Two Convenient Methods to Evaluate Soybean for Resistance to *Sclerotinia sclerotiorum*

Y. Chen, Research Associate, and **D. Wang,** Assistant Professor, Department of Crop and Soil Sciences, Michigan State University, East Lansing 48824

ABSTRACT

Chen, Y., and Wang, D. 2005. Two convenient methods to evaluate soybean for resistance to *Sclerotinia sclerotiorum.* Plant Dis. 89:1268-1272.

Several greenhouse inoculation methods are available to evaluate soybean (*Glycine max* (L.) Merr.) for resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary. Most of these methods are labor intensive and often produce inconsistent results among the tests. The objective of this research was to develop a low-cost and high-efficiency greenhouse inoculation method that can generate a consistent result. We developed a spray-mycelium method in which mycelia were cultured in liquid potato dextrose broth and homogenized before spraying on the soybean leaves. We also developed an inoculation method (the "drop-mycelium" method) in which a drop of homogenized mycelium suspension was dropped on the tips of main stems. Inoculated plants were incubated in a greenhouse chamber with 60 to 80% relative humidity. Plant mortality and area under the wilt progress curve (AUWPC) were used to measure disease severity daily from 3 to 14 days after inoculation (DAI). Eighteen soybean genotypes, including partially resistant line NKS19-90 and susceptible line Resnik, were employed in this study. The spray-mycelium method and the drop-mycelium method were compared with the cut-petiole method in the greenhouse. The three experiments were a randomized complete block design. Twenty-four plants per genotype in each experiment were inoculated at V3 growth stage in the greenhouse. Significant differences ($P < 0.05$) in disease ratings of plant mortality and AUWPC to Sclerotinia stem rot were found among 18 tested genotypes. The results obtained with the spray-mycelium and drop-mycelium inoculation methods were significantly ($R > 0.73$, $P < 0.01$) correlated with the results obtained with the cut-petiole inoculation method for both of the plant mortality and AUWPC. Compared with the cut-petiole method, the spray-mycelium and the drop-mycelium methods used less inoculation time and are less expensive in terms of materials. Both of these new methods are low cost, efficient, and reliable and they can be valuable for large-scale evaluation of germ plasm and breeding lines for resistance to Sclerotinia stem rot in a greenhouse or other similar facilities.

Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a major soybean disease in north-central regions of the United States. In 2004, this disease caused soybean yield loss of 1.63 million tons in the United States and was the second most yield-reducing soybean disease in the country (19). In field environments, ascospores of *S. sclerotiorum* first land on flowers and germinate when moisture is adequate. The fungus then grows and girdles the stem, causing plant death. The typical foliar symptoms of Sclerotinia stem rot include necrotic leaves, bleached stem lesions on stem and pods, white fluffy mycelia, and black sclerotia present on the plant surface and internally in the stems and pods. The infections occur mainly in fields during cool, moist environmental conditions. Cultural factors associated with conditions that favor infection include irrigation, high plant population, plant spacing, lush vegetative growth, plant height, flowering date, and maturity date (1,7).

It has been reported that some soybean genotypes have partial resistance to *S. sclerotiorum* (5,6). Physiological resistance and escape mechanisms are involved in field resistance of soybean to Sclerotinia stem rot (7). Several inoculation methods have been developed for evaluation of soybean for resistance to *S. sclerotiorum* in a greenhouse or laboratory, including cotyledon inoculation (4,9), excised stem or detached leaf assay (2,9–12,15,18), cut-stem inoculation (9,17), oxalic acid assay (8,13,16), and cut-petiole inoculation (3). With the cotyledon inoculation method, cotyledons of 2-week-old seedlings are inoculated with mycelia plugs and the plant usually dies within 3 days after inoculation. This method failed to distinguish susceptible and partially resistant soybean genotypes when either highly or weakly aggressive isolates of *S. sclerotiorum* were used (9). With the excised stem or detached leaf assay, cut apices or detached leaves are inoculated with mycelial disks and lesion lengths are used to determine the susceptibility of the plants. Asynchronous initiation of lesion development could result in failure to detect differences between susceptible and partially resistant soybean genotypes (2). With the cut-stem and the cut-petiole inoculation methods, the plants are wounded prior to inoculation; this method is not be able to detect resistance due to preformed structural barriers. The oxalic acid assay is an indirect evaluation of resistance through measurement of stem response to oxalic acid. Most of these methods are labor intensive and the results can be inconsistent among the tests (2,6,12,18). The objective of this research was to develop a low-cost and high-efficiency inoculation method that can generate consistent results for greenhouse evaluation of soybean for resistance to Sclerotinia stem rot.

MATERIALS AND METHODS

Two inoculation methods, spray-mycelium method and drop-mycelium method, were tested and compared with a commonly accepted inoculation method, the cut-petiole method (3), in a greenhouse on the campus of Michigan State University, East Lansing. The experimental design was a randomized complete block design with three experiments, and 24 plants in each experiment were evaluated. The three experiments were conducted at three different times of year: April to May, May to June, and September to October.

Plant preparation. Eighteen soybean cultivars with different levels of susceptibility to *S. sclerotiorum* were chosen for this experiment (Table 1). Thirty seed per genotype per replication were planted in a greenhouse at Michigan State University in East Lansing. Seed were planted on 9 April, 14 May, and 18 September 2004 for the three replicated tests. Seed were planted in moist planting mix (Baccto High Porosity Professional Planting Mix, Houston, TX) in 20-by-20-cm plastic pots with five seeds per pot. The pots were kept in the greenhouse at a temperature of 18 to 25ºC, with illumination by natural sun light. Four uniform V3-stage plants from each pot were used for inoculation. Six pots or 24 plants per genotype were used for each treatment in each replication.

Isolate and inoculum preparation. The *S. sclerotiorum* isolate 105HT provided by Dr. Glen Hartman (Soybean Pathogen Collection Center at the United

Corresponding author: D. Wang
E-mail: wangdech@msu.edu

This research is partially funded by the National Sclerotinia Initiative.

Accepted for publication 5 July 2005.

DOI: 10.1094/PD-89-1268

States Department of Agriculture–Agricultural Research Service at the University of Illinois) was used for inoculations. The culture was maintained by subculturing every 3 weeks at room temperature on potato dextrose agar (PDA; Sigma P-2182, Sigma-Aldrich, St. Louis) medium and a new culture was started directly from sclerotia every 2 months. All the sclerotia used for starting the new cultures were collected from soybean plants infected with isolate 105HT in the greenhouse and were kept in a refrigerator at 4ºC before use. A single mycelia plug cut from the culture with a 6-mm-diameter cork-borer was placed in the center of a new plate with an approximately 8-mm-thick layer of PDA (39.0 g of PDA/liter) to produce inoculum for the cut-petiole inoculation method. Each seeded petri plate was sealed and incubated for 72 h at 22ºC with 10 h of light daily. Five mycelial plugs cut from the culture also were placed in a 2,000-ml flask containing 1 liter of potato dextrose broth (PDB; 24 g of PDB/liter; Sigma P-6685,) to produce liquid inoculum for the spray-mycelium method and the drop-mycelium method. The inoculated flask was cultured at room temperature (22ºC) for 5 days with shaking on a G10 GYROTORY shaker (Edison, NJ) at a speed of 200 rpm. Before inoculation, the mycelia were homogenized in the culture media by blending for 10 s with Hamilton Beach blender (Hamilton Beach/Proctor-Silex, Inc.) and the absorbance of the suspension was measured at 600 nm with a Gilford RESPONSE spectrophotometer. The optical density of the liquid inoculum was adjusted to an optical density at 600 nm (OD_{600}) value of 1.5 to 2.0 to obtain a uniform mycelium concentration among inocula prepared at different times.

Inoculation methods. In the two new methods, spray-mycelium method and drop-mycelium method, homogenized mycelium suspension was used to inoculate noninjured V3-stage soybean plants. In the spray-mycelium method, homogenized mycelium suspension was evenly sprayed on the leaves of soybean plants with a UIVA+ Micron Sprayer (Bromyard, UK) at a dosage of 2 liters of inoculum per 432 plants in one replication (average 4.6 ml/plant). In the drop-mycelium method, homogenized mycelium suspension was dropped onto the apical meristem of soybean plants at a dosage of approximately 1 ml/plant using a washer bottle or a pipette. A visible drop of the inoculum was retained on the apical meristem while a small portion of the inoculum dispersed downward along the main stem. During the inoculation with both the spray-mycelium and the drop-mycelium methods, the containers containing the mycelium suspension were shaken frequently to keep the mycelium evenly suspended.

In the cut-petiole method, mycelial plugs were used to inoculate injured V3-stage soybean plants. The mycelial plugs were prepared as described above. The inoculation procedure for the cut-petiole method was identical to that described by Del Rio et al. (3).

Inoculated plants were incubated in a plastic chamber with a relative humidity ranging from 60 to 100%. The plastic chamber was assembled on a greenhouse bench 1.2 m wide and 4.9 m long. Metal pipes 1.9 cm in diameter were used to build the frame of the chamber 1.2 m wide, 4.9 m long, and 1.2 m high. The chamber frame was attached to the greenhouse bench by bolts and nuts. A clear plastic sheet cut to appropriate size was put over the frame of the chamber and large binder clips (5 cm size) were used to hold the plastic sheets to the frames. The tops of the greenhouse bench were covered with Dewitt Weed Barrier fabric, which served as the bottom of the chamber. The humidity of the chamber was maintained with two Trion 707TW Centrifugal Atomizers (Attleboro, MA), one at each end of the chamber. A timer was used to turn the atomizers on for 1 min in every 5 min. No artificial illumination was provided in the greenhouse and inside the chamber. The chamber's temperature, relative humidity, and light intensity were monitored by Onset Greenline HOBO U-family Data Loggers & Devices (Bourne, MA). The plants were watered every 2 days.

Disease assessment, data collection, and analysis. Plants were examined for wilt beginning 3 days post inoculation. A plant was considered wilted when the apical meristem fell over and the leaves were flaccid. The number of wilted plants was recorded daily until the 14th day following inoculation. Area under the wilt progress curve (AUWPC) was used to characterize the rate of disease progress. The AUWPC was calculated using the following formula suggested by Shaner and Finney (14):

$$\sum_{i=1}^{n-1}\left[\left(y_i + y_{i+1}\right)/2\right]\left(t_{i+1} - t_i\right)$$

where y_i is the number of wilted plants at time t_i and n is the total number of observations. On the 14th day after inoculation, the total number of wilted plants was counted and plant mortality was calculated.

A single factor analysis of variances was conducted to test homogeneity of the experiment. The GLM procedure of SAS (SAS Institute, Cary, NC) was used to analyze the data collected in the green-

Table 1. Effect of *Sclerotinia sclerotiorum* on soybean plant mortality and area under the wilt progress curve (AUWPC) using three different inoculation methods[a]

	AUWPC			Plant mortality (%)		
Genotypes[b]	Cut-petiole	Spray-mycelium	Drop-mycelium	Cut-petiole	Spray-mycelium	Drop-mycelium
A2506	4.27 (6)	0.90 (1)	1.80 (2)	65 (7)	21 (1)	31 (2)
Chapman	3.60 (2)	1.60 (2)	2.13 (3)	52 (2)	36 (2)	36 (3)
Kenwood 94	4.10 (5)	1.77 (3)	4.13 (6)	60 (5)	36 (2)	67 (7)
NKS 19-90	3.03 (1)	2.00 (6)	2.37 (4)	44 (1)	39 (4)	39 (4)
Corsoy 79	4.27 (6)	1.80 (5)	4.77 (12)	58 (4)	40 (5)	75 (11)
BSR 101	4.00 (4)	1.80 (4)	1.43 (1)	63 (6)	46 (6)	28 (1)
Hardin 91	4.90 (8)	2.87 (9)	4.77 (12)	69 (8)	53 (7)	78 (12)
Vinton 81	4.90 (8)	2.53 (8)	5.30 (15)	77 (11)	54 (8)	83 (14)
Colfax	3.97 (3)	2.37 (7)	3.57 (5)	55 (3)	56 (9)	61 (5)
Conrad 94	5.90 (12)	3.63 (16)	4.33 (8)	81 (13)	58 (10)	72 (9)
Dunbar	5.20 (11)	3.27 (12)	4.37 (9)	73 (9)	61 (11)	64 (6)
Elgin 87	6.27 (16)	3.13 (10)	4.13 (6)	79 (12)	61 (11)	72 (9)
Jack	6.00 (14)	3.13 (10)	4.63 (11)	82 (14)	62 (13)	69 (8)
Olympus	6.10 (15)	3.30 (14)	5.13 (14)	83 (16)	62 (13)	83 (14)
Resnik	6.43 (17)	3.27 (12)	5.53 (17)	87 (17)	63 (15)	89 (17)
Ciba 3253	7.77 (18)	3.30 (14)	4.57 (10)	93 (18)	65 (16)	81 (13)
Felix	5.17 (10)	3.90 (17)	5.37 (16)	74 (10)	67 (17)	83 (14)
Fairbault	5.93 (13)	4.17 (18)	6.27 (18)	82 (14)	68 (18)	95 (18)
$LSD_{0.05}$	3.33	1.66	2.26	28	21	34

[a] Data from the three experiments were combined for analysis; numbers in parentheses indicate the ranking, with 1 being the most resistant.

[b] Genotypes are listed in the order of ranking based on plant mortality with the spray-mycelium method. NKS 19-90 is a resistant check and Resnik is a susceptible check. LSD = least significant difference.

house. Fisher's least significant difference (LSD) at 5% significant level was used to test the differences among genotypes. Cultivars were ranked according to their means of plant mortality and AUPWC and correlation coefficients were calculated from the ranks by the CORR procedure of SAS.

RESULTS

The temperature inside the plastic chamber was not controlled and it ranged from 20 to 38ºC. Light intensity inside the chamber was not controlled, either, and the daytime light intensity averaged 5,824, 4,798, and 7,954 lux/m^2 for the April, May, and September tests, respectively. The humidity inside the chamber was increased intentionally with two large-capacity atomizers throughout the experiment; the humidity ranged from 60 to 100% inside the chamber. The leaf surface of the plants inside the chamber was wet throughout the experiment.

All infected plants showed typical symptoms of Sclerotinia stem rot, with white fluffy mycelia clearly visible on leaves, apical meristems, and the main stems by 10 days after inoculation. The symptoms first appeared 4, 5, and 6 days after inoculation for the spray-mycelia, drop-mycelium, and cut-petiole methods, respectively. On plants inoculated with the cut-petiole method, lesions reached the main stem from the cut petiole and caused wilting when the lesions expanded transversely through the whole diameter of the main stem. On plants inoculated with the spray-mycelia method, the first symptom of Sclerotinia stem rot was gray or light brown spots scattered on the leaves (Fig. 1A). Some of the spots had white mycelia growing on the margin of the spot and others had necrotic spots. The petioles then turned flaccid and the infected leaves either fell off the plant or remained on the main stem. The main stems were affected either by mycelia growing through petioles or secondary infection via infected leaves touching the stem (Fig. 1B). On plants inoculated with the drop-mycelium method, the lesion developed directly downward from the apical meristem where homogenized mycelia were dropped (Fig. 1C). When the margin of lesion reached the first top node, the plant started wilting.

The results of analysis of variance revealed that there was a significant genotype effect, but that the experiment–genotype effect was not significant. Therefore, the data from the three experiments were combined in the final analysis. Significant differences ($P < 0.05$) in plant mortality and AUWPC were found among the 18 genotypes (Table 1). AUWPC ranged from 3.03 (NKS 19-90) to 7.77 (Ciba 3253), 0.90 (A2506) to 4.17 (Fairbault), and 1.43 (BSR 101) to 6.27 (Fairbault) for cut-petiole, spray-mycelium, and drop-mycelium methods, respectively. Plant mortality ranged from 44 (NKS 19-90) to 93% (Ciba 3253), 21 (A2506) to 68% (Fairbault), and 28 (BSR 101) to 95% (Fairbault) for cut-petiole, spray-mycelium, and drop-mycelium methods, respectively (Table 1). The rank correlation coefficients between AUWPC and plant mortality among the three inoculation methods ranged from 0.95 to 0.99 ($P < 0.01$). Correlation coefficients between the plant mortalities or AUPWCs obtained

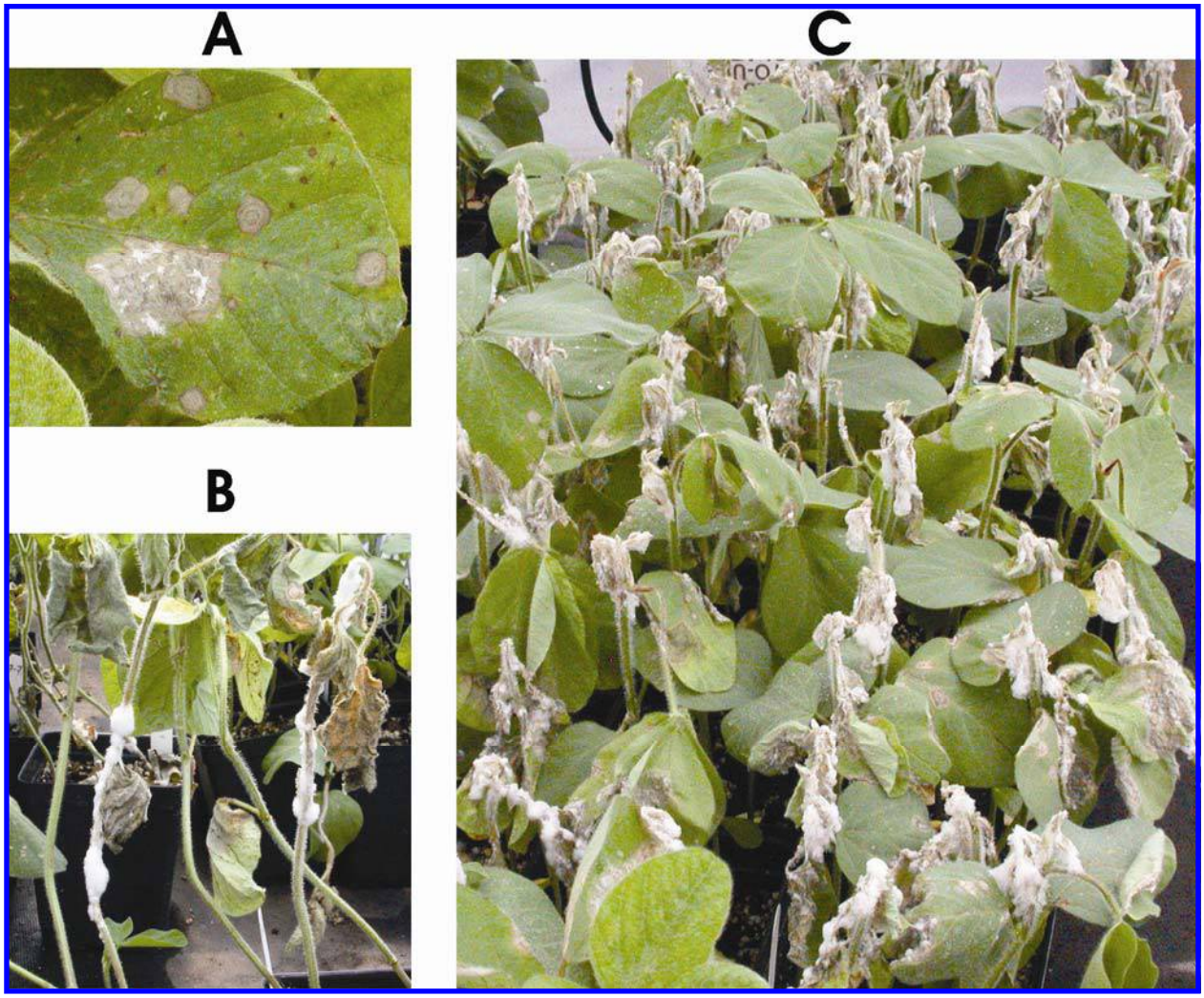

Fig. 1. A, Early symptom of Sclerotinia stem rot on leaves of soybean plants inoculated with the spray-mycelium method (7 days after inoculation). **B,** Secondary infection of the main stems caused by infected leaves that came into contact with the main stem on plants inoculated with the spray-mycelium method (14 days after inoculation). **C,** Typical symptom of Sclerotinia stem rot on plants inoculated with the drop-mycelium method (9 days after inoculation).

with the three inoculation methods were between 0.63 and 0.81 ($P < 0.01$; Table 2). NKS 19-90 (resistant check), BSR 101, and A2506 had consistently lower plant mortality and AUPWC, whereas Resnik (susceptible check), Olympus, and Ciba 3253 had consistently higher plant mortality and AUPWC no matter which inoculation method was used in the evaluation.

DISCUSSION

Infection of soybean plants by *S. sclerotiorum* is affected by many factors such as temperature, light intensity, and humidity (12,18). In this study, the temperature and light intensity were not controlled, but the humidity was intentionally increased. Our success in obtaining high plant mortalities for susceptible genotypes in all three tests indicated that humidity might have played a more important role than other factors in the infection of soybean plants by *S. sclerotiorum*.

Another factor that may affect the infection of soybean plants by *S. sclerotiorum* is the amount of inoculum applied to the plants. No significant differences were observed in the results of the three experiments with both the spray-mycelium and drop-mycelium methods, suggesting that the difference in the amount of inoculum applied to the plants in the three experiments was too small to cause significant difference in fungus infection.

The high correlation between the plant mortalities or AUPWCs obtained with the two new inoculation methods and the plant mortalities or AUPWCs obtained with the cut-petiole method indicated that spray-mycelium and drop-mycelium inoculation methods can substitute for the cut-petiole inoculation method. The major advantage of these two new methods is that they require less time in inoculation and are less expensive than the cut-petiole method in terms of materials. The time needed for a person to inoculate the 18 genotypes with 24 plants per genotype was approximately 0.3, 0.8, and 2.0 h with the spray-mycelium, drop-mycelium, and cut-petiole methods, respectively, in our laboratory. The cost of materials for inoculating the 18 genotypes with 24 plants each was approximately $8, $2, and $22 with the spray-mycelium, drop-mycelium, and cut-petiole methods, respectively. Another advantage of these two new methods is that no wounds to the plants are induced prior to the inoculation. Therefore, resistance due to preformed structural barriers to infection by the fungus can be evaluated with these two new methods.

The spray-mycelium method used about four times more liquid inocula than the drop-mycelium method. When plants were inoculated with the spray-mycelium method, most of the infection started from the leaves. The infection either extended to the main stem or the infected leaves fell and caused secondary infections to the tissue they touched (Fig. 1B). When plants were inoculated with the drop-mycelium method, infection started from the top of the main stem and moved down along the main stem (Fig. 1C); thus, secondary infections were much less frequent than with the spray-mycelium method. Therefore, the drop-mycelium method should be used if secondary infection is undesirable. When a high disease pressure is needed, such as in the case of large-scale screening of germ plasm for resistance, the spray-mycelium method is recommended.

The drop-mycelium method is similar to the cut-stem method (17) in that both methods use the top of the main stem as the inoculation point. One key difference between the two methods is that the cut-stem method wounds the inoculated plants prior to inoculation whereas the drop-mycelium method does not. With the cut-stem method, the response is measured by the lesion length on the main stem, whereas plant mortalities or AUWPC are used with the drop-mycelium method. However, measurement of lesion length on the main stem can be adopted with the drop-mycelium method. Our preliminary study in comparison of the drop-mycelium method with the cut-stem method showed that correlation coefficients between these two methods were 0.64 ($P < 0.01$) and 0.63 ($P < 0.01$) for the lesion length and the plant mortality, respectively (*unpublished data*).

To obtain value for the plant mortality, data need to be collected only at 14 days after inoculation. However, data need to be collected daily from the third day after inoculation until 14 days after inoculation in order to obtain value for the AUWPC. Our results showed that the plant mortality and the AUWPC obtained with the three inoculation methods (cut-petiole, spray-mycelium, and drop-mycelium) were highly correlated with correlation coefficients of 0.63 to 0.81 ($P < 0.01$; Table 2). Therefore, the plant mortality is recommended as the measurement for the plant responses to the disease with both the spray-mycelium method and the drop-mycelium method.

It has been reported that the correlations of Sclerotinia stem rot ratings in soybean between different tests are low (12). In our study, the analysis of variance revealed that there was no significant difference among the three tests conducted at different times with either the spray-mycelium method or the drop-mycelium method, indicating that consistent results can be obtained with both methods. The results obtained with the cut-petiole method also were consistent among the three tests in our study. The plants inoculated with the cut-petiole method were kept together with plants inoculated with other methods in the chamber with high humidity in this study. The high humidity might have helped us obtain consistent results among the different tests.

BSR 101 was included in this study because it was reported to be susceptible to Sclerotinia stem rot (6). However, this cultivar showed partial resistance in our study. Wegulo et al. (19) also found that BSR 101 had partial resistance to Sclerotinia stem rot in greenhouse tests and field trails.

ACKNOWLEDGMENTS

We thank C. R. Grau and R. Hammerschmidt for their critical reviews and comments on the manuscript and B. W. Diers for supplying the 18 soybean genotypes used in this study.

LITERATURE CITED

1. Boland, G. J., and Hall, R. 1987. Evaluating soybean cultivars for resistance to *Sclerotinia sclerotiorum* under field conditions. Plant Dis. 71:934-936.
2. Chun, D., Kao, L. B., Lockwood, J. L., and Isleib, T. G. 1987. Laboratory and field assessment of resistance in soybean to stem rot caused by *Sclerotinia sclerotiorum.* Plant Dis. 71:811-815.
3. Del Rio, L. E., Kurtzweil, N. C., and Grau, C. R. 2001. Petiole inoculation as a tool to screen soybean germ plasm for resistance to *Sclerotinia sclerotiorum*. (Abstr.) Phytopathology 91:S176.

Table 2. Rank correlation coefficients among the data obtained with the three inoculation methods[a]

	AUWPC			Plant mortality (%)		
Methods	**Cut-petiole**	**Spray-mycelium**	**Drop-mycelium**	**Cut-petiole**	**Spray-mycelium**	**Drop-mycelium**
AUWPC						
Cut-petiole	1.00	…	…	…	…	…
Spray-mycelium	0.76**	1.00	…	…	…	…
Drop-mycelium	0.63**	0.72**	1.00	…	…	…
Plant mortality (%)						
Cut-petiole	0.96**	0.78**	0.65**	1.00	…	…
Spray-mycelium	0.81**	0.93**	0.73**	0.81**	1.00	…
Drop-mycelium	0.70**	0.76**	0.96**	0.71**	0.77**	1.00

[a] ** = Significant at the 0.01 probability level.

4. Grau, C. R., and Bissonette, H. L. 1974. Whetzelinia stem rot of soybean in Minnesota. Plant Dis. Rep. 58:693-695.
5. Grau, C. R., Radke, V. L., and Gillespie, F.L. 1982. Resistance of soybean cultivars to *Sclerotinia sclerotiorum*. Plant Dis. 66:506-508.
6. Hoffman, D. D., Diers, B. W., Hartman, G. L., Nickell, C. D., Nelson, R. L., Pedersen, W. L., Cober, E. R., Dorrance, A. E., Graef, G. L., Steadman, J. R., Grau, C. R., Nelson, B. D., Del Rio, L. E., Helms, T., Poysa, V., Rajcan, I., and Stienstra, W. C. 2002. Selected soybean plant introductions with partial resistance to *Sclerotinia sclerotiorum.* Plant Dis. 86:971-980.
7. Kim, H. S., Hartman, G. L., Manandhar, J. B., Graef, G. L., Steadman, J. R., and Diers. B. W. 2000. Reaction of soybean cultivars to Sclerotinia stem rot in field, greenhouse, and laboratory evaluations. Crop Sci. 40:665-669.
8. Kolkman, J. M., and Kelly, J. D. 2000. An indirect test using oxalate to determine physiological resistance to white mold in common bean. Crop Sci. 40:281-285.
9. Kull, L. S., Vuong, T. D., Powers, K. S., Eskridge, K. M., Steadman, J. R., and G. L. Hartman, G. L. 2003. Evaluation of three resistance screening methods using six *Sclerotinia sclerotiorum* isolates and three entries of each soybean and dry bean. Plant Dis. 87:1471-1476.
10. Leone, G., and Tonneijck, A. E. G. 1990. A rapid procedure for screening the resistance of bean cultivars (*Phaseolus vulgaris* L.) to *Botrytis cinerea* and *Sclerotinia sclerotiorum.* Euphytica 48:87-90.
11. Miklas, P. N., Grafton, K. F., and Nelson, B. D. 1992. Screening for partial physiological resistance to white mold in dry bean using excised stems. J. Am. Soc. Hortic. Sci. 117:321-327.
12. Nelson, B. D., Helms, T. C., and Olson, M. A. 1991. Comparison of laboratory and field evaluations of resistance in soybean to *Sclerotinia sclerotiorum.* Plant Dis. 75:662-665.
13. Noyes, R. D., and Hancock, J. G. 1981. Role of oxalic acid in the *Sclerotinia sclerotiorum* wilt of sunflower. Physiol. Plant Pathol. 18:123-132.
14. Shaner E., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow mildewing in Knox wheat. Phytopathology. 67:1051-1056.
15. Steadman, J., Eskridge, K., Costa, J., Grafton, K., Kelly, J., Kmiecik, K., Kolkman, J., Myers, J., and Miklas, P. 2001. Evaluation of sources of resistance to *Sclerotinia sclerotiorum* in common bean with five test methods at multiple locations. Annu. Rep. Bean Improv. Coop. 44:89-90.
16. Tu, J. C. 1985. Tolerance of white bean (*Phaseolus vulgaris*) to white mold (*Sclerotinia sclerotiorum*) associated with tolerance to oxalic acid. Physiol. Plant Pathol. 26:111-117.
17. Vuong, T. D., Hoffman, D. D., Diers, B. W., Miller, J. K., Steadman, J. R., and Hartman, G. L. 2004. Utilization of the cut stem inoculation method to evaluate soybean, dry bean, and sunflower for resistance to *Sclerotinia sclerotiorum*. Crop Sci. 44:777-783.
18. Wegulo, S. N., Yang, X. B., and Martinson, C. A. 1998. Soybean cultivar responses to *Sclerotinia sclerotiorum* in field and controlled environment studies. Plant Dis. 82:1264-1270.
19. Wrather, J. A., and Koenning, S. 2005. Soybean disease loss estimates for the United States, 1996-2004. Missouri Agricultural Experiment Station, Delta Research Center. Online publication.