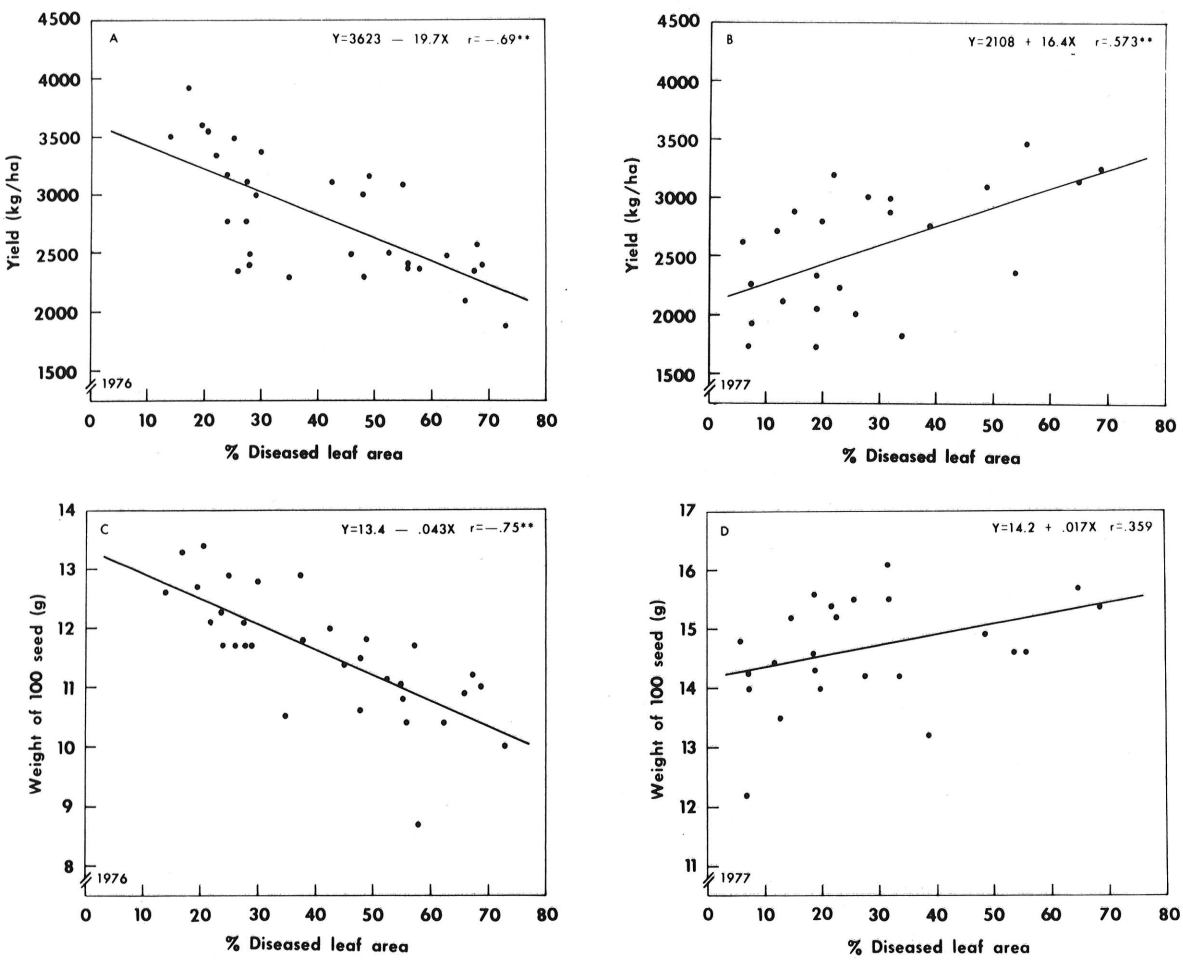

Fig. 1-(A to D). Regression analysis of percent diseased leaf area and yield of Essex soybeans in **A)** 1976 and **B)** 1977 and of percent leaf area diseased and seed size in **C)** 1976 and **D)** 1977. Percent leaf area diseased ratings were made on 13 September 1976 and on 28 September 1977.

TABLE 3. Brown spot development on inoculated leaves of Essex soybeans grown in pots outdoors and inoculated simultaneously at different developmental stages on 25 August 1977

Date of planting	Developmental stage at inoculation	DI[a] at inoculation plus:			
		12 days	21 days	27 days	40 days
3 Aug	Two trifoliolates	75	100	100	100
1 Jul	Pods 1 cm long	16	34	57	100
2 Jun	Beginning pod-fill	12	83	94	100
1 May	Full-pod	58	90	100	100
LSD ($P = 0.05$)		30	18	12	
LSD ($P = 0.01$)		42	26	17	

[a]Disease index (DI) = [% defoliation + % leaves remaining] × [proportion of remaining leaf area diseased].

TABLE 4. Brown spot development on inoculated leaves of field-grown Essex soybeans at various developmental stages 3 wk after inoculations with *Septoria* glycines in 1977

Inoculation date	Developmental stage at inoculation	Disease index[a]
14 Jun	Two trifoliolates	100
Control		0
6 Jul	Eight trifoliolates	33[b]
Control		1
26 Jul	Flowering	0[c]
Control		0
12 Aug	Pods 2-cm long	0[d]
Control		0
8 Sept	Full-pod	49
Control		23

[a]Disease index = [% defoliation + % leaves remaining] × proportion of remaining leaf area diseased].

[b]Differences between inoculated and controls significant, $P = 0.01$.

[c]At 64 days after inoculation disease indices were 86 and 23 for inoculated and control, respectively.

[d]At 47 days after inoculation disease indices were 41 and 23 for inoculated and control, respectively.

made to determine if this correlation occurred within the treatments, blocks and/or error terms. A nonsignificant correlation occurred within each of these terms, but such an analysis of 1976 data was significant within the treatments ($r = -0.98$). Therefore, it was concluded that there was no supporting data for the correlation between yield and percent leaf area diseased in 1977.

Possible explanations for lack of yield reduction in 1977 are that chlorotic symptoms appeared 2 wk later than they did in 1976 and/or that there was more disease in 1976 than 1977 presumably because of the greater abundance of naturally occurring inoculum and more frequent inoculations in 1976. If symptoms develop more rapidly when infected plants are under stress, then infections initiated in July may have developed earlier in 1976 than in 1977 due to water deficiency during August. Plots received only 51 mm of rainfall in August 1976 compared to 204 mm in August 1977. In 1977, the seed dry matter may have accumulated before the disease developed since chlorosis on infected leaves was not observed until 8 September compared to 23 August in 1976.

The slight disease development in Centennial in 1977 accounted for the absence of yield loss, and this may reflect resistance. Disease indices (DI) of inoculated plots of Forrest were equal to those of Essex in some blocks (DI = 68.5 and 64.4 for Forrest and Essex, respectively, in one replicate); therefore, there may be a potential for yield reduction in Forrest in some seasons.

Slower disease development on plants in middle growth stages than on younger or older plants has been reported in potato. In some potato cultivars, leaves intermediate between the top and bottom were resistant to infection by *Phytophthora infestans* (Mont.) d By. during pre- and post-flowering stages (7). At flowering time, the top leaves were less susceptible than at other times. It was not reported whether those leaves developed more symptoms as the plants aged. Other reports of similar changes in susceptibility during middle growth stages have been reported for leaf diseases of other crops (2,3).

Hemmi (1) and Wolf and Lehman (8) reported that brown spot developed rapidly in the fall and "thousands of specks, with no distinctive microscopic feature except their rust brown color" occurred on the leaves. The development of large numbers of lesions in the fall apparently resulted from an accumulation of infections, from the onset of flowering, which did not develop until the plants reached a certain physiological stage coincident with the late productive stages. The accumulation of infections accounted for the rapid development of the disease observed in the fall. If infections accumulate throughout the summer without symptom expression until fall, then protectant fungicides should be applied in July to prevent early infections. Yield loss determinations should take into consideration the plant age when symptoms are expressed. Resistance evaluations should be done in the field after full-pod stage, since a form of resistance may delay symptom development until after dry matter accumulation in the seed has occurred.

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