Comparison of Laboratory and Field Evaluations of Resistance in Soybean to *Sclerotinia sclerotiorum*

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ABSTRACT

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Fifteen commercial soybean (*Glycine max*) cultivars were evaluated for resistance to *Sclerotinia sclerotiorum* in the laboratory, using an excised stem technique, and in the field during 1988 and 1989. In the laboratory tests, significant differences in the length of stem lesions among cultivars were observed. Maple Presto, McCall, and Clay were significantly more resistant to stem decay then Pride B152, Apache, Merit, and Portage. In the field, significant differences in the disease severity index among cultivars were observed. Portage, Maple Presto, and McCall were the most resistant, whereas Merit, Evans, and Simpson were highly susceptible. Evans, although highly susceptible in the field, did not differ significantly from Maple Presto in the laboratory tests. There was no correlation ($P = 0.05$) between laboratory evaluations and the 1988 and 1989 field evaluations. The lack of a correlation between field and laboratory evaluations suggests that a laboratory test measuring stem decay is unlikely to be a reliable method for identifying field resistance to *S. sclerotiorum* and, therefore, would have limited value in a breeding program.

Sclerotinia stem rot of soybean (*Glycine max* (L.) Merr.) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary has become an important soybean disease in North Dakota. Soybean acreage in the state has increased from 81,000 ha to 283,000 ha in the past decade, with the largest hectarage in the Red River Valley, where *S. sclerotiorum* has been a serious problem on other susceptible crops such as sunflower and dry bean (13,17). Soybean growers in North Dakota have reported substantial yield losses from Sclerotinia stem rot when soybean was grown in infested soils under narrow row spacings and irrigation or during wet years. Other northern soybean growing areas have also reported an increase in Sclerotinia stem rot (5-7,10,11). In Wisconsin, Grau and Radke (11) reported that soybean yield reductions due to Sclerotinia stem rot were up to 42% under dryland conditions. Chun et al (6) observed that for every 10% increase in disease from 0 to 52%, yield was reduced 7.8% of the maximum.

Growers are also concerned that contamination of seed with sclerotia of *S. sclerotiorum* may affect overseas exporting of seed. Furthermore, soybean stems infected with *S. sclerotiorum* return sclerotia to the soil, increasing the inoculum density. This can have a profound effect on rotation schedules that include other susceptible crops. Sunflowers, for example, cannot be grown commercially in fields with high inoculum densities (13,16). With the increasing emphasis on crop diversity in North Dakota, there is now a greater chance of other crops susceptible to *S. sclerotiorum* being included in rotations with soybean. The amount of infested land and the level of infestation are important factors affecting decisions of growers to plant soybean and other susceptible crops. Soybean cultivars with resistance to *S. sclerotiorum* would be highly desirable.

Differences among soybean cultivars in resistance to *S. sclerotiorum* are reported from field, greenhouse, and laboratory evaluations (3,4,6,7,12). Some cultivars have shown a high level of resistance under field conditions over several years (4). There is sufficient evidence to suggest that resistance to *S. sclerotiorum* in soybean can be identified.

Development of a reliable controlled-environment method to screen soybeans for physiological resistance would greatly aid a breeding effort, especially in a recurrent selection program. Recurrent selection has shown success in breeding for white mold resistance in snap bean (9). Field testing is often not reliable because environmental conditions conducive to disease development may not occur at the appropriate stage of crop development.

Controlled environment methods for testing resistance to *S. sclerotiorum* have been reported. Cline and Jacobsen (7) found that the limited-term inoculation method of Hunter et al (14) distinguished differences in resistance among 10 soybean cultivars and that the results paralleled observations made on diseased plants in the field, but they did not provide correlation data. Boland and Hall (3) also found that resistance could be evaluated with the limited-term inoculation method but that the results of greenhouse evaluations were not correlated with evaluations in the field (4). Chun et al (6) recently described a laboratory test using excised stems that identified differences in disease reaction among cultivars and provided results with some correlation to field tests. The excised stem method was reported to have several advantages, such as speed and economy, over other methods.

The objectives of this study were to determine 1) if cultivars adapted to the North Dakota environment differed in resistance to *S. sclerotiorum* and 2) if the excised stem method (6) would be a useful procedure to identify resistance that would also be expressed under field conditions. Cultivars were evaluated first with the excised stem method in the laboratory and then under natural field conditions, and the results of the evaluations were correlated.

MATERIALS AND METHODS

Soybean cultivars. Fifteen soybean cultivars were chosen for this study because they were reported to vary in disease reaction to *S. sclerotiorum* (3,4,6,7,10,12) but were in maturity groups for the Northern Great Plains: Ada (00), Agripro 1650 (I), Apache (0), Bicentennial (00), Clay (0), Evans (0), Maple Arrow (00), Maple Presto (000), McCall (00), Merit (0), Norman (00), Ozzie (0), Portage (00), Pride B152 (I), and Simpson (0).

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Greenhouse procedures. Soybean seed was surface-disinfested in 0.5% NaOCl for 3 min, then planted in a pasteurized potting mix (equal parts of Glyndon sandy loam, peat moss, and vermiculite) in 15.5-cm-diameter clay pots. Pots were overseeded, and following seedling emergence, plants were thinned to five seedlings of approximately equal height per pot. Plants were fertilized weekly with 0.04 g of Peters 20-20-20 (N-P-K) (W. R. Grace Co., Fogelsville, PA) plus 0.01 g of an iron chelate and were illuminated with high-pressure sodium lamps (1,000 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 16 hr per day. Greenhouse temperatures ranged from 19 to 28 C. Cultivars were arranged in four randomized complete blocks consisting of two pots of each cultivar. At 5 wk of age, plants were cut off just above their bases, placed in plastic bags, and immediately taken to the laboratory. Within 4 hr, the leaves and growing tips were excised (6) and the stems were inoculated.

Laboratory inoculation procedure. A virulent isolate of *S. sclerotiorum*, ND 9, was maintained as sclerotia at 4 C. The sclerotia were collected from soybeans artificially inoculated with ND 9 in the greenhouse. Inoculum of ND 9 was prepared using the tissue paper technique as described by Nelson (15).

A modification of the excised stem method as described by Chun et al (6) was used. Plastic trays (36 × 46 × 2.5 cm) were lined with clear polyethylene wrap, and each tray was filled with 2 L of pasteurized vermiculite (1.5 cm deep), which was moistened with 1 L of distilled water. Stems were placed on the vermiculite, parallel to the long axis of the tray, and inoculated by wrapping a 5 × 3 mm piece of tissue paper inoculum around the cut apex of each stem. Trays were then covered with a layer of clear polyethylene wrap to maintain high humidity around stems. Trays were placed on racks in the laboratory, and stems were incubated for 7 days at 20 ± 2 C in the dark. Lesion lengths in millimeters were measured as described by Chun et al (6).

The experimental design was a randomized complete block with 40 replications per trial. Cultivars were randomized within replications. Each tray was considered a replication (15 cultivars) and each stem was an experimental unit. The experiment was conducted four times between November 1987 and March 1988. The data were pooled over trials after tests for homogeneity of variance; trials were considered a random effect and cultivars a fixed effect. Data were analyzed by analysis of variance, and Fisher's protected least significant difference (LSD) (19) was used as an a posteriori multiple comparison test. The cultivar mean square was tested using the cultivar × trial mean square as the denominator for the *F* test. The difference among trials was tested using the replication (within trial) mean square as the denominator for the *F* test.

Field experiments. Soybean cultivars were evaluated for reaction to *S. sclerotiorum* in the field during 1988 and 1989 at the Carrington and Oakes irrigated research stations in central and southeastern North Dakota, respectively. The research sites were naturally infested fields that had been planted to susceptible crops in previous years. To ensure an adequate level of inoculum in soil, sclerotia were collected from cull pile screenings obtained from a dry bean processing plant in the fall of 1987. Approximately 100 kg of sclerotia was applied with a fertilizer spreader to each field site (about 0.08 ha) and lightly incorporated with a cultivator.

The experimental design was a randomized complete block with four replications and 15 cultivars. Each replication consisted of a four-row plot, 6 m long on 30.5-cm row spacing. Plant populations were 4.94×10^5 plants per hectare. Experiments at Carrington were planted on 18 May 1988 and 1989 and at Oakes on 26 May 1988 and 10 May 1989. The two experiments at Carrington were furrow-irrigated weekly with approximately 2.5 cm of water. Experiments at Oakes received approximately 1.9 cm of water each 3–4 days by overhead irrigation. Other than irrigation, conventional cultivation practices for soybean production were followed in each experiment.

After closure of the canopy, experiments were monitored biweekly for disease development. Disease was evaluated on 9 September 1988 and 31 August 1989 at the two Carrington sites and on 30 August 1988 and 24 August 1989 at the Oakes sites. Reaction of cultivars to *S. sclerotiorum* was rated on a 0–3 scale, where 0 = no symptoms, 1 = lesion(s) only on lateral stems, 2 = lesion(s) on main stems, and 3 = diseased plant dead (11). Twenty plants chosen at random from the center two rows of each replication were evaluated. For each replication, a disease severity index (DSI) was calculated according to the method of Sherwood and Hagedorn (21): DSI = Σ class × no. of plants in class × 100/total no. of plants × no. of disease classes. In 1989, at both locations, cultivars were rated for lodging severity on a scale of 1–5, where 1 = plants vertical; 2, 3, and 4 = plants lodged at approximately 23, 45, and 65°, respectively; and 5 = plants completely lodged (23).

Data were analyzed by analysis of variance, and Fisher's protected LSD (9) was used as an a posteriori multiple comparison test. Locations were considered a random effect and cultivars a fixed effect. The cultivar mean square was tested using the cultivar × location interaction mean square as the denominator of the *F* test. Homogeneity of variance was determined for experiments within years and between years before pooling of data for analysis.

Correlations. Correlations were evaluated among DSIs from the 1988 and 1989 field evaluations, the laboratory stem decay evaluations, and the lodging scores from field experiments in 1989.

RESULTS

Laboratory inoculations. Differences ($P = 0.01$) in the length of stem lesions caused by *S. sclerotiorum* were observed among cultivars (Table 1). Cultivars did not react the same in each trial relative to each other, as indicated by a significant trial × cultivar interaction. Among trials, the mean lesion lengths, averaged over all cultivars, were significantly different. Maple Presto, McCall, and Clay had significantly smaller lesions than Pride B152, Apache, Merit, and Portage.

Field evaluations. Because the mean square errors between field tests in 1988 and 1989 were not homogeneous, data were not pooled over years. There was homogeneity of variance within years, however, and data were pooled over locations within years. There were no significant cultivar × location interactions in either 1988 or 1989. Averaged over the two sites within each year, significant differences among cultivars in DSI were observed in both 1988 and 1989 (Table 2). Portage, Maple Presto, and McCall were the most resistant, and Merit, Evans, and Simpson were highly susceptible. There were significant differences among cultivars for lodging scores in 1989 (Table 2). Disease was low in 1988 because of an extreme drought with continuous high temperatures, which inhibited activity of *S. sclerotiorum* even in irrigated plots. Rainfall for July and August 1988 was 81 mm at Carrington and 58 mm at Oakes, compared with 188 and 249 mm, respectively, in 1989. The average maximum air temperatures in 1988 during July and August at the two sites ranged from 29 to 34 C.

Correlations. Disease severity indices from field tests in 1988 and 1989 were highly correlated ($r = 0.82$, $P = 0.01$). Lodging scores from 1989 were correlated with DSI in 1989 ($r = 0.60$, $P = 0.05$) but not with DSI in 1988. There was no correlation ($P = 0.05$) between stem lesion length from the laboratory evaluations and DSI in 1989 or 1988.

DISCUSSION

The results of this research are in agreement with other reports of resistance in soybean to *S. sclerotiorum* (3,4,6,10). There was a good correlation between field tests of 1988 and 1989, even though the extreme drought of 1988 limited disease development. Maple Presto and McCall had less disease than most cultivars in both laboratory and

field tests, findings similar to those of Boland and Hall (4) in field tests in Ontario. Portage, a cultivar released in 1964 and not grown commercially, was highly resistant in field tests but not in laboratory tests. Grau and Bissonnette (10) observed that Portage was more resistant than Merit in greenhouse seedling tests, which contrasts with our results of the laboratory tests. Portage apparently has components of resistance other than resistance to stem decay that are expressed in the field.

Maple Arrow, reported as highly resistant during 3 yr of field tests in Ontario (4), was less resistant than Maple Presto and Portage in the 1989 field tests but was similar to those two cultivars in the laboratory test. Ozzie was similar to Maple Arrow in our test but was reported by Boland and Hall (3,4) to be less resistant than Maple Arrow in both field and greenhouse evaluations. Chun et al (6) also reported that Ozzie showed some resistance in field tests.

Of the four most susceptible cultivars in the field—Merit, Evans, Simpson, and Pride B152—only Merit and Pride B152 were highly susceptible in the laboratory tests. Evans and Simpson did not differ significantly from Maple Presto in laboratory tests. In most reports on this disease, Evans has been shown to be a highly to moderately susceptible cultivar (3,4,7,12), but Chun et al (6) found Evans to be one of the cultivars with the least amount of disease in field tests.

In addition to this report, two others compared the results of greenhouse/laboratory testing with field testing. Boland and Hall (4) found no correlation, and Chun et al (6) indicated that only one of eight correlations was statistically significant. The lack of a correlation in our study, plus the evidence from these previous reports, suggests that greenhouse/laboratory testing is unlikely to be a reliable method for identifying field resistance (18) in soybean to *S. sclerotiorum* and, therefore, would have no, or limited, value in a breeding program. A strong correlation is desirable, because the intent of an artificial screening procedure is to identify resistant germ plasm that will perform similarly in the field. For example, the cultivar Evans was highly susceptible in the field but did not differ significantly in the laboratory test from Maple Presto, which is highly resistant in the field. Laboratory testing may, however, be useful in studying the specific nature of resistance (4).

Some components of resistance expressed in the field cannot be evaluated with a laboratory method. Factors such as lodging, cultivar architecture, height, and maturity appear to affect disease escape mechanisms in soybean to *S. sclerotiorum* (4). In this study, increased lodging was correlated with greater disease severity. Lodging results in a dense canopy that promotes a humid environment favorable to disease development (1,2,4). The plant architecture of dry bean has been shown to have a direct effect on disease escape mechanisms to *S. sclerotiorum* (8,20).

A problem in the laboratory testing was that cultivars did not always rank the same among the four trials of the experiment. This same problem was encountered by Boland and Hall (4) and Chun et al (6). The reasons for this are not understood, but growing conditions in the greenhouse before inoculation, especially the intensity of light, could be important factors. Plants grown under the high-pressure sodium lights during the winter were not as robust (smaller stem diameters and etiolated) as plants grown during the spring or summer when there was more sunlight. Light intensity in the greenhouse during the summer, for example, can be approximately 50% greater (1,500 $\mu E \cdot m^{-2} \cdot s^{-1}$) than in the winter. Cline and Jacobsen (7) reported that disease severity ratings of soybean plants inoculated with *S. sclerotiorum* were affected by the light intensity under which the plants were grown. Etiolated plants were more susceptible than non-etiolated plants. Light intensity also is reported to affect disease development in greenhouse evaluations of resistance in dry bean to *S. sclerotiorum* (14).

Differences in light intensity or other environmental factors could also be involved in the lack of a strong correlation between laboratory and field testing. Cultivars may differ in resistance reaction depending on the light intensity during the preinoculation period, as shown in soybean inoculated with *Pseudomonas glycinea* Coerper (22). Chun et al (6), reporting on the excised stem technique, took 10 cultivars grown to maturity in the field and tested the stems with the laboratory technique. They found a correlation between the stem decay assay and field testing, indi-

Table 1. Lesion lengths on 5-wk-old excised stems of soybean cultivars artificially inoculated with *Sclerotinia sclerotiorum* and incubated at 20 ± 2 C for 7 days[a]

Cultivars	Rankings[b]				Mean lesion length (mm)[c]
	Trial 1	Trial 2	Trial 3	Trial 4	
Maple Presto	1	10	1	1	111
McCall	2	1	12	3	116
Clay	3	5	3	6	117
Maple Arrow	6	4	8	5	120
Evans	4	3	10	12	124
Ada	8	8	9	4	124
Simpson	5	2	2	14	124
Ozzie	9	7	5	10	128
Agripro 1650	12	6	7	7	129
Norman	11	13	6	9	130
Bicentennial	10	15	4	8	130
Portage	15	9	13	2	136
Merit	7	12	15	11	139
Apache	13	14	11	13	141
Pride B152	14	11	14	15	152
					LSD = 17.7

[a]One isolate, ND 9, was used for all inoculations. Data from four trials were pooled after homogeneity of variance was determined.
[b]From 1 = shortest lesions to 15 = longest lesions.
[c]Means of pooled data were compared with Fisher's protected least significant difference ($P = 0.05$).

Table 2. Reactions of soybean cultivars to Sclerotinia stem rot and lodging scores from field tests in 1988 and 1989[a]

Cultivar	DSI[b]		Lodging[c]
	1988	1989	1989
Portage	0.1	1.7	2.4
Maple Presto	0.0	2.9	1.6
McCall	0.0	16.5	3.3
Norman	0.0	19.2	3.9
Clay	3.4	21.2	3.0
Ada	0.2	22.3	3.4
Maple Arrow	0.2	25.2	3.8
Ozzie	0.0	27.5	2.5
Apache	1.4	33.1	4.0
Bicentennial	0.9	37.1	4.3
Agripro 1650	2.3	39.4	2.3
Pride B152	6.7	56.3	3.1
Evans	16.4	61.7	4.3
Simpson	7.4	61.9	3.9
Merit	9.6	67.3	4.3
LSD[d]	4.2	18	1.2

[a]Tests were conducted at two locations in North Dakota (Oakes and Carrington) during both years. Data were pooled within each year after homogeneity of variance was determined.
[b]Disease severity index, calculated as: Σ class × no. plants in class × 100/total no. of plants × no. of disease classes. Reaction of cultivars was rated on a 0–3 scale, where 0 = no symptoms, 1 = lesion(s) only on lateral stems, 2 = lesion(s) on main stems, and 3 = diseased plant dead.
[c]Based on a 1–5 scale, where 1 = plants vertical; 2, 3, and 4 = plants lodged at approximately 23, 45, and 65°, respectively; and 5 = plants completely lodged.
[d]DSI and lodging means were compared with Fisher's protected least significant difference ($P = 0.05$).

cating that when cultivars were grown under natural conditions in the field, their physiological resistance to *S. sclerotiorum* in laboratory tests was more similar to that observed the field.

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