

ished (P-GT treatment), the seed flow was smoother, allowing uniform flotation and selection. Other interactions between treatment combinations may be due to the variance of groat percentage within each treatment combination. Another factor is the variation in seed size within a treatment combination. To some extent, each of the selection methods will select for seed size, thus limiting the efficiency of density selection. Large and small seed size fractions have poorer selection efficiency than the medium sized fraction because they sample the tails of the distribution that are more variable than the medium sized fraction. The important conclusion from the CMSS experiment is that different populations and fractions within populations are nonuniform in their response to selection. Peterson et al. (1986) also found significant differences between wheat populations for response to mass selection by seed density, indicating that mass selection may not be effective for all populations.

Groat percentage increased less in the CM and RS experiments than in the CMSS selection experiment. Different populations were used in the CMSS, CM, and RS experiments; interactions between populations and selection methods could be responsible for these differences. Differences in gain from selection between experiments may also be due to interactions between selection and seed size observed in the CMSS experiment. Populations originally were stratified by size to limit selection for seed size while selecting for density. In the CMSS experiment, the stratification was retained in the experimental design to test for interactions between seed size and improvement for groat percentage. In the CM and RS experiments, the seed size classes were selected independently but were recomposited to advance to the next generation. Thus, increased groat percentage in one size class would be masked by lack of progress in other seed size classes.

Factors Limiting Selection Efficiency In the RS and CM experiments, the heritability of groat percentages for individual seeds was low because high groat percentage seeds may not necessarily be derived from genotypes having high groat percentage. For example, selection for seed density increased the frequency of genotypes with high tertiary seed set. The increase in tertiary floret fertility in the RS experiment shows that selection was effective in selecting seed with higher groat percentage, as tertiary seeds have the highest groat percentage of the three floret positions (Youngs and Shands, 1974). However, the increase in tertiary seed set did not, in our research, result in higher overall groat percentages after two cycles of selection. Although it appears contradictory, tertiary seeds with high groat percentage actually reduce the overall groat percentage of a genotype. Palagyi (1983) found that cultivars with higher than average tertiary seed frequency had a lower than average groat percentage. In our research, we also found a significant negative correlation between tertiary fertility and overall groat percentage. A reason for this contradiction is that tertiary seeds are genetically correlated with decreased primary floret fertility (Takeda and Frey, 1980). This indicates a competition for photosynthate between supernumerary florets and primary florets. Primary ker-

nels compose the largest portion of the seed yield; a reduction in fertility or amount of primary kernel filling would significantly reduce a genotype's groat percentage.

Selection among florets (e.g. selection for high tertiary and low primary floret fertility) by density grading may be counteracting selection between high and low groat percentage genotypes, because there is as much seed variation between florets as between genotype means. Youngs and Shands (1974) found 10 to 14 percentage points difference between the groat percentage of the primary and tertiary florets, which is similar to the range of 7 to 11 percentage points difference between the highest and lowest F_5 families within the unselected treatments of the CM and RS experiments.

Improvements for Selection Methodology Based on this research, two modifications to improve efficiency of selection using P-GT or ASP are recommended; i) the population should be limited to more homogeneous seed size subdivisions and ii) tertiary seeds should be eliminated. Effects of tertiary seeds may be eliminated by discarding small seed-size fractions and selecting only in medium- and large-size fractions. Frey's (1967) results from selection for wider oat kernels suggest that discarding small seed should have the added advantage of improving the average grain yield of a population. Some tertiary seeds would be eliminated by increasing the planting density of bulk populations to increase sterility of supernumerary spikelets. These modifications should result in more effective selection by P-GT and ASP to improve the frequency of genotypes having high groat percentage and high test weight in segregating oat populations.

References

- Atkins, R.E. 1943. Factors affecting milling quality in oats. *J. Am. Soc. Agron.* 35:532-539.
- Frey, K.J. 1967. Mass Selection for seed width in oat populations. *Euphytica* 16:341-349.
- Hamond, J.E., N.R. Brandenburg, and L.M. Klein. 1968. Mechanical seed cleaning and handling. USDA-ARS Agriculture Handb. 354. U.S. Gov. Print. Office, Washington, DC.
- Jensen, N.F. 1961. Genetics and inheritance in oats. *In* F.A. Coffman (ed.) Oats and oat improvement. *Agronomy* 8:125-206.
- . 1964. Processing equipment for small grain test weight samples. *Crop Sci.* 4:438-439.
- Murphy, C.F., and K.J. Frey. 1962. Inheritance and heritability of seed weight and its components in oats. *Crop Sci.* 2:509-512.
- Palagyi, A. 1983. Tertiary seed proportion in the grain yield of several oat varieties. *Cereal Res. Commun.* 11:269-274.
- Peek, J.M., and J.M. Poehlman. 1949. Grain size and hull percentage as factors in the milling quality of oats. *Agron. J.* 41:462-466.
- Peterson, C.J., G.T. Liu, P.J. Mattern, V.A. Johnson, and S.L. Kuhr. 1986. Mass selection for increased seed protein concentration of wheat based on density. *Crop Sci.* 26:523-526.
- Pomeranz, Y., G.D. Davis, J.L. Stoops, and F.S. Lai. 1979. Test weight and groat to hull ratio in oats. *Cereal Foods World* 24:600-602.
- Root, W.R. 1979. The influence of oat (*Avena sativa* L.) kernel and caryopsis morphological traits on grain quality characteristics. Ph.D. diss. Univ. of Wisconsin, Madison. (Diss. Abstr. 80-04738).
- SAS Institute. 1982. SAS Users Guide: Basics. SAS Institute, Carry, NC.
- Smith, R.R., and C.R. Weber. 1968. Mass selection by specific gravity for protein and oil in soybean populations. *Crop Sci.* 8:373-377.

- Snedecor, G.W. and W.G. Cochran. 1980. Statistical Methods, 7th ed. Factorial Experiments. p. 339-377. Iowa State Univ. Press. Ames, IA.
- Stuthman, D.D., and R.M. Granger. 1977. Selection for caryopsis percentage in oats. *Crop Sci.* 17:411-414.
- Takeda, K., and K.J. Frey. 1980. Tertiary seed set in oat cultivars.

- Crop Sci.* 20:771-774.
- Wesenberg, D.M., and H.L. Shands. 1973. Heritability of oat caryopsis percentage and other grain quality components. *Crop Sci.* 13:481-484.
- Youngs, V.L., and H.L. Shands. 1974. Variation in oat kernel characteristics within the panicle. *Crop Sci.* 14:578-582.

Response to Selection for Time of Flowering in Soybean

Randall L. Nelson*

ABSTRACT

Previous research demonstrated that the duration of the seed-filling period (SFP) in soybean [*Glycine max* (L.) Merr.] could be genetically modified. Long SFP was associated with early flowering. The objectives of this research were to evaluate the effectiveness of divergent selection for time of flowering while maintaining similar dates of maturity and to determine the influence of such selection on other agronomic characteristics. Genetic male sterility was used to intermate populations selected for time of flowering from a single cross. Pedigree selection was practiced to identify lines with similar maturity and extremes in flowering date. Data from 22 lines were collected on the center two rows of four-row plots and included dates of R1, R5, R7, and R8, lodging, plant height, and seed yield. In each of 2 yr, the experiment was replicated twice at each of two locations. Selected lines flowered significantly earlier or later than the commercial cultivars in the experiment. Lines with similar time to maturity differed by as much as 24 d in time to flowering. The time from R1 to R5 was a nearly constant proportion of the time from R1 to R7. Because of this relationship changes in duration of SFP were much smaller than changes in time to flowering. The mean SFP (R5-R7) for the six earliest-flowering lines was 5 d greater than the mean SFP for the six latest-flowering lines. One line significantly exceeded the SFP and seed yield of 'Williams', the higher yielding parent.

Additional Index Words: *Glycine max* (L.) Merr., Pedigree selection, Genetic male-sterility, Seed yield, Seed-filling period

(1979) concluded, from their study of 119 cultivars, that high yield was associated with both late flowering and late maturity. No range of maturity was reported but Maturity Groups I and III were represented which may explain the advantage associated with later maturity. Reicosky et al. (1982) reported no correlation between the length of seed fill and flowering date but suggested that the time between flowering and maturity could be used as an initial selection criterion for identifying lines with long seed fill.

The objectives of this research were to evaluate the effectiveness of divergent selection for time of flowering while maintaining similar dates of maturity and to determine the influence of such selection on other agronomic characteristics including the duration of SFP and seed yield.

MATERIALS AND METHODS

In 1979, an F₂ population from the cross L74-01 × 'Kanrich' was planted on 10 May and thinned to 20 plants/m⁻² on 6 June. L74-01 was released by R. L. Bernard (personal communication) as an isolate of 'Williams' with the *ms₂* allele. This allele causes complete male sterility with little effect on female fertility (Graybosch et al., 1984). Dates of R1 (Fehr and Caviness, 1977) were recorded for 234 plants. These plants were located in the center of the population to help prevent pollen from outside the population from fertilizing the 63 plants that were male sterile. Fourteen early flowering plants and 20 late-flowering plants were selected from among the male-sterile plants with average dates of R1 of 28 June and 28 July, respectively. In 1980, the 156 seeds from the early flowering, male-sterile plants and the 165 seeds from the late-flowering, male-sterile plants were planted in two isolation blocks similar to the ones described by Nelson and Bernard (1984). No pollen parent was used as a border but 7 m of bare soil separated the blocks. No male-sterile plants were harvested from the outside 2 m of each block. From seven male-sterile plants in the early flowering block, 702 seeds were harvested and 123 seeds were harvested from five male-sterile plants in the late-flowering block. In addition, 11 and 14 male-fertile plants in Maturity Group III were harvested from the early and late-flowering blocks, respectively. Date of flowering was not recorded for any plants in 1980.

In 1981, the seeds harvested from male-sterile plants in the early and late-flowering blocks were planted in separate isolation blocks. Animal damage early in the season destroyed most of the plants in both blocks so nothing was harvested from the early flowering block and only 10 male-

DIVERGENT SELECTION in soybean for duration of the seed-filling period (SFP) among germplasm accessions (Nelson, 1986) and within heterogeneous populations (Smith and Nelson, 1986a) was successful in identifying lines with significant differences in SFP but with no detected differences in time of maturity. Even with adapted, domestic germplasm none of the selected lines exceeded the seed yield or the SFP of commercial cultivars of similar maturity (Smith and Nelson, 1986b). In all cases early flowering was associated with long SFP.

Many studies of the duration of seed fill in soybean (Gay et al., 1980; McBlain and Hume, 1980; Salado-Navarro et al., 1985) have not included time of flowering or its relationship to other traits. Dunphy et al.

USDA-ARS, Dep. of Agronomy, 1102 S. Goodwin Ave., Univ. of Illinois, Urbana, IL 61801. Joint contribution of the USDA-ARS and the Illinois Agric. Exp. Stn. Received 21 Oct. 1987. *Corresponding author.

fertile plants were harvested from the late-flowering block. Seeds from each male-fertile plant harvested in 1980 were planted in two rows 2.4 m long on 25 May 1981. Approximately once a week all rows were checked for flowering and plants that had begun to flower were marked with a plastic tag. A different colored tag was used for each week. Single plants were harvested each week as they reached maturity. Using these approximate dates for flowering and maturity, selection was made for early flowering and late maturity, and for late flowering and early maturity with the goal of developing lines in Maturity Group III with extremes in flowering date. Ninety seven and 14 plants were selected with early flowering and late flowering, respectively. The progenies from each plant were grown in a row 2.4 m long in 1982 and the process was repeated. In addition, seeds from the 10 male-fertile plants harvested from the late-flowering block in 1981 were grown and 32 male-fertile plants were selected with late flowering.

In 1983, 140 and 47 F₃ plants selected for early and late flowering, respectively, were grown in single rows and classified for dates of R1 and R8. From those rows, 16 and 8 homogeneous-appearing lines were selected for early and late flowering, respectively. Many of the rows exhibited heterogeneity and 97 plants were harvested from 11 rows selected for early flowering. The 32 male-fertile plants harvested from the isolation block in 1982 were also grown in rows in 1983 and dates of R1 and R8 were recorded. Fifty seven plants were harvested from six rows with late flowering and Group III maturity. In 1984, all material selected in 1983, either as rows or plants, was evaluated in single rows 2.4 m long for dates of R1 and R8. From these rows, four were selected for late flowering and 10 for early flowering. The late-flowering lines had average dates of R1 and R8 of 10 July and 15 September, respectively. The early flowering lines had average dates of R1 and R8 of 27 June and 13 September. In addition to this selection procedure, reselections were made within early and late-flowering lines which had been previously tested (Smith and Nelson, 1986b). These lines were also derived from a Williams × Kanrich cross. From the evaluation of 163 sublines from nine F₅ lines, two lines were

selected for late flowering and three lines were selected for early flowering.

Thirteen and six lines selected in 1984 for early and late flowering, respectively, were evaluated for 2 yr in plots 3.8 m long and four rows wide. Rows were spaced 0.75 m apart. All data were collected on the center two rows and at maturity those rows were end trimmed to 3.0 m before harvest. Williams was included as the earlier flowering and higher yielding parent, and 'Harper' and 'Pella' were included as two of the highest yielding, publicly developed cultivars available. These three cultivars also provided a wide range of maturity within Maturity Group III. In each year, the experiment was replicated twice in a randomized complete block design at two locations approximately 3 km apart on the Agronomy-Plant Pathology Farm, Urbana, IL. Location 1 was planted on 13 and 8 May in 1985 and 1986, respectively, in a Drummer silty clay loam (fine-silty, mixed, mesic Typic Haplaquall) and Location 2 was planted on 22 and 23 May in 1985 and 1986, respectively, in a Flanagan silt loam (fine, montmorillonitic, mesic Aquic Argiudoll). Data collected included dates of R1, R5, R7, and R8, lodging on a scale of 1 = erect to 5 = prostrate, plant height at maturity measured from ground to stem tip, and seed yield. Growth stages were determined at weekly intervals. From the reproductive stage data, three growth periods were calculated: total reproductive period (TRP) (R1-R8), early reproductive period (ERP) (R1-R5), and SFP (R5-R7). An analysis of variance was conducted for each variable considering all factors except replications as fixed.

RESULTS AND DISCUSSION

The 2 yr in which this experiment was conducted had very different temperatures (Table 1). In 1985 and 1986, the average monthly temperature from May to September was 2.3°C below and 0.7°C above the 90-yr average, respectively. Total rainfall for this period was 97 and 121% of the 90-yr average for 1985 and 1986, respectively. Total precipitation during the months of August and September was vastly different in the 2 yr but the impact on the experiment was probably less than the data in Table 1 would indicate. In 1985, 93% of the August precipitation occurred before the last line reached the R5 growth stage and in 1986 96% of the September precipitation occurred after the last line reached the R7 growth stage. In both years very little precipitation was received during the SFP.

The main effect of years was significant for many of the traits measured (Table 2). R1 and R8 occurred earlier and plants were taller in 1986 than in 1985 probably because of the higher temperatures. The TRP was unchanged between years but the ERP was longer

Table 1. Climatological data for Urbana, IL in 1985 and 1986.

	May	June	July	Aug.	Sept.
	—Precipitation (cm)—				
1985	8.6	12.2	11.0	12.4	1.8
1986	10.9	11.2	13.2	3.7	18.3
90-yr avg.	9.2	10.0	10.8	9.5	7.7
	—Monthly mean temperatures (°C)—				
1985	18.0	20.6	20.0	17.4	17.2
1986	18.0	22.9	25.0	21.2	20.9
90-yr avg.	16.6	21.8	24.0	22.9	19.3

Table 2. Mean squares from the analysis of variance of selected soybean lines for various traits measured over four environments.

Source	df	Mean square							
		Days to flowering	Days to maturity	Early reproductive period	Seed-filling period	Total reproductive period	Lodging	Plant height	Seed yield
Years (Y)	1	1 524.6**	1 542.3**	1 865.5**	2 768.2**	0.05	0.01	992.8**	39 021
Locations (L)	1	1 885.1**	3 170.5**	521.6**	3.3	166.1**	0.001	0.2	9 687 926**
L × Y	1	8.2*	653.0**	35.5**	134.8**	514.8**	0.41*	311.1**	12 482 469**
Lines (G)	21	376.5**	43.1**	111.7**	61.3**	300.7**	2.36**	301.5**	1 289 431**
G × Y	21	4.6**	17.4**	7.3**	6.4**	11.6**	0.65**	55.7*	157 196**
G × L	21	3.0**	6.4**	5.1**	5.0*	12.2**	0.21**	34.9	83 554
G × L × Y	21	3.4**	6.4**	10.0**	3.8	9.9**	0.24**	75.8**	96 300
Error	86	1.2	2.1	2.0	2.6	2.8	0.10	27.5	70 809

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

and the SFP was shorter in 1986. The earlier onset of drought in 1986 plus the higher temperatures may have caused a more rapid maturation than in 1985. Mean seed yield and lodging were not changed between years. Location 2 is generally a less productive site and in both years was planted much later than Location 1 which explains the differences observed in growth stages and seed yield between locations (Table 2). The genotypic differences among the lines were highly significant for all variables. The interactions with years, locations, or both were all relatively small compared to the differences among lines, so only the means across locations and years are presented.

Selection for differences in time of flowering without changing the time of maturity was successful. Of the 19 experimental lines tested in this experiment, data are presented in Table 3 for the 12 lines that either flowered significantly earlier than Harper or significantly later than Williams. LG84-6188 matured only 1 d earlier than LG83-5423 but flowered nearly 24 d earlier. Four lines flowered significantly earlier than Pella, the earliest flowering cultivar, even though two of these lines matured significantly later than Pella. At the other extreme LG83-5423 had a mean maturity date equal to that of Harper but flowered over 15 d later.

As the date of flowering changed, so did the ERP and SFP. Averaged over the six lines in each flowering category (Table 3), the early-flowering lines required 8 d more to advance from R1 to R5. The length of the ERP was more stable if it were expressed as a percentage of the time between R1 and R7. In all of the lines regardless of time of flowering, the ERP was 44 to 48% of the total time between R1 and R7 with

two exceptions. LG84-6109 and LG83-5423, the earliest and latest flowering lines, respectively, averaged 52 and 41% of the time between R1 and R7 in the ERP, respectively. The reason, if any, for this apparent association between these two growth phases is not evident from these data. Selection for time of flowering was effective in changing the length of the SFP. The early flowering lines averaged 5.5 d more in the SFP than did the late-flowering lines (Table 3). The SFP of all but one of the early flowering and one of the late-flowering lines were significantly different from Williams. The SFP of LG84-6177 significantly exceeded that of Harper.

These data indicate that selection for early flowering can have positive indirect effects on seed yield by lengthening SFP and that flowering of Maturity Group III cultivars can be significantly earlier than that of current cultivars. LG84-6193 flowered earlier, had a longer SFP, and yielded more than Williams, the better parent. The significance of this is enhanced by the fact that only 13 early flowering selections were tested from this cross. None of the lines yielded as well as Harper but five of the six early-flowering lines yielded as well as Pella. Selection for time of flowering did not have a strong influence on plant height but did have a major effect on lodging (Table 3).

The data presented in Table 3 indicate relationships among many of the traits. To quantify those relationships phenotypic correlation coefficients were calculated for each pair of traits and those which were highly statistically significant are presented in Table 4. The data from all of the lines in the test were used for these calculations. Time of maturity and plant height were not correlated with any of the other traits. Selection pressure against variability in maturity was applied so it is not surprising that it was not related to the other traits, but no selection for plant height was imposed. These data confirmed the association among time of flowering, lodging, and seed yield previously mentioned. The magnitude of the correlation coefficients between yield and the other traits in Table 4 were similar. Because of the interrelationships among these traits, one cannot conclude from these data which factors are influencing seed yield and which relationships are artifacts. The highest correlation was between time of flowering and ERP. This relationship has been observed before (Nelson, 1986) and is part of the association between ERP and the time between R1 and R7 that was previously discussed. The practical effect of this relationship is demonstrated in the data in Table 3. A mean change in time of flowering between the early flowering lines and the late-flowering lines of

Table 3. Reproductive characteristics and seed yield for selected early-flowering and late-flowering lines and cultivars, means of two locations and two years.

Entry	Flower- ing d after planting	Matu- rity	Early re- pro- ductive period d	Seed- filling period d	Lodg- ing† score	Plant height cm	Yield kg ha ⁻¹
Early flowering							
LG84-6109	42.4	121.6	36.6	33.9	1.8	115	2855
LG84-6188	43.8	125.0	36.0	38.4	1.6	116	2896
LG84-6164	43.9	123.6	34.6	38.2	1.4	115	2700
LG84-6193	44.1	127.9	36.5	39.9	1.7	119	3093
LG84-6177	45.8	126.4	33.0	40.6	2.6	126	2807
LG83-4447	47.5	125.8	33.4	37.1	1.9	129	2292
\bar{X}	44.6	125.1	35.0	38.0	1.8	120	2774
Late flowering							
LG83-5423	67.5	126.2	21.6	30.6	2.6	120	1477
LG84-6072	61.8	128.1	27.6	32.0	2.8	114	2212
LG84-6071	61.6	129.5	27.1	34.6	2.6	116	2169
LG83-4553	60.9	127.9	27.2	32.8	3.6	118	2291
LG84-6062	59.4	126.5	27.9	32.9	2.9	109	2387
LG83-4547	58.1	126.8	28.1	32.2	2.2	119	2428
\bar{X}	61.6	127.5	26.6	32.5	2.8	116	2161
Cultivars							
Pella	46.0	123.2	32.6	36.5	1.6	114	2947
Harper	51.9	126.9	29.6	38.4	1.4	104	3371
Williams	53.1	130.5	33.0	34.8	1.9	122	2703
\bar{X}	50.3	126.9	31.7	36.6	1.6	113	3007
LSD (0.05)	1.1	1.4	1.4	1.6	0.3	5.2	264

† 1 = erect, 5 = prostrate.

Table 4. Selected correlation coefficients among traits measured on 22 soybean lines selected for differences in time of flowering and evaluated in four environments.

	Lodging	Seed- filling period	Early re- productive period	Yield
Days to flowering	0.71†	-0.74	-0.91	-0.75
Early reproductive period	-0.60	0.61	-	0.71
Yield	-0.61	0.66	0.71	-

† All coefficients are statistically significant at the 0.01% level of probability, $n = 88$.

17 d resulted in a corresponding change in the SFP of only 5.5 d. The reasons for this strong, negative relationship are not known but unless this linkage can be broken, the developmental limitations of the plant for substantially earlier flowering may make further lengthening of the SFP difficult.

REFERENCES

- Dunphy, E.J., J.J. Hanway, and D.E. Green. 1979. Soybean yields in relation to days between specific developmental stages. *Agron. J.* 71:917-920.
- Fehr, W.R., and C.R. Caviness. 1977. Stages of soybean development. Iowa State Univ., Coop. Ext. Serv. Spec. Rep. 80.
- Gay, S., D.B. Egli, and D.A. Reicosky. 1980. Physiological aspects of yield improvement in soybeans. *Agron. J.* 72:387-391.
- Graybosch, R.A., R.L. Bernard, C.R. Creemeens, and R.G. Palmer. 1984. Genetic and cytological studies of a male-sterile female-fertile soybean mutant. *J. Hered.* 75:383-388.
- McBlain, B.A., and D.J. Hume. 1980. Physiological studies of higher yield in new, early maturing soybean cultivars. *Can. J. Plant Sci.* 60:1315-1326.
- Nelson, R.L. 1986. The relationship between seed-filling period and seed yield in selected soybean germplasm accessions. *Field Crops Res.* 15:245-251.
- , and R.L. Bernard. 1984. Production and performance of hybrid soybeans. *Crop Sci.* 24:549-553.
- Reicosky, D.A., J.H. Orf, and C. Poneleit. 1982. Soybean germplasm evaluation for length of the seed filling period. *Crop Sci.* 22:319-322.
- Salado-Navarro, L.R., T.R. Sinclair, and K. Hinson. 1985. Comparisons among effective filling period, reproductive period duration, and R5 to R7 in determinate soybeans. *Crop Sci.* 25:1050-1054.
- Smith, J.R., and R.L. Nelson. 1986a. Selection for seed-filling period in soybean. *Crop Sci.* 26:466-469.
- , and ———. 1986b. The relationship between seed-filling period and yield among soybean breeding lines. *Crop Sci.* 26:469-472.

Field and Greenhouse Evaluations of Stem Canker Resistance in Soybean

D. B. Weaver,* S. A. Sedhom, E. F. Smith, and P. A. Backman

ABSTRACT

Greenhouse screening using infested toothpicks was compared to field evaluation of soybean [*Glycine max* (L.) Merr.] breeding lines for resistance to stem canker disease, caused by southern strains of *Diaporthe phaseolorum* (Cke. & Ell. (Sacc.) var. *caulivora* Athow and Caldwell (*Dpc*). Field screenings are reliable indicators of resistance, but often the disease does not develop naturally in field screening nurseries. Thirty-seven random F_{4:6} lines from the cross 'Hutton' (susceptible) × 'Tracy M' (resistant) were evaluated for their reaction to *Dpc* in the field (two locations, 2 yr) under natural infestation and infection conditions, and in the greenhouse (three experiments) with artificial inoculation using infested toothpicks. Our objectives were to compare field and greenhouse screening and to determine the usefulness of greenhouse inoculation in predicting the yield and disease reaction of breeding lines when these lines were subjected to natural field infection conditions. Field screening based on symptoms and yield was highly effective in identifying resistant genotypes. Heritabilities for yield and disease ratings in the field were 87 and 92%, respectively. The toothpick inoculation procedure used in the greenhouse was effective with each of three *Dpc* isolates (different in geographic origin from the field location) in identifying the genotypes that showed highest levels of disease resistance in the field. Phenotypic correlations between greenhouse ratings and yield in the infested field ranged from -0.71 to -0.61. Results indicated that selection based on greenhouse screening can be an effective alternative to field screening when resistance is derived from Tracy M.

Additional Index Words: *Glycine max* L., Correlated response to selection, Genetic resistance, Heritability, Response to selection.

STEM CANKER DISEASE of soybean caused by southern strains of *Diaporthe phaseolorum* (Cke. & Ell.) (Sacc.) var. *caulivora* (Athow and Caldwell) (*Dpc*), has

D.B. Weaver and S.A. Sedhom, Dep. of Agronomy and Soils, 202 Funchess Hall, and E.F. Smith and P.A. Backman, Dep. of Plant Pathology, 139 Funchess Hall, Auburn University, Auburn, AL 36849. Contribution from the Alabama Agric. Exp. Stn. Journal Series no. 3-871352. Received 22 Sept. 1987. *Corresponding author.

Published in *Crop Sci.* 28:626-630 (1988).

in recent years become a serious threat to soybean production in the southern USA (Backman et al., 1985). Although it has been demonstrated that the use of genetic resistance can be an effective control strategy (Weaver et al., 1984; Harville et al., 1986), there is little information on the effectiveness of artificial techniques for screening genotypes for resistance to *Dpc*, nor their relationship to yield and disease development under conditions of natural (field) infestation.

Several studies have shown a strong relationship between expression of disease symptoms and soybean yield under conditions of natural infestation (Weaver et al., 1984; Backman et al., 1985; Harville et al., 1986). Backman et al. (1985) found a linear relationship between yield and visual estimates of late-season (R6 development stage; Fehr and Caviness, 1977) disease severity when these ratings were converted to a pre-transformed arcsin scale. Therefore, evaluation in the field would be the method of choice among breeders for evaluation of breeding lines and cultivars. However, as many researchers have observed and Ploetz and Shokes (1987) have pointed out, the occurrence of the disease in the field is sporadic and unpredictable. Recent epidemiological studies have been conducted that provide an explanation, primarily based on environmental conditions during early vegetative growth stages, for the year-to-year variation seen in *Dpc* outbreaks (Smith and Backman, 1988). Moreover, stem canker is a disease that has a long latent period in the field, with infection taking place at early vegetative growth stages and subsequent symptoms not being expressed until the R5 or R6 development stages (Backman et al., 1985).

Keeling (1982) suggested the toothpick inoculation technique as a means of differentiating between resistant and susceptible genotypes. It has been used successfully in three inheritance studies. Kilen et al., (1985), and Kilen and Hartwig (1987), concluded that 'Tracy M' has two dominant genes for resistance rel-

ative to the highly susceptible breeding line J77-339. Wendel and Allen (1986) found only one major gene difference between Tracy M and 'Bragg', a susceptible cultivar. However, the usefulness of the toothpick inoculation technique as a tool for screening breeding lines, and its ability to routinely predict performance of breeding lines in the field under conditions of natural infestation, have not been demonstrated. Because of the unreliability of natural disease occurrence for field screening, a large percentage of the elite soybean germplasm in the South is at least moderately susceptible to *Dpc*, as shown by the large number of lines observed to be severely damaged by *Dpc* under natural infestation at Beaumont, TX during 1986 in the USDA Cooperative Soybean Tests (Hartwig and Edwards, 1987). This in spite of the widespread and recognized threat of stem canker and the fact that independent studies have shown that resistance to stem canker is simply inherited (Kilen et al., 1985; Wendel and Allen, 1986). Ploetz and Shokes (1986) warned of the danger of growing susceptible cultivars, even in areas where the disease has not been observed, due to the ability of *Dpc* to infect plants and produce sport inoculum regardless of resistance level and without development of symptoms and associated yield loss.

The objectives of this research were to compare the effectiveness of two methods of evaluating breeding lines for resistance to *Dpc*; screening under conditions of natural infestation in the field, and artificial inoculation with *Dpc* infested toothpicks (Keeling, 1982). An effective artificial inoculation technique would allow breeders to evaluate their elite breeding lines in years when environmental conditions do not favor *Dpc* disease development, or in locations where the disease is not endemic.

MATERIALS AND METHODS

Field experiments

Field experiments were conducted at Marion Junction, AL, on a Sumter clay (fine-loamy, carbonatic, Thermic, Rendollic Eutrochrepts) and at Tallassee, AL, on a Cahaba fine sandy loam (fine-loamy, siliceous, thermic Typic Hapludults) during 1985 and 1986. Both sites had a high incidence of stem canker in 1983 and 1984. Experimental genotypes were random F_4 -derived F_6 ($F_{4,6}$) lines from the cross 'Hutton' (susceptible) (Weaver et al., 1984) \times Tracy M (highly resistant) (Weaver et al., 1984; Kilen et al., 1985). Individual F_2 plants were advanced to the F_4 generation by single-seed descent. The F_4 plants were individually threshed, planted in F_5 rows, and F_6 seeds were harvested in bulk from each row to provide adequate seed for yield testing and disease evaluation. Thirty-seven $F_{4,6}$ lines, along with the control genotypes Hutton, Tracy M, and 'Centennial', were planted in four row plots in a randomized complete block design with three replications at each location. Plots were four rows, 4.9 m long, with 1 m and 0.9 m between rows at Tallassee and Marion Junction, respectively. The two center rows were harvested to determine yield after end trimming to a plot length of 3.7 m. Planting dates were 13 May and 16 May at Marion Junction and Tallassee, respectively in 1985, and 15 May and 3 June at the two locations, respectively, in 1986. Field ratings for stem canker severity were made using a scale suggested by Backman et al. (1985) as follows: A plot with no dead or dying plants was rated 0, one with 10%

dead or dying plants was rated 1.0, 35% was rated 2.0, 65% was rated 3.0, 90% was rated 4.0, and 100% was rated 5.0. Damage was estimated to the nearest 5% and converted to the rating scale accordingly. Thus, a plot judged to have 25% dead or dying plants was rated 1.6. This pretransformed arcsin scale describes a linear relationship between disease severity and yield loss when ratings are made during the R6 development stage (Backman et al., 1985). Plots were rated four times (once each week during the season, beginning when the lines had reached the R4 stage of development (late August), and continuing through late September when lines were at the late R5 or early R6 development stage. All plots were rated on the same date regardless of development stage to avoid confounding ratings with rating dates, and because a breeder is more likely to rate material in a disease evaluation nursery all on the same date, rather than by growth stage. Maturity date was recorded (at the Tallassee location only) as the time when 95% of the pods had reached their mature pod color.

Greenhouse experiments

Three separate greenhouse experiments were conducted during the winter in 1986 and 1987. Experiment 1 was conducted using two *Dpc* isolates, one obtained from diseased Bragg soybean in Geneva county, AL (Isolate 1), and the other from diseased Bragg soybean in Tallassee (Isolate 2). Exp. 2 and 3 were identical, but were conducted using a single isolate obtained from diseased Bragg soybean near Headland, AL (Isolate 3). All three isolates were obtained from geographical locations that were different from the field experiments, with the exception of Isolate 2. No attempt was made to associate these isolates with races of *Dpc* as tentatively proposed by Keeling (1984). Later research by Keeling (1985) has indicated that southern *Dpc* isolates may differ only in virulence. The pathogen was isolated by placing surface-sterilized stems on acidified potato dextrose agar (PDA). After 7 to 14 d, mycelium was removed from the margin of the expanding colonies and maintained on PDA. To prepare inoculum, mycelial plugs were transferred to vials containing sterile toothpicks (flat) that had been soaked in potato dextrose broth. Toothpicks had been previously prepared for inoculation as described by Keeling (1982). Toothpick cultures were allowed to grow for 14 d at 30 °C before use.

The same genotypes (with the exception of Centennial in Exp. 1) used for field experiments were inoculated by piercing the hypocotyl of 10-d-old seedlings 1 cm below the cotyledonary node with a dissecting needle. Through this wound, a 5-mm section cut from the tip of an infested toothpick was inserted, and the wound was sealed with petroleum jelly. Individual plants were grown in Cone-Tainers (Ray Leach Cone-Tainer Nursery, Canby, OR), 21 cm long and 4 cm diam. (total volume = 0.16 L). In Exp. 1, the experimental design was a split plot randomized complete block, with isolates assigned to whole plots and genotypes assigned to the subplots. Two plants were used as an experimental unit, with five replications. The experimental design for Exp. 2 and 3 was a randomized complete block with three replications; an experimental unit was seven Cone-Tainers each containing one plant. In addition to the *Dpc*-inoculated treatments, Tracy M and Hutton inoculated with sterile toothpicks were also included in Exp. 1, but neither genotype exhibited any disease development. These checks were not included in Exp. 2 and 3. Plants were grown for 30 d after inoculation under a constant 14-h daylength to maintain them in a vegetative state. Greenhouse temperature was maintained at approximately 31 °C during the day and 27 °C at night.