Evaluation of Soybean, Dry Bean, and Sunflower for Resistance to *Sclerotinia sclerotiorum*

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ABSTRACT

Many inoculation methods have been used to evaluate resistance of different crops to *Sclerotinia sclerotiorum* (Lib.) de Bary. Only a few of these methods have been used to evaluate more than one crop. This study compared disease evaluations of soybean [*Glycine max* (L.) Merr.], dry bean (*Phaseolus vulgaris* L.), and sunflower (*Helianthus annuus* L.) inoculated in the greenhouse (cut stem inoculation method) to field evaluations. In one experiment, stems of two soybean cultivars, Williams 82 (susceptible) and NKS19-90 (partially resistant), were severed and inoculated with a colonized mycelial plug of *S. sclerotiorum* placed on top of the plant at the cut point of the stem. Stem lesion lengths on these two cultivars were used to determine what effect plant age and post-infection temperature had on disease development. There was a significant ($P < 0.05$) difference in lesion lengths between inoculated 5-wk-old plants compared with 6- or 7-wk-old plants within each cultivar. At different post-infection temperatures, lesions developed at 25°C but not at 30°C. In another experiment, disease rating of 15 soybean cultivars evaluated in the greenhouse and field had significant ($P < 0.05$) correlation coefficients from 0.53 to 0.79. In addition to soybean, two experiments were completed on dry bean and sunflower. There were significant ($P < 0.05$) differences in lesion lengths among 14 genotypes within dry bean and sunflower. The correlation between greenhouse and field evaluations of dry bean and sunflower were 0.74 and 0.50 ($P < 0.05$), respectively. In summary, disease assessments from the cut stem inoculation compared favorably with disease assessments in the field for soybean, dry bean, and sunflower.

Sclerotinia stem rot of soybean is caused by the fungal pathogen *S. sclerotiorum*. On soybean, it is a major disease that causes substantial yield losses in the north central states of the USA. (Hartman et al., 1999, p. 46–48). Varietal differences in resistance to *S. sclerotiorum* in soybean have been reported from field, greenhouse, and laboratory evaluations (Boland and Hall, 1987; Chun et al., 1987; Kim et al., 2000; Nelson et al., 1991). Under field conditions, reaction of cultivars to *S. sclerotiorum* is the result of physiological resistance and escape mechanisms (Boland and Hall, 1987). Disease escape, due in part to open plant architecture and early maturity, caused inconsistent disease ratings in the field (Kim et al., 2000). In contrast, disease reactions in the greenhouse or laboratory evaluations are due to physiological resistance with little chance of escape mechanisms (Grau and Bissonette, 1974; Nelson et al., 1991).

Many inoculation methods have been developed for evaluating resistance to *S. sclerotiorum*. A method using cotyledon inoculation was first reported in soybean (Grau and Bissonette, 1974) and has been used by others (Hartman et al., 2000; Kim et al., 2000, Kull et al., 2003). Another common technique has been the inoculation of excised stems or detached leaves of dry bean or soybean with *S. sclerotiorum* mycelium (Chun et al., 1987; Kull et al., 2003; Leone and Tonneijck, 1990; Miklas et al., 1992; Nelson et al., 1991; Steadman et al., 1997; Wegulo et al., 1998). Intact plants, but with the main stem severed (cut stem method) have been used in soybean to compare inoculation methods and evaluate resistant sources (Kull et al., 2003; Vuong and Hartman, 2002). Some investigators have utilized oxalic acid to evaluate cultivars for resistance (Kolkman and Kelly, 2000; Noyes and Hancock, 1981; Tu, 1985), since it has been associated with pathogenesis by *S. sclerotiorum* (Ferrar and Walker, 1993). In soybean, Wegulo et al. (1998) measured pink pigments dissolved in oxalic acid from soybean stems after incubation of infected tissue at 20°C for 48 h.

Most greenhouse inoculation techniques are destructive to inoculated plants, although several nondestructive techniques have been used to evaluate resistance in dry bean (Petzoldt and Dickson, 1996) and sunflower (Koehler and Friedt, 1999) to *S. sclerotiorum.* The objective of this study was to compare disease evaluations of soybean, dry bean, and sunflower inoculated in the greenhouse by the cut stem inoculation method to field evaluations.

MATERIALS AND METHODS

Isolate and Inoculum Preparation

The *S. sclerotiorum* isolate 105HT was cultured from a soybean seed originating from Story City, IA, in 1996. The culture, stored at the Soybean Pathogen Collection Center at the University of Illinois, was maintained by subculturing in the dark at 4°C on potato dextrose agar (PDA) medium. To produce inoculum for greenhouse tests, a single mycelial plug cut from the culture with a 3-mm-diam cork-borer was placed in the center of a new PDA plate. The fresh culture was incubated at 20°C for 2 or 3 d in the dark. Three plugs from the margin of the growing colony were transferred to a new PDA plate and incubated at 20°C for 24 h in the dark. Mycelial plugs were cut from the margin and used to inoculate plants.

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Abbreviations: DAI, days after inoculation; DSI, disease severity index; FLSD, Fisher's protected least significant difference; PI, plant introduction; RCB, randomized complete-block.

Infested sorghum grain was used for inoculating soybean plants in the field. Sorghum grain was soaked in water overnight. After removing floating debris and draining the water, grain was rinsed several times. About 4 kg of clean grain was placed in 60- by 90-cm polypropylene bags (Fisher Scientific, Hanover Park, IL) and autoclaved for 1 h. It was then cooled at room temperature overnight and autoclaved again the next day. The cooled grain bags were inoculated with 100 g of previously infested sorghum grain inoculum which had been incubated at 22 ± 1°C for a week, shaken daily, and then dried at 32°C for 2 d. Infested dried grain was ground with a Wiley mill using a 3-mm screen, and stored in a cold room until needed.

Soybean Plant Preparation, Inoculation, and Disease Assessment

Seven soybean seeds of each entry were germinated in 15-cm clay pots containing a 1:1:1 mixture of soil:perlite: torpedo sand. Each entry was planted in three replicate pots placed in a greenhouse at 25 ± 1°C and 16-h photoperiod under 300 μmol m^{-2} s^{-1} light intensity. Seven-day-old seedlings were thinned to five plants per pot and allowed to develop to growth stages V5 to R1 depending upon the experiment (Fehr et al., 1971).

For greenhouse inoculations, the main stems of plants were horizontally severed with a sterile razor blade 0.5 cm above either the fourth or fifth node. A single mycelial plug was carefully placed mycelial-side down on the cut stem. Inoculated plants were incubated in a greenhouse mist chamber with about 80% relative humidity. The chamber was maintained at 20 ± 1°C and covered with black mesh cloth to reduce light intensity to less than 18.4 μmol m^{-2} s^{-1}. After 2 d, infected plants were transferred to another greenhouse room at 25 ± 1°C with the same photoperiod and light intensity as before inoculation. Lesion length (cm) on each plant was measured daily until 14 DAI.

Soybean plants were inoculated in the field at the R1 growth stage (Fehr et al., 1971) by broadcasting the infested ground sorghum grain on stems and branches. Inoculated plants were immediately mist-irrigated for several days. The inoculation was repeated three times at weekly intervals to ensure successful infection. At harvest maturity (growth stage R8), disease severity was evaluated using a DSI where 0 = no symptoms, 1 = lesions only found on lateral branches, 2 = small lesions on main stem not affecting pod fill, and 3 = lesions on main stem resulting in poor pod fill. A DSI ranging from 0 to 100 was calculated for each plot by the following formula: DSI = {[sum of ratings of each plant]/[3 × number of plants rated]} × 100 (Hoffman et al., 2002).

Plant Age

Two soybean cultivars, susceptible Williams 82 and partially resistant NKS19-90 (Kim and Diers, 2000) were planted in clay pots as described earlier. Three plantings were conducted a week apart to obtain plants at different ages. Plants were inoculated when they were 5, 6, and 7 wk old, corresponding to V5, V7, and V8/R1 growth stages (Fehr et al., 1971), respectively. Inoculated plants were incubated in a mist chamber for 48 h. Infected plants were then transferred to an adjacent greenhouse room for lesion development. Lesion length (cm) was measured daily until 14 DAI. Sclerotia that formed in the stems were collected at 14 DAI by splitting the infected segments, and the average number of sclerotia per plant was calculated. The experiment was a factorial design (cultivar × plant age) with three replications. Each pot was an experimental unit consisting of five plants. The experiment was repeated once.

Post-infection Temperatures

Six-week-old plants (five plants per experimental unit) of four soybean cultivars, Williams 82, NKS19-90, A2242, and AG2506, were planted in pots inoculated and incubated for 48 h as previously described. After incubation, plants were placed in temperature-controlled growth chambers at 25 and 30 ± 1°C under 400 μmol m^{-2} s^{-1} light intensity. Disease development was observed and lesion lengths on the main stems were measured daily until 14 DAI. The experimental design was a split-plot with three replicated blocks. Temperature was the main plot and cultivar was the subplot. The experiment was repeated once.

Greenhouse and Field Evaluations of Fifteen Soybean Cultivars

Fifteen soybean cultivars (Table 1) of different maturity groups were evaluated in a greenhouse test and two field tests at the Crop Sciences Research and Education Center, University of Illinois, Urbana, IL, in 1999. For the greenhouse test, seed planting and stem inoculations were performed using procedures previously described. Inoculated 6-wk-old plants were incubated for 48 h at 20°C and postincubated at 25 ± 1°C for disease development. Lesion lengths on diseased stems were measured daily from 7 to 14 DAI.

For one field-testing site (named the rain shelter), soybean seeds were sown with 10 seeds per hill, 20 cm apart in a row, and 75 cm between rows. The experimental design was a RCB with three replications. For the other field test (disease nursery), soybean seeds were sown in 4-row plots (2.4 m length and 75-cm row spacing) with the two center rows used for data collection. Plants were inoculated as described in the first field test and plants were misted with a sprinkler irrigation system. The inoculation was repeated three times. The experimental design was a RCB with two replications. Plants at approximately the R1 growth stage (Fehr et al., 1971) were dusted with infested sorghum grains. Plants were misted for

Table 1. Lesion length and disease severity of 15 soybean cultivars evaluated in the greenhouse by cut stem inoculation and at two field locations by sorghum grain infested inoculation. The cultivars NKS19-90 and A2242 were resistant and susceptible checks, respectively.

Cultivar	Lesion length (cm)† (greenhouse)	DSI‡ (rain shelter)	DSI‡ (disease nursery)
NKS19-90	9.1	19	55
Corsoy 79	10.4	14	41
Hardin	11.1	13	41
AG1901	11.4	33	52
AG2001	11.8	27	60
Athow	11.8	48	56
DSR 218	11.8	59	52
NE 3001	11.9	57	62
AG2501	12.0	44	58
DSR 215RR	12.0	66	57
CM 2012 RR	12.2	36	67
FFR HS3471	12.4	59	76
Conrad 94	12.5	62	65
XB29X00	12.8	68	71
A2242	12.8	69	65
FLSD	1.4	31	21

† Lesion length (cm) was measured on diseased main stems at 14 DAI.
‡ A disease severity index (DSI) ranging from 0 to 100 was calculated for each plot by the following formula: DSI = {[sum of ratings of each plant]/[3 × number of plants rated]} × 100.

5 min every 45 min with a misting irrigation system to maintain high humidity.

Evaluation of Resistance in Dry Bean and Sunflower Entries

Fourteen dry bean cultivars and 14 sunflower lines (Tables 2 and 3, respectively) were evaluated for resistance to *S. sclerotiorum* in the greenhouse. Entries were selected on the basis of differences in field disease severity ratings with Beryl used as a susceptible check and no definite line selected specifically as a resistant check (Steadman et al., 2001). Each entry was sown in three replicates in clay pots following the procedure described earlier for soybean with minor modifications. Seven-day-old seedlings were thinned to three plants per pot and inoculated when plants were 5 wk old. *S. sclerotiorum* isolate 105HT was used to inoculate plants as previously described for soybean. Inoculated plants were incubated for 48 h at 20 ± 1°C. Post-infection was performed at 25 ± 1°C as earlier described. Disease was allowed to develop and lesion lengths (cm) were measured daily until 14 DAI. The experimental design was a RCB with three replications and the experiments, dry bean and sunflower, were each repeated once.

For dry bean field tests, each entry was grown in 2-row plots (4.6 m long) adjacent to one row of a common susceptible genotype. There were three replicated plots for each entry arranged in a randomized complete block design. Experiments were conducted in Michigan, Washington, and Wisconsin. Because of the differences in disease assessments between locations, the entries were ranked from most resistant (1) to most susceptible (12) in each test. A Spearman's rank correlation was used to compare entry ranking. Part of the data from these tests was previously published (Steadman et al., 2001).

For the sunflower field evaluation, 14 sunflower hybrids were planted in a RCB design with three replications. Hybrid-9 was considered to be the susceptible check with no definite hybrid as the resistant check. Plots were 6-m-long single rows with 0.75 m between rows. Plants within rows were thinned to 50 000 plants ha^{-1}. Ten plants per plot were selected for inoculation when pollen shed had begun in the outer ring of disk flowers. Five mL of a suspension containing 5000 ascospores/mL were sprayed on each head. A misting system was used to apply moisture to heads after inoculation, with the plots misted 3 min every half hour for 35 d. After 35 d, plants were evaluated for incidence and severity of Sclerotinia head rot. Incidence was the average number of plants infected with the fungus per 10 plants. Severity was based on the following scale: 0 = no symptoms, 1 = mycelium growing in the floral parts, 2 = first tan spots appearing on the dorsal surface, 3 = more and larger tan spots, 4 = severe damage to the head, and 5 = head completely destroyed.

Table 2. Mean lesion lengths were measured at 14 DAI on dry bean stems inoculated with the *S. sclerotiorum* isolate 105HT in the greenhouse; mean ranking of dry bean lines/cultivars were evaluated in multilocation tests (Steadman et al., 2001).

	Greenhouse	Multilocation
Line–cultivar	Lesion length (cm)†	Ranking
MO 162	2.3	5.5
L 192	3.0	4.8
G 122	3.3	5.3
NY6020-5	3.5	6.3
B7354	4.2	4.5
PC-50	4.2	6.1
N 97774	5.7	8.3
NG 8025	6.3	‡
Prosperity	6.7	8.0
I9365-25	7.0	4.5
Bunsi	7.5	7.7
Great Northern§	9.1	‡
ND89-151-46-02	9.4	7.8
Beryl	10.9	9.7
FLSD	2.2	2.8

† Mean lesion length was measured on diseased main stems at 14 DAI over two runs.
‡ Great Northern and NG 8025 were not used in three state multi-location tests.
§ Market class, not a cultivar.

Statistical Analysis

Statistical analysis was performed by PROC GLM (SAS Release Version 8.0, SAS Institute, Cary, NC) with linear models appropriate for experimental designs in each experiment. Those inoculated plants that did not show symptoms and the controls in greenhouse tests were not included in the analysis. FLSD was used to determine significant ($P = 0.05$) differences among mean values. Correlation analysis was used to determine the relationship between the disease response of entries evaluated under greenhouse and field conditions.

Table 3. Disease reaction of 14 sunflower hybrids evaluated in greenhouse and field conditions. Lesion lengths of diseased stems were measured in the greenhouse evaluation; incidence and severity of disease on inoculated heads was estimated for plants in the field evaluation.

Code	Hybrid	Lesion lengths†	Incidence‡	Severity§
		cm		
Hybrid-1	cms 412/RHA 377	8.1	0.7	0.2
Hybrid-3	cms 411/RHA 377	8.7	1.7	0.3
Hybrid-2	cms 412/RHA 373	9.0	1.7	0.4
Hybrid-11	Cargill SF 187	9.0	5.7	1.4
Hybrid-6	cms 406//RHA377/AS3211-3	9.5	2.0	0.8
Hybrid-8	cms 406//RHA377/AS3211-2	10.8	1.7	0.5
Hybrid-13	Mycogen 8242	10.8	5.6	2.6
Hybrid-12	IS Hysun 450	10.9	4.6	1.3
Hybrid-7	cms 406//RHA377/AS3211-1	11.0	0.7	0.2
Hybrid-4	cms 411/RHA 373	11.2	3.0	1.0
Hybrid-14	Pioneer 63M80	11.4	2.7	0.8
Hybrid-10	Novartis 278	11.8	1.7	0.6
Hybrid-9	Mycogen 924	14.3	6.0	2.8
Hybrid-5	cms 406//RHA 377/AS3211-4	14.8	3.0	1.1
FLSD		2.8	0.96	0.32

† Mean lesion length was measured on diseased stems at 14 DAI over two runs.
‡ Incidence was the number of plants infected for every 10 plants inoculated; measured at 35 DAI.
§ Severity based on a scale from 0 (no symptoms) to 5 (head completely destroyed).

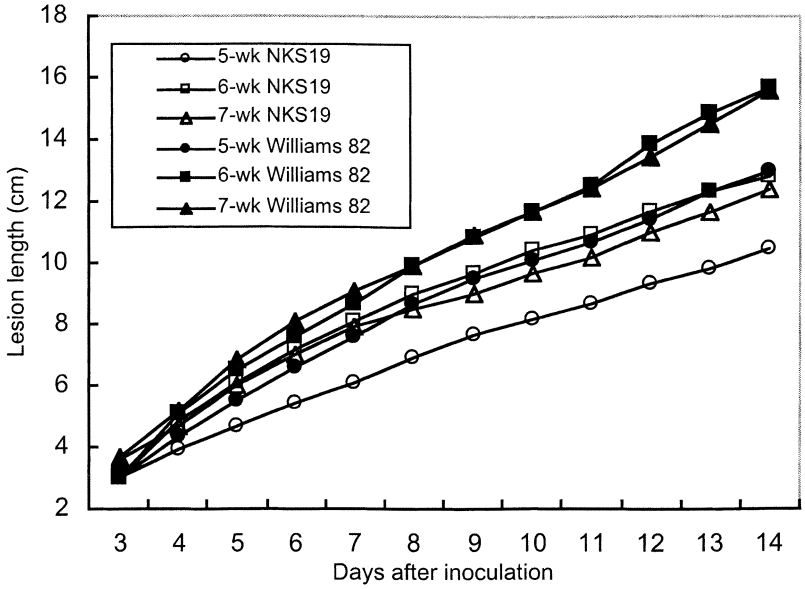

Fig. 1. Lesion length (cm) from 3 to 14 DAI on soybean plants that were 5-, 6-, and 7 wk old at inoculation. A partially resistant cultivar, NKS19-90, and susceptible cultivar, Williams 82, were inoculated with *S. sclerotiorum* isolate 105HT, incubated for 48 h, and postincubated at 25 ± 1°C to observe disease development. FLSD values were 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, and 1.3, respectively, for 8, 9, 10, 11, 12, 13, and 14 DAI.

RESULTS

Plant Age

Of 270 soybean plants inoculated, 98% showed typical symptoms of Sclerotinia stem rot with a bleached white segment clearly visible on the main stem. Lesion lengths were 2 to 3 cm long 3 DAI, developing from the point of inoculation downward. When the margin of lesion reached a node, leaves wilted and died the next day.

The cultivar × plant age interaction was not significant. With respect to plant age within each cultivar there were no differences between 6- (V6) and 7-wk-old (V7) plants for stem lesion lengths at any disease rating time, but there was significant difference when 5 wk old plants were compared to older inoculated plants (Fig. 1). At the end of the experiment, 14 DAI, mean lesion lengths combined over the 6- and 7-wk-old plants were 10.2 and 14.8 cm long in NKS19-90 and Williams 82, respectively. There were significantly more sclerotia formed inside diseased stems of Williams 82 (4.5 per plant) than in NKS19-90 (1.2 per plant) averaged over the three different plant ages.

Effects of Post-Infection Temperatures

There were no interactions of post-incubation temperature × cultivars. In the first 4 DAI, there were no significant differences in lesion development under the two temperatures. However, from 5 DAI onward, disease lesions were larger at 25°C than at 30°C, and the differences remained significant for all cultivars through 14 DAI. Lesion lengths of all cultivars at 30°C were similar, 3.0 cm at 3 DAI and 4.0 cm at 14 DAI; while lesion lengths at 25°C increased from 3.0 cm at 3 DAI to 12.7 cm at 14 DAI with NKS19-90 having significantly smaller lesions than the other three cultivars by as early as 4 DAI (Fig. 2).

Greenhouse and Field Evaluations of 15 Soybean Cultivars

There were significant ($P < 0.05$) differences among soybean cultivars evaluated in both greenhouse and field evaluations (Table 1). In the greenhouse test, lesion lengths (cm) at 14 DAI varied from 9.1 cm for NKS19-90 (partially resistant check) to 12.8 cm for A2242 (susceptible check). In the rain shelter evaluation, DSI among cultivars ranged from 13 to 70. These indices of 'Corsoy 79' and 'Hardin' were not significantly different from NKS19-90, which had a DSI of 19. The susceptible check, A2242, had the highest DSI of 69. In the disease nursery, DSI among cultivars ranged from 41 to 76. The cultivars Corsoy 79 and Hardin were lowest, both with a DSI of 41, while the DSI for NKS19-90 was 55. The DSI of the susceptible check, A2242, was 65.

Coefficients of correlation between lesion length measured from 7 to 14 DAI in the greenhouse test and DSI from the field varied from 0.47 to 0.80 (Table 4). At 14 DAI, the correlations between lesion length and DSI in the rain shelter and disease nursery were 0.78 and 0.65, respectively. The correlation coefficient for the two field evaluations was 0.67 ($P < 0.01$).

Evaluation of Resistance in Dry Bean and Sunflower Entries

Of 250 dry bean and sunflower plants inoculated, over 98% showed disease symptoms. Infected plants had water-soaked stem lesions. During the early period of post-infection, lesions developed relatively slowly. Differentiation among entries was recorded at 14 DAI.

For dry bean plants in the greenhouse, lesion development was slower than that observed in soybean. At 14 DAI, the dry bean cultivars, MO 162, L 192, and G 122 (Table 2) had lesions of 2.3, 3.0, and 3.3 cm, respectively. The cultivar PC-50, a resistant check, had a lesion length

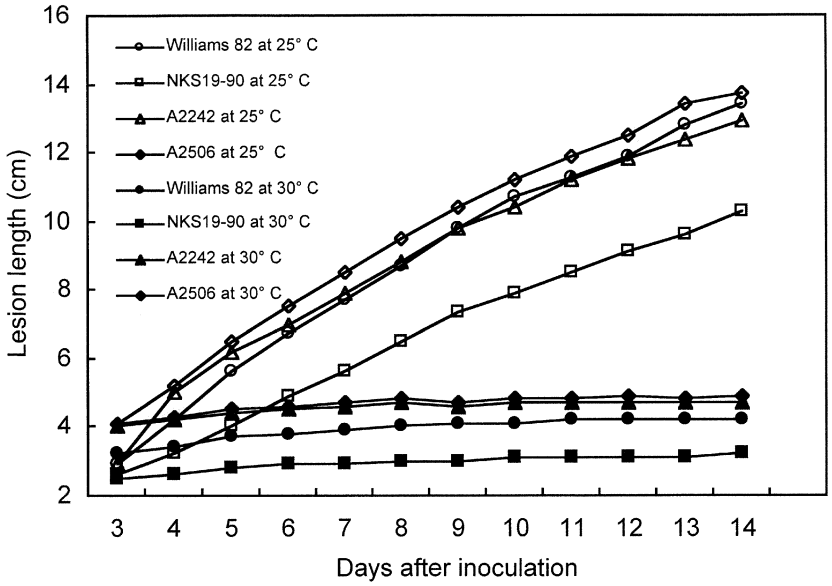

Fig. 2. Effect of postincubation temperatures on disease response of four soybean cultivars evaluated. Inoculated stems were incubated for 48 h followed by an exposure at 25 and 30°C for 12 d. FLSD values were 0.2, 0.2, 0.3, 0.4, 0.5, 0.6, 0.6, 0.6, 0.7, and 0.8, respectively, for 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 DAI.

of 4.2 cm, which was significantly less than ND 89-151-46-02 and Beryl. In field evaluations, there were significant differences among genotypes (Table 2). The cultivars B7354 and I9365-25 had the lowest disease ratings, but L192 and MO 162, G122, PC-50 and NY6020-5 also had significantly less disease than the susceptible control Beryl. The correlation coefficient (r = 0.74) of lesion length measured in the greenhouse and field ranking was highly significant.

Lesions on sunflower plants in the greenhouse developed faster than in dry beans. Lesion lengths were longer and ranged from 8.1 cm for Hybrid-1 to 14.9 cm for Hybrid-5 (Table 3). There were significant differences among hybrids for lesion development. In the field, hybrid-1 had the lowest incidence and severity, 0.67 and 0.20, respectively, and had the lowest lesion length in the greenhouse (Table 3). There was no correlation of lesion length measured in the greenhouse and field incidence, and a significant but low correlation (r = 0.50) between lesion length and field severity.

DISCUSSION

Many greenhouse and laboratory inoculation methods have been developed to evaluate various crop plants for resistance to *S. sclerotiorum*. Some of these methods have drawbacks such as the need for complete plant destruction (Kolkman and Kelly, 2000; Wegulo et al., 1998), and low correlations with field tests (Kim et al., 2000). Consistency and reliability of an evaluation method in the greenhouse is important so that disease assessments can be predicted as under field conditions. Moreover, a nondestructive approach may be useful in genetic studies and breeding programs where progeny tests to measure seed productivity are often required.

Chun et al. (1987) studied effects of plant age on lesion development of excised stems from 3- to 7-wk-old soybean plants of Corsoy and concluded that lesion lengths tended to decrease in older plants. In contrast, the lesion lengths of 6- and 7-wk-old plants were significantly greater than 5-wk-old plants in our study. Moreover, there were no differences in lesion lengths between 6- and 7-wk-old plants. Although statistically significant differences were observed, disease progression manifested the same pattern in both cultivars at different plant ages throughout the period of study. Also, the plant stage × cultivar interaction was not significant. These results indicated that plant age did not affect the disease response and the differentiation between susceptible or resistant genotypes.

In a preliminary experiment, we investigated temperature effects on the growth of the 105HT isolate on PDA and found that the fungus grew slowly at 15°C, very fast at 20 and 25°C, and was suppressed at 30°C (data not shown). The results on plants in the present study were similar in that lesions developed at 25°C and were suppressed at 30°C. These findings agree with a previous study (Chun et al., 1987), in which excised

Table 4. Correlation coefficients between the disease responses of 15 soybean cultivars evaluated in the greenhouse and in the two field tests. Lesion lengths (cm) were measured at 7, 9, 11, 13, and 14 DAI in the greenhouse evaluation. Disease severity index (DSI) was estimated at the R7 growth stage in field tests.

Lesion length† (greenhouse)	DSI‡ (rain shelter)	DSI‡ (disease nursery)
7 DAI	0.47ns	0.51ns
9 DAI	0.53*	0.59*
11 DAI	0.63*	0.66**
13 DAI	0.67**	0.80**
14 DAI	0.78**	0.65**

* Significant at the 0.05 level of probability.
** Significant at the 0.01 level of probability.
ns: not significant
† Lesion length (cm) was measured on diseased main stems at 7, 9, 11, 13, and 14 DAI.
‡ A disease severity index (DSI) ranging from 0 to 100 was calculated for each plot using the following formula: DSI = {[sum of ratings of each plant]/[3 × number of plants rated]} × 100.

stems incubated at 30°C had significantly shorter lesions than those incubated at 20 or 25°C. Temperature plays an important role in infection and symptom development, and this may explain why Sclerotinia stem rot of soybean is less severe in the southern versus the northern soybean production region.

Correlations of Sclerotinia stem rot ratings in soybean between the greenhouse, laboratory and field tests have been reported (Chun et al., 1987; Kim et al., 2000; Nelson et al., 1991), and some of these results indicate that there is a low correlation or inconsistency among the tests. The combined factors of physiological resistance, pathogen distribution, and escape mechanisms are important in field ratings (Boland and Hall, 1987). For instance, plant height, date of flowering, and maturity data were significantly correlated with levels of resistance to *S. sclerotiorum* in soybean (Boland and Hall, 1987; Kim and Diers, 2000), but these factors were not significantly correlated with DSI in another field test (Kim et al., 2000). Furthermore, relatively large error variances (Kim et al., 2000) in field tests occur, whereas coefficients of variance (CV) of most of our experiments in the greenhouse were less than 10%.

In a previous study of dry bean (Petzoldt and Dickson, 1996), a drinking straw containing a mycelial plug was placed over the cut stem to inoculate dry bean, and disease severity was rated on a 1-to-9 scale. In our study, plants were inoculated with a single mycelial plug and quantitative measurements were recorded. Disease reactions of dry bean entries were differentiated according to higher levels of resistance, such as MO 162, L 192, and G 122, and moderately-resistant, PC-50 (Table 2).

The cut stem technique had not been previously used on sunflower. Unlike soybean or dry bean plants with at least five internodes at the time of inoculation, sunflower seedlings had only two internodes. When inoculation was performed at the second internode, the cut stem technique enabled us to differentiate sunflower genotypes according to physiological resistance to *S. sclerotiorum*. However, given that symptoms of Sclerotinia stem rot in the field also appear on the sunflower heads, it is interesting to see that there was low correlation between the results of the cut stem technique and the evaluation of sunflower heads for resistance. Additional studies need to be conducted to determine if such stem resistance is similar to the head rot resistance in all cases (Hahn et al., 2001).

The cut stem inoculation technique may require more time and space than would be practical for applied breeding programs where large numbers of genotypes are screened for resistance. However, it is necessary to note several positive features of the technique that are equally important for geneticists and breeders. First, several reports have indicated low reproducibility between experiments when evaluating soybean for resistance (Boland and Hall, 1987; Chun et al., 1987). In our study, the standard deviation for lesion length measurements taken from greenhouse inoculated plants were low, varying from 0.2 to 0.8 cm. Second, the technique enabled us to directly evaluate the response of individual genotypes to disease infection on the basis of quantitative measurements. These quantitative measurements reflected the nature of multigenic inheritance of resistance to *S. sclerotiorum* (Vuong et al., 2001). Therefore, the technique may be highly suitable for evaluating resistance of small plant populations in genetic studies. Third, though upper stems were severed for inoculation and stem segments invaded, removal of the diseased segments allowed branches from lower stems to grow and the plants to produce seeds. Because individual plants with disease resistance can be saved for seed production, the technique may prove useful for crop breeding programs requiring offspring generation for progeny tests.

Recently, Kull et al. (2003) used three inoculation methods (detached leaf, cotyledon, and cut stem) and six isolates of *S. sclerotiorum* to compare the response of dry beans and soybean under controlled environments. Using several statistical procedures, these authors concluded that the cut stem inoculation method was statistically better than the other two methods for evaluating resistance in soybean and dry bean cultivars. The cut stem technique was used to identify two soybean plant introductions, PI194634 and PI194639, which expressed greater levels of resistance to *S. sclerotiorum* than the partially resistant soybean cultivar NKS19-90 (Vuong and Hartman, 2002). Additional research is needed to determine if this cut stem technique could also be useful for other crops like canola (*Brassica napus* L. and *B. rapa* L.) and potato (*Solanum tuberosum* L.).

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