Comparison of Soybean Genotypes for Resistance to and Agronomic Performance in the Presence of Brown Stem Rot

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ABSTRACT

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Soybean genotypes selected for resistance to *Phialophora gregata* in Illinois and Iowa showed moderate to high levels of resistance to *P. gregata* in naturally infested field plots in Wisconsin. Proportion of internal stem discoloration and severity of foliar symptoms were better measures of resistance than was percent disease incidence (based on internal stem discoloration). Mean yield of resistant lines was 17% more than yield of susceptible cultivars. Seed weight per plant was influenced more by the number of seed-bearing pods than by seed size. Host resistance could be measured in the field and was related to increased yield in the presence of brown stem rot.

Brown stem rot of soybean (*Glycine max* (L.) Merr.), a disease of major economic importance (1,2,8,9,16) caused by the fungus *Phialophora gregata* (Allington & Chamberlain) Gams (7), traditionally has been controlled by crop rotation (4,11,22). Resistance to *P. gregata* is available (3,20) and has been incorporated into agronomically acceptable cultivars (17,18). Tachibana (17) reported a 30% yield advantage for the brown stem rot-resistant cultivar BSR 301 over susceptible cultivars of similar maturity when grown side by side in fields naturally infested with *P. gregata*. Resistant cultivars (17,18), supplemented by crop rotation (4,11), can provide effective control of brown stem rot. However, more information is needed on how soybean genotypes react to *P. gregata* in a diversity of soil and climatic environments. Measures of brown stem rot that best characterize inherent resistance within soybean genotypes (15,20) need to be refined and related to agronomic performance (9,10,19).

The objectives of this study were to evaluate and compare advanced soybean lines for resistance to and agronomic performance in the presence of *P. gregata* in Wisconsin and to compare methods for evaluating soybean lines for resistance to *P. gregata* in a natural agroecosystem.

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MATERIALS AND METHODS

Field plots were established at the University of Wisconsin Experimental Station near Hancock in May 1983. The plot area consisted of a Plainfield loamy sand (typic udipsamments) soil that had been cropped to soybean for three consecutive years. The previous year's soybean crop had a 100% incidence of brown stem rot. The susceptible cultivars Corsoy 79 and Century and genotypes selected for brown stem rot resistance, LN80-7532, LN80-7579, and L78-4094 (developed at the University of Illinois) and BSR-201, A79-331022, A80-149020, and A80-349006 (developed at Iowa State University), were planted in a randomized complete block design with three replicates. Seeds were planted at the rate of 20–30/m of row using a four-row John Deere Max-Emerge 7000 planter with cone attachments, manually set blade furrow openers, and a hydraulic depth-control system. Fertilizer (6-24-24) was broadcast at the rate of 10.9 q/ha before planting. Alachlor (Lasso) herbicide was applied at the rate of 4.7 L/ha immediately after planting. Each plot consisted of four rows 9 m long and 0.76 m apart. Irrigation, based on evapotranspiration data, was applied from the VC to the R8 growth stages (6).

Sampling methods for disease assessment. Ten plants were sampled for disease assessment from each plot at the V2, V4, V7, R2, R5, and R7 growth stages every 3 wk from the date of planting. The following disease severity and incidence measurements were recorded:

1. Proportion of internal stem discoloration to total plant height (PISD) was assessed by splitting the stem longitudinally and measuring the greatest length of discoloration attributed to brown stem rot.

2. Severity of foliar symptoms for each plot was based on the Horsfall-Barratt scale of 0–11, where 0 = no foliar symptoms, 1 = 0–3%, 2 = 3–6%, 3 = 6–12%, 4 = 12–25%, 5 = 25–50%, 6 = 50–75%, 7 = 75–87%, 8 = 87–94%, 9 = 94–97%, 10 = 97–100% and 11 = 100% leaf necrosis. Two ratings were given for each plot by assessing the severity of foliar symptoms from two sides. Ratings were later converted to standardized percentage values, using conversion tables provided by Elanco Products Company (Division of Eli Lilly, Indianapolis, IN). Because foliar symptoms did not appear until 6 wk before maturity (R5 stage), severity of foliar symptoms was measured weekly from the date of symptom onset.

3. Disease incidence was based on the percentage of plants with internal stem discoloration symptomatic of brown stem rot.

Isolation of *P. gregata*. Ten stem and 10 root pieces were excised from each of five plants per plot per sampling period. Pieces of host tissue were excised 5 cm above and below the soil line, surface-disinfested by immersion in a 0.25% NaOCl solution for 2 min, and blotted dry. Stem and root tissues were plated separately on acidified potato-dextrose agar and incubated at 20 C for 10 days.

Indirect assessment of brown stem rot. Area under the disease progress curve (AUDPC) was calculated for severity of foliar symptoms, PISD, and incidence based on internal stem discoloration using the following formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2][t_{i+1} - t_i],$$

where X_i = cumulative disease incidence or severity, expressed as a proportion of the i^{th} observation, t_i = time (days after

planting) at the i^{th} observation, and n = total number of observations of brown stem rot severity or incidence (per unit) at the i^{th} observation (21).

Lodging. Lodging was recorded for each genotype at the R8 growth stage based on a scale of 1–5, where 1 = almost all plants erect; 2 = all plants leaning slightly or few plants prostrate; 3 = all plants leaning moderately (45°), or 25–50% of the plants prostrate; 4 = all plants leaning considerably, or 50–80% of the plants prostrate; and 5 = all plants prostrate.

Yield, yield components, and yield loss estimates. Estimates of yield were obtained by harvesting 6.1 m of the two center rows of each four-row plot with a plot combine. Total seed yields and 100-seed weights were determined after harvested seed were dried to uniform moisture content (12%). The numbers of seedless and seed-bearing pods per plant were determined for five plants sampled from each plot before harvest; the plants were then threshed to determine yield on a per-plant basis.

RESULTS

Measurement of brown stem rot. Seven soybean genotypes, selected for resistance to *P. gregata* in Illinois and Iowa, were less diseased than two nonselected genotypes when grown in a naturally infested soil in Wisconsin. Soybean lines selected for resistance to *P. gregata* were distinguished from non-selected lines, Corsoy 79 and Century, by percent disease incidence based on internal stem symptoms (Fig. 1A). The maximum difference between susceptible and resistant genotypes occurred at the R5 (97 days) growth stage (Fig. 1A). Separation of resistant and susceptible genotypes became less evident by the R7 (119 days) growth stage. However, PISD and especially severity of foliar symptoms separated susceptible and resistant genotypes better than disease incidence (Fig. 1B,C).

An AUDPC value was calculated for percent disease incidence, PISD, and severity of foliar symptoms to compare phenotypic reactions to *P. gregata* over a growing season rather than at one point in time. The AUDPCs for each disease measurement were much lower for resistant than for susceptible cultivars and breeding lines (Table 1). Severity of foliar symptoms best differentiated selected and nonselected genotypes, although no statistically significant (P = 0.05) differences occurred within each group. Differences for PISD were measured between BSR 201 and other experimental lines. A statistically significant difference among selected lines for percent disease incidence was not obtained using the raw data, but statistical significance occurred when an AUDPC was calculated for disease incidence (Table 1). Disease measurement (AUDPC) over time was a more sensitive method for evaluating differences between experimental lines for resistance to brown stem rot.

Of the nine soybean lines tested, Corsoy 79 and Century showed the greatest disease incidence, PISD, and foliar symptoms at the R7 growth stage (Table 1). BSR 201 was not significantly different from the susceptible cultivars

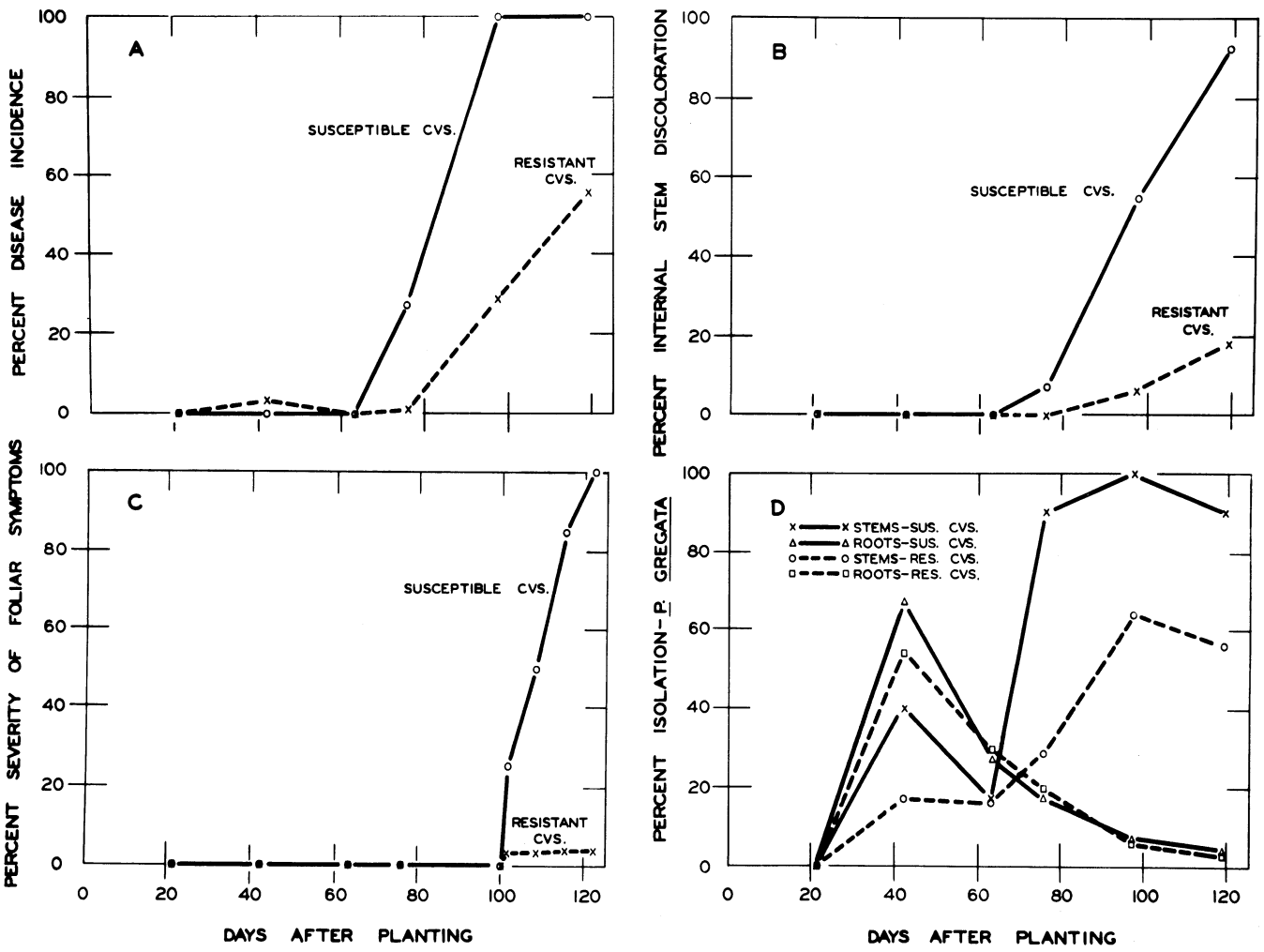

Fig. 1. Combined means of cultivars selected or not selected for resistance to *Phialophora gregata* for **(A)** percent disease incidence, **(B)** percent internal stem discoloration, **(C)** percent severity of foliar symptoms, and **(D)** percentage of plants from which *P. gregata* was isolated from roots and stems. Disease measurements and isolations were made at the V2, V4, V7, R2, R5, and R7 growth stages.

Corsoy 79 and Century when disease incidence was based on internal stem symptoms (Table 1). PISD values for Corsoy 79 and Century were 99 and 84%, respectively, at the R7 growth stage. The cultivar BSR 201 had a PISD lower than Corsoy 79 and Century but greater than the experimental lines. The lines A80-349006, LN80-7579, L78-4094, and A79-331022 had PISD values of 9, 11, 11, and 12%, respectively. The PISD values obtained for these lines were significantly lower than those of BSR 201, LN80-7532, and A80-14020. Among the nine soybean lines evaluated (Table 1), Corsoy 79 and Century showed the greatest severity of foliar symptoms at the R7 growth stage. Foliar symptoms progressed rapidly after the R5 growth stage and most dramatically separated resistant and susceptible lines (Fig. 1C).

Isolation of *P. gregata*. *P. gregata* was more frequently isolated from roots than from stems up to the R2 (63 days) growth stage but was isolated more frequently from the stems than from roots after the R4 (76 days) growth stage (Fig. 1D). Although recovery from stems was high between the R4 and R7 (76–119 days) growth stages, the stage for maximum recovery varied among cultivars. The fungus was isolated more readily from stems of nonselected genotypes than from genotypes selected for resistance. However, the fungus was isolated at similar frequencies from roots of both genotypic groups. The pathogen was first isolated 42 days after planting (Fig. 1D), 34 days before internal stem symptoms (Fig. 1A) and 59 days before foliar symptoms were readily observed (Fig. 1C).

Yield and other agronomic measurements. The mean yield for the resistant cultivars was 27.8 compared with 23.0 q/ha for susceptible cultivars (Table 2). Collectively, the resistant cultivars had a 17% yield advantage over the susceptible cultivars when grown in *P. gregata*-infested soil. The yield advantage of the resistant cultivars over Corsoy 79 ranged from 8 to 24% and from 19 to 33% for Century. The line A79-331022 (group III) was damaged by a late-season frost, possibly explaining its low yield.

Yield per plant was influenced more by number of seed-bearing pods than by seed size, as measured by 100-seed weight (Table 2). Both Corsoy 79 and Century had more seedless pods than all other genotypes except A79-331022, which was affected by a late-season frost. Yield per plant ranged from 10.7 to 15.4 g per plant, and 100-seed weights ranged from 15.6 to 20.8 g. The susceptible lines Corsoy 79 and Century produced 11.6 and 11.4 g of seed per plant, respectively, whereas the resistant lines averaged 13.8 g of seed per plant. The two top-yielding lines, A80-149020 and LN80 7579, had significantly lower lodging scores than the susceptible lines Corsoy 79 and Century. Taller cultivars had a correspondingly higher lodging score.

DISCUSSION

P. gregata was isolated from roots of susceptible and resistant lines at similar frequencies but was more readily recovered from stems of susceptible than from resistant lines. Thus, resistant cultivars could conceivably reduce inoculum of the pathogen in a manner similar to a nonhost crop such as corn (4). The fungus also was recovered earlier in the season than previously reported (14). Enhanced recovery of *P. gregata* after growth stage R1 was preceded by an increase in disease incidence and advancement of internal stem discoloration. The activity of the pathogen prior to symptom expression could influence its impact on yield (22).

Slight differences in disease incidence and severity were measured among the genotypes selected for resistance. Genotypes that ranked high for resistance, as measured by severity of foliar symptoms, also ranked high for resistance based on PISD. Selection based on internal stem symptoms (Iowa lines) resulted in genotypes that showed very minimal foliar symptoms, and selection based on foliar symptoms (Illinois lines) resulted in genotypes with reduced internal discoloration. Sebastian et al (15) report higher heritability estimates for resistance measured by foliar symptoms than by stem symptoms. It is apparent that foliar symptoms can be used in breeding programs to improve resistance to the foliar and stem phases of this disease. The greater ease of measuring foliar symptoms makes this method of disease assessment very attractive. Simultaneous selection for yield and resistance to brown stem rot using stem symptoms is reported to be difficult, and selection for both traits needs to be done independently (5). Whether this relationship also is true when foliar symptoms are used to assess

Table 1. Disease incidence (%) based on internal stem symptoms, percent internal stem discoloration (PISD), and percent severity of foliar symptoms 119 days after planting (R7 growth stage) and areas under the disease progress curves (AUDPC) for each measure of disease for nine soybean genotypes grown in a field naturally infested with *Phialophora gregata* at Hancock, WI, in 1983

	Disease measurement criteria[w]					
	Disease incidence (%)		PISD		Foliar severity (%)[x]	
Genotype	R7	AUDPC[y]	R7	AUDPC	R7	AUDPC
Corsoy 79	100 a[z]	34 a	99 a	22 a	100 a	1,155 a
Century	100 a	40 a	84 b	24 a	94 b	830 a
BSR 201	80 ab	20 b	32 c	6 b	6 c	53 c
LN80-7532	67 abc	13 bcd	23 cde	3 bc	4 c	35 c
LN80-7579	40 cd	9 cd	11 ef	3 bc	4 c	32 c
A79-331022	27 d	6 d	12 def	2 c	3 c	7 c
A80-149020	73 abc	17 bc	25 cd	2 c	3 c	32 c
A80-349006	47 bcd	14 bcd	9 f	2 c	3 c	33 c
L78-4094	60 bcd	15 bcd	11 ef	2 c	3 c	16 c

[w] Plants were sampled 21 (V1), 42 (V3), 63 (R2), 76 (R4), 97 (R5), and 119 (R7) days after planting.
[x] Percent foliar severity based on conversion of the Horsfall-Barrett scale.
[y] AUDPC = $\Sigma_{i=1}^{n-1} [(X_{i+1} + X_i)/2] [t_{i+1} - t_i]$, in which X_i = cumulative disease incidence or severity expressed as a proportion of the i^{th} observation, t_i = time (days after planting) at the i^{th} observation, and n = total number of observations (18).
[z] Means followed by the same letter within a column are not significantly different from each other ($P = 0.05$) according to Fisher's least significant difference.

Table 2. Yield, 100-seed weight, yield per plant, number of seed-bearing or seedless pods per plant, plant height at maturity, and lodging scores for nine soybean genotypes grown in a field plot naturally infested with *Phialophora gregata* at Hancock, WI, in 1983

Cultivars	Yield (q/ha)	100-Seed weight (g)	Yield per plant (g)	No. pods with seeds	No. pods with no seeds	Plant height at maturity (cm)	Lodging[a]
Corsoy 79	24.4	15.6	11.6	29	12	109	4.0
Century	21.6	18.0	11.4	27	10	113	4.0
BSR 201	28.8	16.8	15.2	35	2	100	2.0
LN80-7532	27.2	18.7	13.9	33	4	108	2.0
LN80-7579	29.5	20.7	14.8	29	2	102	1.7
A79-331022	22.9	18.8	10.7	28	9	110	3.3
A80-149020	32.1	18.5	15.4	29	2	101	1.7
A80-349006	26.6	19.0	15.3	37	4	122	3.3
L78-4094	27.4	20.8	11.4	26	3	103	1.7
$FLSD_{0.05}$[b]	4.0	1.1	3.2	10	4	7	1.2

[a] Lodging score: 1 = almost all plants erect; 2 = all plants leaning slightly or a few plants down; 3 = all plants leaning moderately (45°), or 25–50% of the plants down; 4 = all plants leaning considerably, or 50–80% of the plants prostrate; and 5 = all plants prostrate.
[b] Fisher's least significant difference ($P = 0.05$) within cultivar means for agronomic parameters.

resistance needs to be investigated. Lower severity of foliar symptoms was associated with higher yields in our study. The stage of soybean development when internal stem symptoms are assessed may influence the relationship between stem symptoms and yield. Ertl and Fehr (5) assessed stem symptoms at maturity, growth stage R8. Our findings indicate that the R5–R6 growth stage is an optimal time if disease measurements involving stems are recorded only once during the growing season. However, factors such as inoculum levels (4), pathotypes of *P. gregata* (8), and soil moisture conditions (12) could influence the severity of stem and foliar symptoms at specific growth stages.

Resistance to brown stem rot functioned in a rate-reducing manner (13) based on AUDPCs for stem and foliar disease measurements. Methodology for measuring resistance needs to be adaptable to naturally occurring populations of *P. gregata* under a range of climatic conditions. Further inheritance studies are needed to determine if different host genetic systems govern expression of foliar and stem symptoms (3,5,15,20). A greater understanding of the genetics governing resistance to brown stem rot is needed for the prudent management and deployment of genes that govern resistance to *P. gregata* (17).

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LITERATURE CITED

1. Abel, G. H. 1977. Brown stem rot of soybean *Cephalosporium gregatum*. Rev. Plant Pathol. 56:1065-1076.
2. Athow, K. L. 1973. Fungal diseases. Pages 461-462 in: Soybeans: Improvement, Production, and Uses. B. E. Caldwell, ed. American Society of Agronomy, Madison, WI.
3. Chamberlain, D. W., and Bernard, R. L. 1968. Resistance to brown stem rot in soybeans. Crop Sci. 8:728-729.
4. Dunleavy, J. M., and Weber, C. R. 1967. Control of brown stem rot of soybeans with corn-soybean rotations. Phytopathology 57:114-117.
5. Ertl, D. S., and Fehr, W. R. 1983. Simultaneous selection for yield and resistance to brown stem rot of soybean in hill plots. Crop Sci. 23:680-682.
6. Fehr, R., Caviness, C. E., Burmood, D. T., and Pennington, J. S. 1971. Stages of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11:929-931.
7. Gams, W. 1971. *Cephalosporium* artige Schimmelpilze (Hypomycetes). Gustav Fischer Verlag, Stuttgart. 262 pp.
8. Gray, L. E. 1972. Effect of *Cephalosporium gregatum* on soybean yield. Plant Dis. Rep. 56:580-581.
9. Gray, L. E., and Sinclair, J. B. 1973. The incidence, development and yield effects of *Cephalosporium gregatum* on soybeans in Illinois. Plant Dis. Rep. 57:853-854.
10. Gray, L. E., Thapliyal, P. N., and Sinclair, J. B. 1970. A rating system for determining soybean yield reduction by *Cephalosporium gregatum*. Phytopathology 60:1024.
11. Kennedy, B. W., and Lambert, J. W. 1981. Influence of brown stem rot and cropping history on soybean performance. Plant Dis. 65:896-897.
12. Mengistu, A. 1985. Epidemiology of brown stem rot of soybeans in Wisconsin. Ph.D. thesis. University of Wisconin-Madison. 171 pp.
13. Parlevliet, J. E. 1979. Components of resistance that reduce the rate of epidemic development. Annu. Rev. Phytopathol. 17:203-222.
14. Schneider, R. W., Sinclair, J. B., and Gray, L. E. 1972. Etiology of *Cephalosporium gregatum* in soybean. Phytopathology 62:345-349.
15. Sebastian, S. A., Nickell, C. D., and Gray, L. E. 1984. Efficient selection for brown stem rot resistance in soybeans under greenhouse screening conditions. Crop Sci. 25:753-757.
16. Sinclair, J. B., ed. 1982. Compendium of Soybean Diseases. American Phytopathological Society, St. Paul, MN. 104 pp.
17. Tachibana, H. 1982. Prescribed resistant cultivars for controlling brown stem rot of soybean and managing resistant genes. Plant Dis. 66:271-274.
18. Tachibana, H., Bahrenfus, J. B., and Fehr, W. R. 1983. Registration of BSR 201 soybean. Crop Sci. 23:186.
19. Tachibana, H., and Card, L. C. 1972. Correlation coefficients between various criteria for evaluating brown stem rot resistance in soybean. Phytopathology. (Abstr.) 62:1112-1113.
20. Tachibana, H., and Card, L. C. 1979. Field evaluation of soybean resistant to brown stem rot. Plant Dis. Rep. 63:1042-1045.
21. Tooley, P. W., and Grau, C. R. 1984. Field characterization of rate-reducing resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybean. Phytopathology 74:1201-1208.
22. Weber, C. R., Dunleavy J. M., and Fehr, W. R. 1966. Influence of brown stem rot on agronomic performance of soybean. Agron. J. 58:519-520.