

Inoculum Rate Influences Selection for Field Resistance to Soybean Sudden Death Syndrome in the Greenhouse

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ABSTRACT

Effective selection of field resistance to soybean sudden death syndrome (SDS) caused by *Fusarium solani* (Mart.) Sacc. f. sp. *glycines* (*Fsg*) (Roy, 1997), measured by disease index (DX), requires multiple environments. Current greenhouse assays reduce genotype \times environment interactions, but fail to predict field resistance. Our objective was to compare selection for field resistance to SDS in the greenhouse among recombinant inbred lines (RILs) inoculated with *Fsg* at three rates. Thirty soybean [*Glycine max* (L.) Merr.] RILs with characterized field resistance to SDS were evaluated in the greenhouse for scorch severity at three inoculum rates in four experiments. Ten cultivars with characterized field resistance were compared using disease severity (DS) readings from one experiment at one inoculum rate. The heritability of DS among RILs in the greenhouse was 46% at the low, 66% at the moderate, and 37% at the high inoculum rates. Reduced inoculum rates in the greenhouse (3500 to 5000 spores cm^{-3} plant growth medium) provided DS values that explained $\approx 65\%$ of variation in the field DX. Using a *Fsg* inoculum rate of 5000 spores cm^{-3} plant growth medium and greenhouse midparent DS as criterion for selection, the number of lines potentially resistant to SDS within a segregating population could be reduced by 53%. Errors caused $\approx 10\%$ of field resistant lines to be eliminated. Among unrelated soybean cultivars, greenhouse DS values from an inoculum rate of 4000 spores cm^{-3} plant growth medium explained 81 and 73% of variations in field DS and DX, respectively. Therefore, the method is an effective tool for inheritance studies and cultivar evaluation for SDS.

SOYBEAN SUDDEN DEATH SYNDROME significantly reduces soybean yield in the midwestern USA and South America (Wrather et al., 1997; Roy et al., 1997; Njiti et al., 1998b). Protection against yield loss derives from the use of SDS-resistant cultivars (Gibson et al., 1994; Njiti et al., 1998b).

In the greenhouse, monogenic resistance to leaf scorch was identified in 'Ripley' (Stephens et al., 1993a) and bigenic resistance to leaf scorch in 'P9451' (Ringler and Nickell, 1996). Resistance to SDS in the field is partial (Njiti et al., 1996, 1997, 1998a; Iqbal et al., 2001), multi-genic (Hnetkovsky et al., 1996; Chang et al., 1996), and derived from both root resistance loci (Njiti et al., 1997, 1998a; Prabhu et al., 1999) and leaf scorch resistance loci (Gibson et al., 1994; Meksem et al., 1999). Selection for SDS resistance in the field is complicated by the quantitative nature of the trait and interactions between resistance loci and the environment (Njiti et al., 1996). Selection for stable and durable resistance to SDS might be improved using controlled environmental conditions in the greenhouse or growth chambers (Ste-

phens et al., 1993a,b). However, the existing greenhouse assays with seedlings have not accurately predicted field responses of mature plants in inheritance studies (Torto et al., 1996).

The two most common greenhouse assays for SDS inoculate seedling roots with *Fsg*-infested oat seeds (Lim and Jin, 1991; Hartman et al., 1997) or a *Fsg*-infested sand and cornmeal mixture (Killebrew et al., 1988; Gray and Achenbach, 1996). In both assays, disease is rated in seedlings 2 wk after inoculation (Stephens et al., 1993a,b; Melgar and Roy, 1994; Torto et al., 1996). In the field, plants are exposed to the pathogen from planting through the reproductive period before leaf symptoms appear (Gibson et al., 1994; Njiti et al., 1997). The latent period of the disease differs between the field and greenhouse because the inoculum rate is higher ($>10\,000$ spores cm^{-3} plant growth medium) in the greenhouse (Torto et al., 1996) than in the field (<5000 spores cm^{-3} plant soil; Roy et al., 1997). High pathogen rates overcome both partial and complete plant resistance to disease pathogens (Parleviet, 1979; Tooley and Grau, 1982) including the soybean-*Fsg* interaction (Torto et al., 1996; Gray and Achenbach, 1996; Hartman et al., 1997). The objective of this study was to compare selection for field resistance to SDS in the greenhouse by leaf DS at three inoculum rates.

MATERIALS AND METHODS

The genetic material included 30 $F_{5:10}$ RILs (a subset of a population of 100 lines) from the cross of 'Essex' (Smith and Camper, 1973) \times 'Forrest' (Hartwig and Epps, 1973) and 10 soybean cultivars of diverse genetic background. Essex is susceptible, while Forrest is resistant to SDS (Gibson et al., 1994; Hnetkovsky et al., 1996). One of the RILs (ExF78) has been released as germplasm under the name LS-G96 (Schmidt et al., 1999). LS-G96 is resistant to the soybean cyst nematode (*Heterodera glycine* Ichinohe) and soybean SDS. The RILs were selected by DX mean (Gibson et al., 1994) to include three classes (10 RILs per class) that contrasted for SDS DX and DS scores by the mean of five field environments. The classes were (i) field resistant, the 10 most resistant of the 100 lines, eight of which were significantly more resistant than Forrest; (ii) field partially-resistant, the 10 lines around the population median; and (iii) field SDS-susceptible; the 10 least resistant lines, all of which were more susceptible than Essex (Hnetkovsky et al., 1996). Although SDS resistance in the field is an incomplete resistance in all cultivars and is controlled by several genes, resistance and partial resistance will be used to refer to SDS Classes 1 and 2, respectively, in this paper. The 10 diverse soybean genotypes included four cultivars that were susceptible to SDS [Flyer (McBain et al., 1990), Spencer (Wilcox et al., 1989), A5403, and CM497]; five cultivars and one

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Abbreviations: DS, disease severity; DX, disease index; *Fsg*, *Fusarium solani* f. sp. *glycines*; RIL, recombinant inbred line; SDS, sudden death syndrome.

plant introduction that were resistant to SDS in the field [Ripley (Cooper et al., 1990), 'Jack' (Nickell et al., 1990a), 'Manokin' (Kenworthy et al., 1996), 'Hartwig' (Anand, 1992), 'Hamilton' (Nickell et al., 1990b), and PI 520733].

The *Fsg* isolate (ST90) was isolated from SDS-infected roots of the soybean cultivar Spencer in Stonington, IL, in 1990 by single spore isolation (Stephens et al., 1993a). The strain, stored on Bilays medium, was subcultured on potato dextrose agar medium and used to infest a 1:1 (v/v) mixture of cornmeal and SiO₂. After incubation at room temperature for 10 d (O'Donnell and Gray, 1995), 5 cm³ of the inoculum was added to 250 mL of sterile water, and the average count of spores (in 10 samples of 1 mL) determined on a hemocytometer under a microscope. Spore counts were used to calculate the volume of culture necessary for each inoculum rate. The plant growth medium consisted of a 1:1 (v/v) mixture of sterile sand and soil in all experiments.

Five experiments were conducted in the greenhouse at the Southern Illinois University Horticulture Research Center in Carbondale, IL. To evaluate the effect of inoculum rate on leaf scorch resistance by SDS DS among RILs, four experiments were conducted using three inoculum rates (high = 10⁴, moderate = 5 × 10³, and low = 3.3 × 10³ spores cm⁻³ plant growth medium). Two experiments were replicated two times and two were replicated five times, for a total of 14 replications per genotype per treatment. To test the robustness of the greenhouse assay, ten soybean genotypes of diverse genetic background were evaluated at 4000 spores cm⁻³ plant growth medium in a fifth experiment. Experiments were conducted between 1 Nov. 1996 and 15 Mar. 2000. Plants were grown with a 14-h photoperiod, and the air temperature ranged from 20 ± 2°C at night to 27 ± 2°C during the day in the greenhouse.

All greenhouse experiments were planted in a randomized complete block design. Parents and noninoculated control plants were included in the experiments. Two-week-old seedlings were transplanted onto *Fsg*-infested plant growth medium in four-inch styrofoam cups, and kept saturated to 5.08 cm with water for 4 wk. Sudden death syndrome DS was rated at 21 d after inoculation, determined on the basis of the degree of leaf damage (chlorosis/necrosis) on each plant, and was rated on a scale of 1 to 9 (1 = 0–10%/1–5%, 2 = 10–20%/6–10%, 3 = 20–40%/10–20%, 4 = 40–60%/20–40%, 5 = >60%/>40% of leaf surface chlorosis/necrosis, respectively, 6 = up to 33% premature defoliation, 7 = up to 66% premature defoliation, 8 = >66% premature defoliation, and 9 = premature death of plant).

Field resistance was previously evaluated in Villa Ridge in 1990 with soil type Bonnie silt loam (Fine-silty, mixed, active, acid, mesic Typic Fluvaquents); Pulaski in 1991 with soil type Dupo silt loam (Coarse-silty over clayey, mixed over smectitic, superactive, nonacid, mesic Aquic Udifluvents); Cora in 1991 with soil type Gorham silty clay loam (Fine-silty, mixed, superactive, mesic Fluvaquentic Endoaquolls); Cora in 1992 with soil type Dupo silt loam (Coarse-silty over clayey, mixed over smectitic, superactive, nonacid, mesic Aquic Udifluvents); and Ridgway in 1993 with soil type Patton silty clay loam (Fine-silty, mixed, superactive, mesic Typic Endoaquolls); for a total of five southern Illinois environments. Plots were rated for SDS disease incidence and DS. Disease incidence was the percentage of plants in the plot with visible SDS leaf symptoms. Disease severity was rated using the same scale as for greenhouse-grown plants, except it was average over all disease plants in each plot. Disease index was then calculated as (DI*DS)/9, with a possible range of 0 (no disease) to 100 (death of all plants).

Data were subjected to ANOVA (SAS Institute, Cary,

NC)¹. The interaction terms (experiment × inoculum rate, experiment × SDS class, and experiment × inoculum rate × SDS class) were tested to determine pooling data for mean comparisons. The heritability (*h*²) of DS within inoculum rate was calculated from variance component (Nyquist, 1991) as

$$h^2 = \frac{\sigma_g^2}{(\sigma_e^2/R + \sigma_{gE}^2/E + \sigma_g^2)}, \quad [1]$$

where σ_g^2 = genotypic variance, σ_e^2 = plant-to-plant error variance, σ_{gE}^2 = Genotype × experiment interaction error variance, R = total number of replications, and E = number of experiments. Mean comparisons were made by LSD (Gomez and Gomez, 1984). A simple regression analysis (SAS Institute, Cary, NC) of greenhouse DS on field DX was used to derive predictive equations for field DX at each inoculum rate.

RESULTS AND DISCUSSION

Sudden death syndrome leaf symptoms were not observed on control plants grown on non-*Fsg*-infested plant growth medium. Among plants grown on infested media, there was a significant effect of inoculum rate (Table 1). Disease severity means were 1.6, 2.6, and 4.4 for low, moderate, and high inoculum rates, respectively. Higher *Fsg* inoculum rates were expected to cause more severe SDS leaf symptoms and greater levels of root rot (Gray and Achenbach, 1996). Means of DS at the high inoculum rate in the first experiment could not be determined because of the death of numerous plants. Soil

Table 1. Mean squares of sudden death syndrome disease severity in 30 soybean lines across four greenhouse experiments using three inoculum rates.

Source of variation	df	MS	Divisor	
MS Across Inoculum Rates				
Experiment	3	233***	4	
Inoculum rate	2	840***	3	
Experiment × inoculum rate	5	35*	4	
Error a	10	6.1*	9	
Class	2	164***	6	
Experiment × class	6	6	10	
Inoculum rate × class	4	17	8	
Experiment × inoculum rate × class	10	11.8***	13	
Error b	58	2.5*	14	
Line (class)	27	5.3	11	
Experiment × line (class)	81	3.5	13	
Inoculum rate × line (class)	54	4.8***	13	
Experiment × inoculum rate × line (class)	134	2.6***	14	
Error c	761	1.7		
MS Within Inoculum Rates				
Source of variation	df	Low	Medium	High
Experiment	3	44***	119***	276 (2†)***
Replication (experiment)	10	3.7	4.0	12 (9†)*
Class	2	23.9	48.8***	69
Experiment × class	6	7.0***	2.3	25 (4†)***
Error b	20	1.7***	2.1***	4 (18†)
Lines (class)	27	1.6	4.7*	7
Experiment × lines (class)	81	1.4***	2.8***	5 (54†)***
Error c	287	0.8	1.5	3 (232†)

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Missing degrees of freedom resulting from missing treatment.

¹The USDA neither guarantees nor warrants the standard of the product and the use of the name by USDA implies no approval of the product to the exclusion of the others that may also be suitable.

Table 2. Disease severity (DS) means among sudden death syndrome (SDS) resistance classes within and across four greenhouse experiments using 30 soybean lines.

Field SDS class†	DS means for inoculum rates‡		
	Low	Moderate	High
Experiment 1			
Resistant	2.3A	3.7A	§
Partially resistant	2.4A	3.7A	§
Susceptible	3.7B	4.8B	§
Experiment 2			
Resistant	1.5A	3.8A	7.3A
Partially resistant	1.5A	3.7A	6.7A
Susceptible	3.2B	4.4A	7.4A
Experiment 3			
Resistant	1.1A	1.1A	2.8A
Partially resistant	1.1A	1.3A	2.8A
Susceptible	1.2A	2.6B	5.5B
Experiment 4			
Resistant	1.5A	1.6A	3.0A
Partially resistant	1.5A	2.2B	4.3AB
Susceptible	1.5A	3.5C	4.7B
Mean DS	1.6	2.6	4.4

† SDS classes based on field disease index (five field environments).
 ‡ Means in the same column within experiment followed by the same letter are not significantly different by LSD ($\alpha \leq 0.05$).
 § Missing data (treatment was lost).

compaction contributed to the death of this set of plants (treatments were planted by different individuals), and was monitored closely in subsequent experiments.

Analysis of variance across experiments and inoculum rates found a significant interaction between inoculum rate and experiment (Table 1). Therefore, comparison of inoculum rates was limited to within experiment (Table 2). No significant interaction was found between experiment and SDS class or inoculum rate and SDS class. However, there was a significant three-way interaction of experiment \times inoculum rate \times SDS class (Table 1). Therefore, comparison of SDS class means was conducted within experiment and inoculum rate (Table 2). This interaction resulted from the differential response of the first two experiments compared with the second two experiments. At a given inoculum rate, DS was higher for Experiments 1 and 2 (conducted earlier) than for Experiments 3 and 4 (conducted later). These sets of experiments were conducted 4 yr apart, using the same isolate of *Fsg*. Factors such as, but not limited to, soil temperature, effective inoculum rate, and changes in the pathogen's ability to cause damage may be responsible for the reduced DS observed in Experiments 3 and 4.

Analysis of variance across experiments within inoculum rate indicated that at the moderate inoculum rate (5000 spores cm^{-3} of plant growth medium), no significant interaction occurred between SDS class and experiment. In addition, only the moderate inoculum rate produced significant variation among SDS classes. However, comparison of SDS class means within experiments indicated that while the low inoculum rate was effective at separating SDS classes in Experiments 1 and 2, the moderate inoculum rate was effective in Experiments 1, 3, and 4. The spore rate in the low inoculum treatment (3300 spores cm^{-3} of plant growth medium) was similar to that in field hot spots (Roy et al., 1997).

Table 3. Variance components and heritabilities of soybean sudden death syndrome (SDS) disease severity among recombinant inbred lines at three *Fusarium solani* f. sp. *glycines* inoculum rates and four experiments in the greenhouse.

Inoculum rate†	$\sigma_e^2‡$	$\sigma_{ge}^2§$	$\sigma_g^2 $	R#	E††	$h^2‡‡$
						%
low (3.3×10^3)	0.8	0.24	0.10	14	4	46.1
moderate (5×10^3)	1.5	0.32	0.37	14	4	66.4
high (10^4)	3.1	1.18	0.38	12	3	36.8

† Number of spores cm^{-3} of plant growth medium.
 ‡ σ_e^2 = error variance (plant to plant).
 § σ_{ge}^2 = genotype \times experiment interaction variance.
 || σ_g^2 = genotypic variance.
 # R = number of replications in all experiments combined.
 †† E = number of experiments.
 ‡‡ h^2 = heritability = $\sigma_g^2 / (\sigma_g^2/R + \sigma_{ge}^2/E + \sigma_e^2)$.

Lines within SDS class did not vary significantly in SDS DS in the greenhouse (Table 1). Since class members were selected by similarity of response to SDS in the field, low variation within class was expected for an effective assay. There was a significant interaction between inoculum rate and line within SDS class. The ability of a resistant cultivar to withstand high pathogen populations in the field may depend on the number of beneficial alleles contributing to the resistance (Iqbal et al., 2001).

Variance component heritabilities of SDS DS for seedlings in the greenhouse were 46, 66, and 37% for low, medium, and high inoculum rates, respectively (Table 3). These estimates were all lower than those obtained from replicated field studies for plants at the R6 growth stage (Hnetkovsky et al., 1996) using the Essex \times Forrest RIL population. The modest heritability ($\leq 66\%$) indicated that the greenhouse assay did not control all environmental and developmental factors that can influence SDS occurrence and severity in seedlings. Air temperature varied ($\pm 2^\circ\text{C}$) from bench to bench, and could have influenced soil temperature, which has been a major factor influencing SDS occurrence and severity in the field (Rupe et al., 1993). The use of growth chambers and water baths may enable us to test the effect of soil temperature on heritability.

Within each SDS class, SDS DS increased with increasing inoculum rate (Table 4). At the high inoculum rate, all three classes were highly susceptible (Table 4), as expected (Stephens et al., 1993b; Torto et al., 1996; Gray and Achenbach, 1996). Therefore, high inoculum rates may be responsible for the breakdown of field SDS resistance in the greenhouse (Torto et al., 1996; Stephens et al., 1993b; Gray and Achenbach, 1996; Hartman et al., 1997).

At the low inoculum rate in Experiments 1 and 2, and moderate inoculum rate in Experiments 3 and 4 (Table 2), the DS means of the field SDS-susceptible class were significantly higher than those of both the partially-resistant and the resistant classes. However, the field partially-resistant and resistant classes were not significantly different from each other (Table 2), although in the field, the class of genotype with partial resistance to SDS had a significantly higher DX mean (7.4%) than the class of genotypes with resistance to SDS (1.2%). Therefore, the greenhouse assay was not as effective as

Table 4. Comparison of individual soybean line means for disease severity (DS) at three inoculum rates in the greenhouse with sudden death syndrome (SDS) field disease index (DX), and field DS.

Genotype	Greenhouse SDS class†	Field DX	Field DS	Greenhouse DS		
				low	moderate	high
Field resistant class						
LS-G96	R	0.1	1.1	1.6	1.6	4.2
(ExF) 23	R	0.5	1.1	1.1	2.1	4.4
(ExF) 59	R	0.5	1.2	1.6	1.6	3.2
(ExF) 67	R	1.1	1.2	1.5	1.8	2.8
(ExF) 57	R	1.1	1.3	1.3	2.1	4.5
(ExF) 44	R	1.1	1.3	1.9	2.1	3.2
(ExF) 20	R	1.2	1.2	1.2	2.1	3.9
(ExF) 47	S	1.5	1.2	1.9	3.0	2.8
(ExF) 37	R	1.9	1.2	1.3	1.9	1.9
(ExF) 55	S	3.0	1.1	1.4	2.3	5.7
‘Forrest’				1.2	1.3	2.9
Field partially resistant class						
(ExF) 46	S	5.4	1.4	1.2	2.4	3.4
(ExF) 14	R	6.3	1.3	1.7	1.7	4.2
(ExF) 91	R	6.4	1.3	1.4	2.1	3.6
(ExF) 75	S	6.4	1.4	1.6	2.6	5.3
(ExF) 49	R	6.9	1.3	1.2	2.0	4.0
(ExF) 26	R	7.2	1.4	1.4	2.1	4.4
(ExF) 6	R	8.2	1.5	1.3	2.0	4.2
(ExF) 73	S	8.6	1.5	1.8	2.7	3.6
(ExF) 97	S	9.1	1.4	1.6	3.1	3.7
(ExF) 45	S	9.7	1.4	1.7	2.7	4.6
Field SDS susceptible class						
‘Essex’				2.2	3.0	4.6
(ExF) 39	S	15.7	2.3	2.1	2.6	6.6
(ExF) 51	S	16.0	2.0	2.2	3.4	5.6
(ExF) 10	S	17.5	2.0	2.0	2.9	6.1
(ExF) 68	S	18.2	2.1	1.3	2.9	4.7
(ExF) 18	S	18.6	2.1	2.1	2.3	4.4
(ExF) 83	S	18.7	1.9	2.2	3.6	5.4
(ExF) 76	S	18.7	2.3	1.6	2.9	6.1
(ExF) 85	S	19.8	2.3	2.3	4.5	5.8
(ExF) 80	S	20.2	2.1	1.9	4.8	6.0
(ExF) 7	S	20.3	2.2	2.0	4.7	4.2
Mid-parent value				1.7	2.2	3.6

† SDS resistance class based on greenhouse disease severity at moderate inoculum rate (R = SDS-resistant and S = SDS-susceptible).

an equally-replicated field study for separating cultivars with partial resistance from those with resistance.

Simple regression analyses of greenhouse SDS DS with field DX indicated that the amount of variation in field DX explained by greenhouse DS was 40, 60, and 42% at the low, moderate, and high inoculum rates, respectively (Fig. 1). Predicted values of field DX were calculated for each inoculum rate (Fig. 1) using the predictive equation:

$$Y^1 = a + bx \quad [2]$$

where Y^1 = the predicted value of field DX, a = y-intercept, b = slope of the regression line, and x = greenhouse DS. The y-intercepts were 12.7, -8.2, and -9.9 for low, moderate, and high inoculum rates, respectively. The slopes of regression lines were 13.3, 6.6, and 4.3 for low, moderate, and high inoculum rates, respectively.

When DS values from the low and moderate inoculum rates were pooled for each genotype, the amount of variation in field DX explained by greenhouse DS was 65%. Therefore, reduced inoculum rates (3500–5000 spores cm^{-3} plant growth medium) will provide greenhouse DS values that are a better predictor of field DX. Field DX was held constant for all three regres-

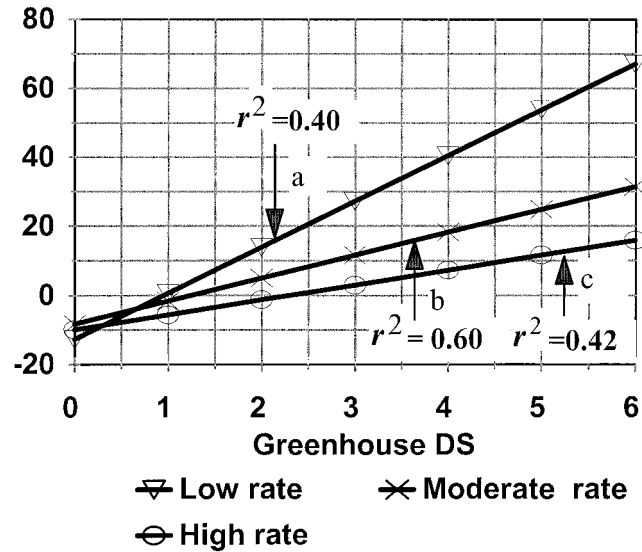


Fig. 1. Regression lines for soybean sudden death syndrome disease index (DX) from field experiments with disease severity (DS) from four greenhouse experiments among 30 soybean lines. The greenhouse experiments were conducted at three *Fusarium solani* f. sp. *glycines* inoculum rates: (a) low (3.3×10^3), (b) moderate inoculum rate (5×10^3), and (c) high (10^4). While the moderate inoculum rate explained more variation in field DX and produced predicted values that were close to those observed in the field, the low and high inoculum rates produced predicted field DX values that were higher and lower than those observed in the field in this population, respectively.

sion lines. However, greenhouse DS depended on inoculum rate, causing differences in slopes among regression lines (Fig. 1).

The strong association between field and greenhouse disease severities contrasted with the absence of correlations between field and greenhouse DS in previous tests (Torto et al., 1996).

The midparent value of DS within inoculum rate was used as the cut-off point for resistance in the greenhouse. At the low inoculum rate, while 20% of field resistant lines were eliminated, there was a high rate of retention of field partially-resistant (90%) and field susceptible (20%) lines. At the moderate inoculum rate, only 10% of field resistant lines were eliminated, and the proportion of lines retained was lower for field partially-resistant (50%) and field susceptible (0%). At the high inoculum rate, the lower proportion of field partially-resistant (30%) and susceptible lines (0%) retained was offset by a higher proportion of field resistant lines eliminated (Table 4). Therefore, the highest selection efficiency of 42% [(selected field resistant/total selected) * 100] was achieved with the moderate inoculum rate.

CONCLUSIONS

While significant variability was observed among SDS classes at the low inoculum rate in Experiments 1 and 2 and at moderate inoculum rate in Experiments 2, 3, and 4, the moderate inoculum rate was more consistent in separating SDS classes. The moderate inoculum rate also resulted in more efficient selection with the lowest rate of elimination (10%) of field resistant lines. Hence,

Table 5. Comparison of soybean cultivars for sudden death syndrome (SDS) following seedling inoculation by *Fusarium solani* f. sp. *glycines* at low inoculum rate (about 4000 spores per cm³ of plant growth medium) in the greenhouse and SDS disease index (DX) and DS from field experiments.

Cultivar	Field DX	Field DS	Greenhouse DS
'Ripley'	1.6	1.09	1.40
PI 520 733	0.2	1.15	1.52
'Jack'	1.5	1.10	1.83
'Manokin'	1.8	1.09	1.90
'Hartwig'	0.6	1.12	2.20
'Hamilton'	2.2	1.15	2.54
'Spencer'	33.3	2.72	2.78
'A5403'	18.0	2.10	3.20
'CM497'	30.1	3.01	4.40
'Flyer'	35.7	3.06	4.90
LSD ($\alpha = 0.05$)	13.1	1.11	1.53

it is recommended that inoculum rates of 4000 to 5000 spores cm⁻³ of plant growth medium be used in greenhouse assays to predict field performance for SDS.

In a greenhouse cultivar trial, two out of four field susceptible genotypes were significantly more susceptible than all six field resistant genotypes. Three field SDS-susceptible cultivars showed significantly ($P \leq 0.05$) higher SDS DS means than Ripley when evaluated in the greenhouse on plant growth medium infested with ≈ 4000 *Fsg* spores cm⁻³ (Table 5). Among these 10 cultivars, greenhouse DS was significantly associated with field DS ($r^2 = 0.81$) and field DX ($r^2 = 0.74$). Given that most of these cultivars are not directly related to Essex and Forrest, and because of the diverse germplasm they contain, we conclude that the low *Fsg* inoculum rate greenhouse assay can select for field SDS resistance in many genetic backgrounds.

The assay described above will allow for multiple cycles of testing per season and reduce the time to both produce and verify new resistant cultivars. The assay will reduce the costs of data loss due to field variability. Finally, the assay will facilitate the isolation of SDS resistance genes by rapid characterization of recombinants during fine mapping.

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Genetic Background and Environment Influence Palmitate Content of Soybean Seed Oil

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ABSTRACT

Dietary concerns over high saturates contained in edible vegetable oils has stimulated development of soybean [*Glycine max* (L.) Merr.] cultivars with reduced palmitate content. Little is known of factors that might influence phenotypic expression of palmitate content among soybean populations varying for presence of a major reduced palmitate allele. The objective of this study was to investigate how environment and genetic background influence palmitate content when introducing the reduced palmitate trait into adapted backgrounds. Crosses were made between reduced palmitate germplasm, N87-2122-4 (53 g kg⁻¹ palmitate) and normal palmitate cultivars, A3733, Burlison, Kenwood, P9273, and P9341 (103–123 g kg⁻¹ palmitate). For each cross, F₄₆ lines homozygous for major reduced or normal palmitate alleles were bulked separately into Maturity Groups (MG) II, III, IV, and V, and evaluated in 10 contrasting field environments during 1993. Palmitate content varied between 82 and 90 g kg⁻¹ across southern U.S. and Puerto Rican environments. Much of this environmental variation was associated with changes in minimum temperature during the growing season. Genetic background effects were highly significant ($P < 0.01$) with cross means for palmitate content ranging between 81 and 93 g kg⁻¹. Across different maturity groups, palmitate content of the progeny was correlated ($r = 0.94-0.99$, $P < 0.05$) with mean content of the normal palmitate parent, such that for every 1 g kg⁻¹ palmitate increase in the normal palmitate parent there was a 0.32 to 0.51 g kg⁻¹ palmitate increase in the progeny. Genetic background effects were presumed to be associated with action of minor alleles transmitted from the normal palmitate parent. Presence of the reduced palmitate allele was associated with significantly ($P < 0.01$) lower stearate (-6 to -13%) and higher oleate (+4 to +10%) contents across all maturity groups. Selection of low palmitate, high-yielding parents should further decrease palmitate content and produce correlated improvements in stearate and oleate contents to improve overall oil quality in progeny containing reduced palmitate alleles.

EPIDEMIOLOGICAL EVIDENCE and clinical studies alike have demonstrated that higher saturated fatty acid

intake contributes to raised blood serum cholesterol levels, thus increasing the risk of coronary heart disease (Willett, 1994; Uusitalo et al., 1996). Public perception of this potential health issue has provoked a strong trend towards greater consumption of foods containing lower levels of saturated fats (Wilson, 1991; Uusitalo et al., 1996). In turn, the U.S. Food and Drug Authority has proposed labeling regulations indicating "low saturate" vegetable oils must contain no greater than 70 g kg⁻¹ total saturates. Although soybean oil is relatively low in total saturates ($\approx 120-200$ g kg⁻¹), at least a minimum 50% reduction in saturated fat is needed to enhance the utility of soybean oil in this new market.

Palmitate is the predominant saturated fatty acid in soybean and most other vegetable oils (Weiss, 1983). Major alleles conditioning reduced palmitate content are available in soybean germplasm obtained from recurrent selection (Burton et al., 1994) and chemical mutagenesis (Horejsi et al., 1994; Wilcox et al., 1994). In response to consumer demand for healthful oils, soybean breeders are now actively incorporating the reduced palmitate trait into cultivar development programs (Burton et al., 1996). Given the need to transfer reduced palmitate alleles into a range of adapted, high-yielding genetic backgrounds (Wilson, 1991; Burton et al., 1996), it would be helpful to understand whether palmitate expression is contingent upon reduced palmitate genes contributed by the gene donor only, or genes present within both donor and adapted parents. The interaction of target genes with genes present within recipient genetic backgrounds can be an important consideration when introducing new traits into a commercial breeding program (Hallauer and Miranda, 1988). Further, it would be useful to document the influence of genetic background on expression of the reduced palmitate trait in different maturity zones and soybean production regions of the USA.

The influence of parental selection and genetic background on phenotypic expression for reduced palmitate content has not been reported for soybean. Earlier studies (Horejsi et al., 1994; Rebetzke et al., 1998) showed that palmitate content measured in different soybean

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