Environmental Effects on the Development of Brown Stem Rot in Soybean

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ABSTRACT

Waller, R. S., Nickell, C. D., and Gray, L. E. 1992. Environmental effects on the development of brown stem rot in soybean. Plant Dis. 76:454-457.

Brown stem rot of soybean (*Glycine max*), caused by *Phialophora gregata*, can cause significant yield reductions under favorable environmental conditions. Leaf symptoms may not always be present in the field; therefore, field evaluation must rely on stem symptoms. Eleven genotypes from maturity groups I, II, and III were planted in hill plots (three plants per hill) in two environments with different fertility and crop rotations for two years, 1988 and 1989, with and without inoculation with *P. gregata*. All genotypes showed greater development of brown stem rot stem symptoms under low fertility and in inoculated plots. Significant increases in stem symptoms were observed for three of six susceptible genotypes in the two environments. Field evaluations can be highly variable from year to year and within years. Inoculation caused a significant increase in disease development for all susceptible genotypes. Field evaluation and selection for brown stem rot resistance may be more efficient in areas with low fertility and high levels of inoculum of *P. gregata*.

Brown stem rot (BSR) of soybean, (*Glycine max* (L.) Merr.), caused by *Phialophora gregata* (Allington & D. W. Chamberlain) W. Gams, occurs frequently in the north central United States (2). Isolates of the pathogen have been categorized as Type I (causing both stem browning and foliar chlorosis and necrosis) and Type II (causing only stem browning) (10). Gray (11) found that inoculation with Type I isolates significantly reduced yields compared to control and Type II isolate-inoculated plants. Yield loss estimates from 12 to 38% have been associated with infestations of *P. gregata*, with susceptible cultivars, and with environmental conditions that favor disease development (11,18).

Variability in symptom severity caused by Type I isolates has been reported (15,23). This variability will affect breeding for resistance to BSR. All public cultivars registered as BSR-resistant derive their resistance from PI 84946-2. New sources of resistance need to be incorporated into cultivars to provide a broader genetic base for resistance to BSR.

Increased levels of fertility, especially levels of nitrogen and potassium, affect the incidence and severity of fungal diseases in several crop species (21). Jeffers et al (12) discovered that supplemental potassium decreased the incidence of Phomopsis seed decay in

Accepted for publication 12 December 1991.

soybean, caused by *Phomopsis longicolla* T. W. Hobbs. The incidence of pod and stem blight, caused by *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. var. *sojae* (S. G. Lehman) Wehmeyer, on soybean was reduced by increased potassium fertilization (5,14). Phytophthora root rot of soybean, caused by *Phytophthora megasperma* Drechs. f. sp. *glycinea* T. Kuan & D. C. Erwin, increased significantly with increased levels of nitrogen, phosphorus, and potassium (6). These results indicate the potential for different levels of fertility to modify disease development in soybean. Knowledge of the relationship between fertility and disease development could enhance production practices and aid breeders in determining the proper environment for selection of resistant plants.

The objectives of this study were to determine the effect of two environments with different fertility and crop rotations and inoculation treatments on the development of BSR on soybean in the field.

MATERIALS AND METHODS

Two fields at the Agronomy and Plant Pathology South Farm, Urbana, Illinois, were selected in 1988 and 1989 because of previous management practices that affected fertility levels. In environment 1 (E1), the soil had mean values of pH 6.28 ± 0.04 (standard error), 445.95 ± 29.58 kg/ha of phosphorus (Bray no. 1 method), and 1,111.04 ± 26.29 kg/ha of potassium (exchangeable). In environment 2 (E2), the soil averaged pH 5.73 ± 0.05, 136.64 ± 4.76 kg/ha of phosphorus, and 501.76 ± 11.37 kg/ha of potassium. The E1 field is in a corn-soybean rotation. The E2 field was in an oats, wheat, soybean, and corn rotation. The soil type in both fields was a Flanagan silt loam.

Soybean genotypes resistant (R) and susceptible (S) to *P. gregata* were used for this study. The resistant lines were included as a comparative standard for the susceptible lines and because of the occurrence of slight stem browning that may be observed in resistant genotypes. Three maturity groups (MG) were represented. MG I consisted of BSR 101 (R) (20), Hardin (S) (8), and Hodgson 78 (S) (13); MG II consisted of BSR 201 (R) (19), Burlison (S) (17), and Century 84 (S) (22); and MG III consisted of Asgrow A3733 (R), Chamberlain (R) (16), Fayette (R-Intermediate) (4), Cumberland (S) (3), and PI 437654 (S). Seeds of each genotype were germinated in sand-filled plastic pots 10 cm in diameter. The roots of unifoliolate seedlings were rinsed in water and blotted dry with a paper towel. Three healthy plants were selected and dipped in 45 ml of inoculum of *P. gregata*. Following inoculation, the plants were transplanted into 355-ml paper cups filled with a steam-sterilized mixture of sand and topsoil (1:1, v/v), and the excess inoculum was added. Another three plants of the same genotype were transplanted without inoculum for each replication.

Inoculum for the root-dip assay was prepared by placing 1-cm-diam. mycelial plugs of *P. gregata* grown on Century 84 soybean stem agar (1) plates into sterile Century 84 soybean seed broth (85 g of seed per liter of distilled water). Cultures were incubated in the dark at 20 C for 4 wk. The broth and resultant mycelial mat were blended at high speed in a blender for 90 sec, the final inoculum concentration was adjusted to 1.2×10^6 propagules per milliliter, and 0.5% (w/v) methylcellulose was added.

After 3 days in the greenhouse, the plants were removed from the paper cups and transplanted into the E1 and E2 fields in hills spaced 1 m × 1 m apart. A completely randomized design was used with six replications. The fields were irrigated with trickle irrigation tubes placed next to each row of hill plots. In 1988, the fields were irrigated weekly, and in 1989 only as needed in order to keep the soil moist. Plants were rated at the early R7 growth stage (9) for stem symptoms in 1988 and for stem and leaf symptoms in 1989. Severity ratings for both stem and leaf symptom were calculated by (number of nodes with symptoms) ÷ (total number of nodes)

Table 1. Analysis of variance mean squares and *F* tests for brown stem rot stem symptom data combined over 2 yr (1988–1989)

		Stem symptom severity[a]	
Source of variation	df	Mean square	*F* test[b]
Year (Y)	1	120.93	NS
Inoculation (I)	1	99,435.58	**
Environment (E)	1	25,249.84	**
Maturity group (MG)	2	2,851.97	**
Genotype (Ge) within MG	8	17,006.27	**
Y × I	1	6,231.71	**
Y × E	1	307.82	NS
Y × MG	2	11,953.30	**
Y × Ge/MG	8	3,340.33	**
I × E	1	1,911.82	**
I × MG	2	2,393.89	**
I × Ge/MG	8	5,575.08	**
E × MG	2	942.89	**
E × Ge/MG	8	957.75	**
Y × I × E	1	18.67	NS
Y × I × MG	2	948.14	**
Y × I × Ge/MG	8	1,608.04	**
Y × E × MG	2	576.47	*
Y × E × Ge/MG	8	484.72	**
I × E × MG	2	253.66	NS
I × E × Ge/MG	8	831.94	**
Y × I × E × MG	2	547.20	*
Y × I × E × Ge/MG	8	440.55	**
Error	1,361	161.28	···

[a] Expressed as percent stem tissue damaged.
[b] Significant at $P < 0.05$ (*) or $P < 0.01$ (**), or nonsignificant (NS).

Table 2. Analysis of variance *F* tests[a] for brown stem rot stem symptom data separated by maturity group (MG)[b]

		Stem symptom severity[c]			
Source of variation	df	MG I	MG II	df	MG III
Year (Y)	1	**	NS	1	**
Inoculation (I)	1	**	**	1	**
Environment (E)	1	**	**	1	**
Genotype (Ge)	2	**	**	4	**
Y × I	1	NS	**	1	**
Y × E	1	NS	NS	1	NS
Y × Ge	2	**	**	4	**
I × E	1	NS	**	1	NS
I × Ge	2	**	**	4	**
E × Ge	2	**	*	4	**
Y × I × E	1	*	NS	1	NS
Y × I × Ge	2	NS	**	4	**
Y × E × Ge	2	NS	NS	4	**
I × E × Ge	2	*	**	4	*
Y × I × E × Ge	2	**	NS	4	NS

[a] Significant at $P < 0.05$ (*) or $P < 0.01$ (**), or nonsignificant (NS).
[b] Three genotypes in MG I and II, five in MG III.
[c] Expressed as percent stem tissue damaged.

× symptom severity. Severity ratings were based on a 0–5 scale, with 0 = 0%, 1 = 20%, 2 = 40%, 3 = 60%, 4 = 80%, 5 = 100% damaged tissue.

Data were analyzed over both years for stem symptom severity and for one year (1989) for leaf symptom severity with the main effects of inoculation, environment, maturity group, and genotype within maturity group and their interactions. The main effects were considered fixed. The overall error term was used to test for significance of main effects and interactions. Data were analyzed with SAS procedure GLM. Plants of Burlison in E1 in 1989 died at approximately the V3 stage of development from metribuzin sensitivity.

RESULTS AND DISCUSSION

The main effect of year was nonsignificant for stem symptom severity (Table 1). Differences in stem symptom severity were observed between the two years, primarily in Century 84, Cumberland, and PI 437654, with higher severity ratings in 1989, when environmental conditions (lower temperatures and more rainfall) were more conducive to disease development. The main effects other than year contributed more to the overall variation among stem severity ratings. When the data were separated by maturity group (because of the significant year × maturity group interaction), the year effect became significant for groups I and III (Table 2). The year × environment interaction was nonsignificant for the combined and separated data.

Inoculation had a significant impact on differentiating resistant and susceptible genotypes (Table 3). This was highly evident in the E1 field. The E2 field had higher stem severity ratings for both inoculated and uninoculated hill plots than the E1 field. Inoculation was beneficial in increasing disease development, but the method used in the study was time- and labor-intensive. Less intensive inoculation methods, such as application of soybean straw infected with *P. gregata*, may prove useful in creating field situations for differentiating resistant and susceptible plants. Dunleavy (7) demonstrated the effect of applying diseased soybean straw on a field free of *P. gregata*. Incidence of BSR on soybean following the application was 67%, and in the second year all plants became infected.

Disease was more severe on plants in field E2 in both susceptible and resistant genotypes than in E1. For several genotypes (e.g., Hardin), stem severity ratings for the uninoculated treatment in E2 did not differ greatly from ratings for the E1 inoculated treatment. The crop rotation in effect for the E2 field would not be conducive to inoculum increase for *P. gregata*; therefore, the increase in disease severity may not be related to lower levels of potassium or phosphorus in the soil. Thus, fields with reduced levels of fertility may aid field selection for resistant plants in segregating populations.

Maturity group (MG) had an effect on severity ratings, primarily because of the early maturity of MG I, before the pathogen had sufficient time to colonize plant tissues. Early-maturing plants may not have as much disease development as later-maturing plants, because of the onset of favorable conditions late in the season. The fungus invades plant roots early in the season and grows slowly until favorable environmental conditions (cool, wet periods) allow for greater disease development (1). This was more evident for leaf symptoms in 1989 when MG I genotypes showed no or very little leaf symptoms. Ratings for stem severity were lowest for MG I among the susceptible genotypes. Field screening for early-maturing plants resistant to BSR may have greater success in areas of adaptation. The area of this study is more suitable for MG II and III; however, differences between resistant and susceptible genotypes were evident from stem symptoms for MG I.

Leaf symptom expression is more dependent on year-to-year environmental conditions, whereas stem symptom expression is usually observed every year. No leaf symptoms were observed in 1988, probably because of abnormally high temperatures. Except for MG I genotypes, which were nearing maturity when leaf symptoms were developing on later-maturing genotypes, the correlation between severity of leaf and stem symptoms was high (MG II: $r = 0.88$; MG III: $r = 0.89$). Progress in selection of resistant plants can be made in the absence of leaf symptoms. In 1989, leaf symptom severity was more dependent on inoculation than environment.

Efficient selection for plants resistant

Table 3. Means of percent stem tissue damaged by brown stem rot during 1988–1989 and leaf tissue damaged in 1989 in inoculated and uninoculated plants in two environments

Maturity group	Genotype	Inoculation[a]	Damaged tissue (%)			
			Environment 1		Environment 2	
			Stem	Leaf	Stem	Leaf
I	BSR 101	I	5.45	0.00	11.17	0.00
		U	1.38	0.00	3.01	0.00
I	Hardin	I	27.90	0.00	36.73	0.29
		U	5.04	0.00	24.30	0.00
I	Hodgson 78	I	27.65	0.00	38.63	1.52
		U	4.25	0.00	20.52	0.00
II	BSR 201	I	6.53	0.00	15.36	0.00
		U	1.83	0.00	5.72	0.00
II	Burlison[b]	I	33.14	...	37.34	6.17
		U	10.19	...	17.34	0.00
II	Century 84	I	56.79	67.46	59.61	70.01
		U	6.07	0.00	33.57	5.61
III	Chamberlain	I	14.99	0.00	15.04	0.00
		U	1.80	0.00	3.65	0.00
III	A3733	I	2.96	0.00	11.67	0.00
		U	1.81	0.00	3.05	0.00
III	Fayette	I	7.24	0.00	9.19	0.00
		U	3.11	0.00	5.84	0.00
III	Cumberland	I	44.79	65.36	47.81	72.09
		U	2.54	0.00	14.90	13.50
III	PI 437.654	I	20.51	15.76	33.81	48.01
		U	5.82	0.00	21.81	18.37
Total means		...	12.44	6.97	21.14	10.79
Treatment means		I	21.46	14.34	28.34	18.16
		U	3.73	0.00	13.91	3.10
LSD[c] (0.05)		...	7.71	8.43	9.36	9.65
LSD[d] (0.05)		I	9.99	11.69	9.99	11.69
		U	7.18	5.74	7.18	5.74

[a] I = inoculated, U = uninoculated.

[b] Burlison plants died of metribuzin injury in 1989.

[c] LSD ($P = 0.05$) for comparison of values within environment 1 and environment 2 for stem and leaf tissue damage; comparison between environments LSD ($P = 0.05$) = 8.58 for stem and 9.06 for leaf tissue damage.

[d] LSD ($P = 0.05$) for comparison of values within inoculated and uninoculated treatments for stem and leaf tissue damage; comparison between inoculation treatments LSD ($P = 0.05$) = 8.70 for stem and 9.21 for leaf tissue damage.

to BSR can be made based on stem symptom severity. The year effect for the overall data was nonsignificant, but differentiation between resistant and susceptible plants will be easier in years favoring BSR disease development. Some method of increasing inoculum levels will also aid selection. The selection of genotypes with a greater level of resistance may be enhanced by the use of fields with reduced levels of fertility. Progress in the selection of BSR-resistant genotypes may be affected by the field (and its cropping history and fertility) used for selection. Selection of the field itself is an important decision that may not receive enough attention.

ACKNOWLEDGMENTS

Research supported in part by Illinois Soybean Program Operating Board and the Illinois Crop Improvement Association.

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