Evaluation of Resistance Screening Methods for Sclerotinia Stem Rot of Soybean and Dry Bean

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ABSTRACT

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Three methods to identify levels of resistance to *Sclerotinia sclerotiorum* in soybean (*Glycine max*) and dry bean (*Phaseolus vulgaris*) were compared using multiple data analyses. The three methods were mycelial plug inoculations of cotyledons, cut stems, and detached leaves. Six *S. sclerotiorum* isolates of known relative aggressiveness were inoculated on each of three soybean and dry bean cultivars with varied response to *S. sclerotiorum*. For soybean, all three inoculation methods accurately identified isolate aggressiveness irrespective of cultivar, but identification of susceptible and partially resistant soybean cultivars was influenced by isolate. For dry bean, the cotyledon and cut stem methods accurately identified isolate aggressiveness, but identification of susceptible and partially resistant dry bean cultivars was influenced by isolate and inoculation method. The cut stem method had the smallest coefficient of variation and was more precise for detecting interactions. When considering root mean square residual error combined over species and experiments, coefficient of variation based on residual error, significance of isolate-by-cultivar interaction from ANOVA, rank correlation between pairs of methods, and sensitivity ratio for the three resistance screening methods under controlled environmental conditions, the cut stem method was statistically better than the cotyledon and detached leaf methods for evaluating resistance in soybean and dry bean cultivars.

Additional keywords: germ plasm screening, isolate variability, multiple data analysis, white mold

The fungal phytopathogen *Sclerotinia sclerotiorum* (Lib.) de Bary has a wide geographic distribution and a diverse host range including many agronomic crops (2). The pathogen can cause major limitations to soybean (*Glycine max*) and dry bean (*Phaseolus vulgaris*) production. On soybean, *S. sclerotiorum* causes Sclerotinia stem rot (8) and was shown to be an important disease in parts of Illinois (9). Sclerotinia stem rot is a yield-limiting disease (11), with high levels of resistance difficult to find in most hosts, including soybean and dry bean. Extensive field evaluations to assess soybean resistance to *S. sclerotiorum* have been reported (10,13,14), but various inoculation techniques and controlled-environment screening methods have not consistently predicted field reactions to *S. sclerotiorum* on soybean (13,18,24) and dry bean (22). This may, in part, be due to variability in isolate aggressiveness (3,16).

Most inoculation techniques use a mycelial-infested substrate (agar plug, carrot, celery, and oat) instead of ascospores. Ascospores are a common form of primary inocula that germinate and colonize flower petals, the primary infection site of many hosts including soybean and dry bean. Ascospores can be difficult to manipulate and produce in vitro, and inoculation with ascospores in laboratory and greenhouse environments has resulted in sporadic infection (5). Therefore, ascospores have not been extensively used as an inoculation technique to evaluate resistance in soybean.

Some investigators have used limited term inoculation methods with various modifications. Limited term methods utilize a mycelial-infested medium that is inoculated onto the plant and then removed from the plant after a specified time. Using intact soybean plants, Cline and Jacobson (5) compared two limited term inoculation techniques in which either mycelial-infested celery was placed in the second or third node of soybean plants or colonized carrot was placed onto center leaflets of growth stage V4-V5 (6) plants. Hunter et al. (12) used mycelial-infested celery inoculated onto dry bean stems, and disease data of dry bean cultivars agreed with other greenhouse and field observations. Boland and Hall (1) employed a limited term inoculation technique to evaluate soybean resistance and did not find a significant correlation with field results. Alternatively, the limited term inoculation technique using mycelial plug inoculated soybean cotyledons was significantly correlated with field disease severity index at one of two field locations (13).

Researchers have compared results of several controlled environment evaluation methods for resistance in soybean (4,18,24). In each paper, excised stems were inoculated with *S. sclerotiorum* mycelia, and stem lesion lengths were measured. All reports indicated that screening results were not consistently correlated with field performance, and that repeated experiments were frequently inconsistent. The detached (excised) leaf inoculation method has shown significant correlations with field performance in two reports (4,13), but only one test out of five was correlated with field performance. Additionally, Wegulo et al. (24) conducted two different screening methods based on stem response to oxalic acid, a pathogenicity determinant (7), and reported that results were repeatable between experiments and correlated with field performance.

Steadman et al. (22) tested 11 common bean genotypes for putative resistance to *S. sclerotiorum* at seven sites in North and South America using both field tests and various laboratory and greenhouse screening methods including the straw test, an oxalate test, and detached leaf methods. The field tests and greenhouse straw tests were highly associated by Spearman's rank correlation. One detached leaf and one

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oxalate test were included, but neither correlated with the field results.

The emphasis in development of screening techniques has been placed on consistency to identify partial resistance and on repeatable high correlations between the screening method results and field disease ratings. Little emphasis has been placed on types of statistical approaches to detect cultivar differences, to reveal cultivar-by-isolate interactions, or to compare the precision of different inoculation techniques to separate resistant and susceptible hosts. To promote correct greenhouse assessment of host resistance and test whether cultivar-by-isolate interactions occur, three screening methods were compared, including the cotyledon inoculation technique, the cut stem method, and the detached (excised) leaf test. The cut stem method is an advantageous protocol allowing nondestructive, repeatable testing for resistance (23). The cotyledon inoculation technique and detached leaf test are rapid testing protocols that produce repeatable results. The detached leaf test is nondestructive and allows testing of any plant whether grown in the field or under controlled environment conditions. Previously characterized *S. sclerotiorum* isolates and cultivars with consistently observed field performance to *S. sclerotiorum* were selected. Six isolates of known relative aggressiveness and three dry bean and three soybean cultivars that varied in their response (susceptible, moderately susceptible, and partially resistant) to *S. sclerotiorum* were utilized in this study. The objectives were (i) to compare efficacy of resistance screening methods to identify resistant and susceptible soybean and dry bean cultivars, (ii) to ascertain the effectiveness and sensitivity of screening methods to consistently indicate levels of isolate aggressiveness on hosts, and (iii) to determine if a cultivar-by-isolate interaction exists.

MATERIALS AND METHODS

***S. sclerotiorum* isolates.** Six isolates were selected based on previously determined aggressiveness reactions on either soybean or dry bean hosts (Table 1). Isolates 3, 105, and 110 were selected from the *S. sclerotiorum* pathogen collection maintained at the National Soybean Research Center at the University of Illinois in Urbana. Isolates 143, 277, and 279 were selected from the *S. sclerotiorum* collection at the University of Nebraska, Lincoln. All isolates were hyphal-tipped and maintained as pure mycelial cultures on potato dextrose agar (PDA) at 4°C. Prior to inoculations for each experimental run, all mycelial cultures were transferred from storage onto new PDA plates and incubated in the dark at 20 ± 2°C to allow renewed growth. From these actively growing cultures, mycelial plugs were removed from the advancing mycelial edge and used to set up the inoculum culture plates that were incubated in the dark at 20 ± 2°C for 24 h just prior to plant inoculations. The aggressiveness of isolates 3, 105, and 110 was initially determined using the cotyledon inoculation method on soybeans and was confirmed by the cut stem method on soybeans. Relative aggressiveness of isolates 143, 277, and 279 was determined using the detached leaf method on dry beans.

Soybean and dry bean cultivars. Three cultivars of soybean and three cultivars of dry bean were selected based on reported severity of field symptoms initiated by ascospores of *S. sclerotiorum*. Based on field evaluations, soybean cultivars Williams 82, Bell, and NKS19-90 were reported to be susceptible (10,24), moderately susceptible (24), and partially resistant (14,24), respectively; and dry bean cultivars Beryl, PC-50, and B7354 were reported (22) to be susceptible, moderately susceptible, and partially resistant, respectively.

Cotyledon inoculation method. Soybeans and dry beans were grown in a soil:sand mix (1:1) in planting trays (27 × 54 × 8 cm) in the greenhouse under a 16-h photoperiod and watered daily. Each tray was divided into 12 rows with four plants per row. Dry beans were planted 3 days after soybeans in order to synchronize trifoliolate expansion of both hosts and to allow for the completion of the experiment prior to cotyledon abscission by dry bean plants. Both soybean and dry bean plants were inoculated when the first trifoliolate was fully expanded on soybean plants. A randomized complete block design with three replications and four plants per replication was used for each species. The experiment was completed twice.

Mycelial cultures were established from stored stock cultures as previously described. To inoculate plants, 3 mm^2 mycelial plugs were removed from the advancing mycelial edge of newly produced 24-h colony grown on PDA and singly placed mycelia-side down on one cotyledon adjacent to and touching the stem. Inoculated plants were hand-misted with water, incubated in the dark in dew chambers (Percival, Boone, Iowa) maintained at 20°C ambient temperature (13°C wall and 34°C water temperature) for 24 h, transferred to a greenhouse bench, and maintained under two layers of 80% filtration greenhouse shade cloth (Hummert International, Earth City, MO) in an air-conditioned greenhouse room at 20 ± 2°C. Approximately 24 h after trays were transferred from dew chambers, the number of dead plants per row was recorded twice per day for 2 to 3 days, and the area under disease progress curve (AUDPC) (19) was calculated.

Cut stem method. Seeds of soybean and dry bean were germinated in 15-cm clay pots containing an equal mixture (1:1:1) of soil:perlite:torpedo sand. Each entry was planted in three replicate pots placed in a greenhouse at 27 ± 1°C and 16-h day length. To promote plant uniformity, soybean seedlings were thinned to five plants per pot and dry bean seedlings were thinned to three plants per pot. The experiment was a split plot in a randomized complete block with three replications. Each pot was an experimental unit. Isolates were the main plot and cultivars were randomized within isolates as a split plot. The experiment was completed twice.

Mycelial cultures were established from stored stock cultures as previously described. Mycelial plugs (3 mm^2) were cut from the margin of actively growing mycelial colonies and used to inoculate plants. Main stems of 5-week-old plants (fifth to sixth trifoliolate leaf fully expanded) were horizontally severed with a sterile razor blade 0.5 cm above either the fourth or fifth node. A single mycelial plug was placed mycelial-side down on the cut stem. Inoculated plants were incubated in a mist chamber with the relative humidity maintained over 80%. The chamber was maintained at 20 ± 1°C and covered with black mesh cloth (80% light reduction). After 24 h, infected plants were transferred to an adjacent room at 25 ± 1°C and disease symptoms were allowed to develop. Disease development was observed, and lesion length (cm) on the main stem was measured 14 days after inoculation.

Detached leaf method. Four seeds of each soybean and dry bean entry were germinated in 13-cm-diameter pots containing an equal mixture (1:1:1:1) of peat:soil:sand:vermiculite and grown under greenhouse conditions of 27 ± 1.5°C (day) and 25 ± 1.5°C (night) for a 16 h day length. Each entry was planted in six replicate pots and arranged in an α-lattice de-

Table 1. *Sclerotinia sclerotiorum* isolates, relative aggressiveness, host, location, and source

Isolate	Relative aggressiveness[x]	Host[y]	Location[z]	Source
3	Intermediate	Soybean	North Carolina	D. Shew
105	High	Soybean	Iowa	G. Cook
110	Low	Pear	Oregon	R. A. Spotts
143	Intermediate	Soybean	Colorado	J. R. Steadman
277	Low	Sunflower	Great Britain	J. R. Steadman
279	High	Pinto	North Carolina	J. R. Steadman

[x] Aggressiveness of isolates 3, 105, and 110 was determined using the cotyledon inoculation method on soybean. Aggressiveness of isolates 143, 277, and 279 was determined using the detached leaf method on dry bean.
[y] Host from which each isolate was taken.
[z] Location from which each isolate was originally isolated.

sign with four adjacent pots as incomplete blocks. Planting dates of dry bean and soybean were staggered so leaf cuttings could be made at the same time for both hosts.

The youngest fully expanded trifoliolate leaves of 3-week-old dry beans and 4-week-old soybeans were cut at the stem, placed in a labeled, moistened paper towel, bagged, and transported to the laboratory. Four trifoliolates were labeled and assigned to aluminum pans as incomplete blocks according to the same α-lattice design of the plants in the greenhouse with 36 treatments (six cultivars × six isolates), four units per incomplete block, and two complete replicates per experiment. Four folded paper towels were placed in the bottom of each aluminum pan (26 × 46 cm and 8 cm deep). Four glass petri dishes were placed upside down in each pan on towels to serve as platforms for detached leaves. Orchid tubes (polypropylene plastic tubes and plastic caps) were filled with tap water, capped, and placed in pans with one tube placed under each petri dish. Petioles were pushed through the orchid tube cap until the cut end reached the water. Four trifoliolates and orchid tubes were placed per pan with the middle leaf of each trifoliolate positioned on the petri dish. If the leaf did not stay flat, labeling tape was used to hold it down.

Mycelial cultures were established from stored stock cultures as previously described. Using aseptic technique, 8 mm^2 plugs were cut 1 cm back from the advancing margin of mycelial growth on a 48-h-old PDA culture maintained in the dark at 20 ± 2°C. Mycelial plugs were placed fungus-side down centered on one side of the middle trifoliolate leaf between the main leaf vein and the leaf edge and gently pressed to ensure good contact with the leaf surface. To each pan, 300 ml of tap water was added, and each pan was wrapped with plastic wrap to maintain humidity. Pans containing inoculated leaves were incubated on a lab bench and maintained at 20 ± 2°C. After 48 h, both lesion length and width were measured. If the lesion reached the edge of the leaf, the radius from the center of the plug to the edge of the lesion was measured and doubled to estimate the lesion diameter. The lesion length and width were used to calculate the lesion area of an ellipse in square centimeters. The experiment was completed twice.

Data analysis. For the cotyledon inoculation method, an analysis of variance (ANOVA) for combined randomized complete block experiments was used to evaluate cultivar-by-isolate interactions, to compare cultivars within isolates, and to evaluate isolate main effects. There was an experiment-by-cultivar and isolate interaction that primarily was due to a change in magnitude and not rank, so experiments were combined. Means were compared by least significant differences (LSD) at $P = 0.05$. PROC GLM (SAS Release Version 8.0, SAS Institute, Cary, NC) was used for calculations.

For the cut stem method, an ANOVA for the combined split plot experiments was conducted to evaluate cultivar-by-isolate interactions, to compare cultivars within isolates, and to compare isolate main effects. There was an experiment-by-cultivar and isolate interaction that primarily was due to a change in magnitude and not rank, so experiments were combined. Means were compared using the least significant differences as previously described. PROC GLM (SAS Release Version 8.0) was used for calculations.

For the detached leaf method, an ANOVA was conducted using PROC MIXED (SAS Version 8.0) with incomplete blocks as random effects. The interaction between the cultivar and isolate factors was determined, and differences between adjusted treatment means were tested using the lsmeans statement and the pdiff option.

The three screening methods to identify resistant cultivars were compared for both soybean and dry bean hosts using the root mean square residual error from each method's ANOVA combined over host species and experiments, the coefficient of variation based on residual error, the significance of the isolate-by-cultivar interaction from the ANOVA, the rank correlation between pairs of methods based on the 36 isolate-by-cultivar means, and the sensitivity ratio (17).

To develop the sensitivity ratio, assume a method M is used to estimate a particular property Q where M is a function of Q, M(Q). Q is defined as $(dM/dQ)/\sigma_M$, where Q = M(Q). Similar results will hold for another method N used to measure Q. Using results for calculus, it can be shown that the sensitivity ratio for the two methods M and N is:

$$SR(M/N) = |dM/dN|/(\sigma_M/\sigma_N)$$

where the relationship between the methods is dM/dN.

We assumed a linear relationship between any two (M and N) of the three methods and that dM/dN was adequately estimated by the slope of a simple linear regression of the means of M regressed on the means of N. We also used the ANOVA root mean square residual error of a method as an estimate of the method's standard deviation. The rank correlations and the sensitivity ratios were estimated by host (soybean and dry bean) for 36 isolate-by-cultivar combinations.

RESULTS

Soybean. The initial disease symptoms were leaf wilting on Williams 82 seedlings 24 h after inoculating the cotyledons with isolates 105, 143, and 279. Wilted plants died the following day. A significant ($P = 0.04$) isolate-by-cultivar interaction was detected (Table 2). Isolates 3, 105, 110, and 277 did not differ among the three cultivars, while isolates 143 and 279 discriminated resistant NKS19-90 from susceptible Williams 82. Differences in AUDPC values for resistant and susceptible cultivars were greatest when inoculated with isolate 279. AUDPC isolate means differed ($P \leq 0.05$) among the six isolates (Table 3). Comparing relative aggressiveness among isolates inoculated onto cotyledons, 105 was the most aggressive, 279 and 143 were intermediately aggressive, and isolates 3, 110, and 277 were the least aggressive.

Cut stem-inoculated plants showed typical water-soaked symptoms of Sclerotinia stem rot 3 days after inoculation. Water-soaked lesions were visible from the point of inoculation downward. When the margins of lesions reached stem nodes, leaves wilted and died the next day. A significant ($P = 0.01$) isolate-by-cultivar interaction was detected (Tables 2 and 4). Comparing disease response of soybean cultivars, five of six isolates differed ($P < 0.001$) in lesion lengths. Isolate 277 did not distinguish resistant and susceptible cultivars (Table 2). The differences in lesion lengths on susceptible versus resistant cultivars were greatest when inoculated with isolates 3, 143, and 279. Lesion lengths were not different between NKS19-90 and Williams 82 when inoculated with weakly aggressive isolate 277 or with highly aggressive isolate 105 (Table 2). Isolate mean lesion lengths differed ($P \leq 0.05$) (Table 3). Of these, isolates 105 and 279 caused the greatest average lesion lengths, 11.8 and 11.0 cm, respectively; while isolate 277 caused the smallest average lesion length of 6.4 cm. The three remaining isolates (3, 110, and 143) produced lesion lengths ranging from 7.9 to 10.3 cm (Table 3).

After 24 h, lesions on detached leaves became visible under the plug as water-soaking and leaf necrosis, which expanded out from the plug after 36 h. At 48 h, the water-soaking and necrotic regions reached the leaf margin in some leaves. Partially resistant and susceptible cultivars did not differ in response to the six *S. sclerotiorum* isolates (Table 2). Isolate mean lesion area values differed ($P \leq 0.05$) (Table 3). Comparing relative aggressiveness among isolates inoculated onto detached leaves, isolates 105 and 279 were the most aggressive, 3 and 10 were intermediately aggressive, and 143 and 277 were the least aggressive.

Dry bean. Symptoms were first observed 24 h after inoculation of cotyledons as the loss of stem integrity and an abrupt bend in the stem at the point of inoculation. This loss of stem integrity was first observed with isolate 105 on Beryl and PC-50. Dry bean cultivars did not differ in response to the six *S. sclerotiorum* isolates (Table 2). AUDPC isolate mean values differed ($P \leq 0.05$) among the six isolates

(Table 3). Comparing relative aggressiveness among isolates, 105 was the most aggressive, 143 and 279 were intermediately aggressive, and 3, 110, and 277 were the least aggressive (Table 3).

Cut stem-inoculated plants had water-soaked stem lesions similar to the symptoms observed in soybean. However, overall stem lesion lengths were less than in soybean. Differences ($P \leq 0.05$) in lesion lengths between cultivars were observed within all isolates tested (Table 2). Partially resistant and susceptible cultivars were accurately distinguished by all isolates (Table 2). The differences in lesion length values for partially resistant versus susceptible cultivars were greatest when inoculated with isolate 110 and least when inoculated with 105 (Table 2). Isolate lesion lengths differed ($P \leq 0.05$) among *S. sclerotiorum* isolates (Table 3). Isolates 143 and 105 caused the longest lesion lengths, 4.6 and 4.5 cm, respectively. Isolate 277 produced the shortest lesion length, 2.5 cm.

Symptoms of detached leaves progressed in the same manner and followed approximately the same time line as on soybean leaves, except the lesion areas and regions of water-soaking were larger on the larger leaf of the dry bean. With respect to disease response of dry bean cultivars inoculated with each isolate, differences ($P \leq 0.05$) in leaf lesion areas were observed with isolates 3, 105, 110, and 279 (Table 2). Partially resistant (PC-50) and susceptible (Beryl) cultivars were not distinguished by isolates 3, 105, and 279, and the intermediately resistant cultivar (B7354) appeared more resistant when inoculated with isolates 3, 105, and 279. Isolate leaf lesion areas differed ($P \leq 0.05$) among *S. sclerotiorum* isolates (Table 3). Isolate 279 caused the greatest leaf lesion area (11.2 cm^2), and isolates 143 and 277 produced the smallest lesion areas (5.2 and 4.9 cm^2, respectively).

Comparisons of the three screening methods and the isolate-by-cultivar interactions. Comparing the root mean square errors and coefficient of variation from lesser to greater values, respectively, the methods were ordered as (i) the cut stem method, (ii) the detached leaf method, and (iii) the cotyledon inoculation method (Table 4). Isolate-by-cultivar interaction within both host species was highly ($P = 0.01$) significant for the cut stem method, but was not significant for either of the other methods (Table 4).

Rank correlation computed for soybean and dry bean showed how closely the screening methods ranked isolate-by-cultivar combinations (Table 4). In soybean, the cut stem method and the detached leaf method had the highest rank correlations ($r_s = 0.76$, $P < 0.01$), while the cut stem and cotyledon methods were nearly as strongly correlated ($r_s = 0.54$, $P \leq 0.05$). In dry bean, the cut stem and cotyledon inoculation methods were moderately correlated ($r_s = 0.54$, $P \leq 0.05$), while the detached leaf method was not significantly correlated with either of the other two methods.

For both hosts, simple linear regression slopes of the isolate-by-cultivar means of the cut stem method regressed on the detached leaf and cotyledon inoculation methods individually showed that both slopes for both methods were positive (Table 5). The slopes for the cotyledon inoculation method were highly significant ($P < 0.01$), whereas the slopes for the detached leaf method were not (Table 5).

Table 2. Disease ratings for three soybean and three dry bean cultivars inoculated with six *Sclerotinia sclerotiorum* isolates that vary in aggressiveness using mycelial plug inoculation of cotyledons, cut stems, and detached leaves

Inoc. method[u]	Isolate[v]					
Cultivars	277	143	279	110	3	105
Soybean						
Cotyledon						
Williams 82	0.0[w] a[x]	296.3 b	288.0 b	95.2 a	26.7 a	273.0 a
Bell	0.0 a	136.0 a	127.0 a	49.0 a	60.0 a	289.0 a
NKS19-90	9.5 a	151.0 a	97.5 a	23.0 a	9.5 a	255.0 a
Cut stem						
Williams 82	7.1[y] a	14.0 b	14.7 c	9.8 b	13.7 b	13.6 b
Bell	5.3 a	8.0 a	7.6 a	6.8 a	6.6 a	9.2 a
NKS19-90	6.8 a	8.6 a	10.5 b	6.9 a	7.9 a	12.3 b
Detached leaf						
Williams 82	2.4[z] a	2.7 a	5.8 a	3.8 a	4.0 a	4.8 a
Bell	1.5 a	2.6 a	3.9 a	2.9 a	2.4 a	4.0 a
NKS19-90	2.1 a	2.3 a	5.3 a	2.0 a	2.0 a	3.9 a
Dry bean						
Cotyledon						
Beryl	12.5 a	189.7 a	174.0 a	23.0 a	17.0 a	238.0 a
B7354	0.0 a	199.0 a	168.0 a	40.0 a	87.5 a	215.0 a
PC-50	31.0 a	146.0 a	208.0 a	6.2 a	43.7 a	277.0 a
Cut stem						
Beryl	3.6 b	5.5 b	5.5 b	4.8 b	4.4 b	5.0 b
B7354	2.1 a	4.5 ab	3.4 a	2.8 a	3.0 a	5.2 b
PC-50	1.7 a	3.6 a	3.6 a	2.3 a	2.2 a	3.4 a
Detached leaf						
Beryl	4.4 a	4.3 a	11.3 ab	7.5 a	7.7 ab	9.2 ab
B7354	4.9 a	4.9 a	9.0 a	8.2 ab	7.1 a	8.7 a
PC-50	5.2 a	6.1 a	13.2 b	10.4 b	9.9 b	11.5 b

[u] Within soybean tests, the cotyledon and cut stem inoculation methods had significant isolate-by-cultivar interactions at $P = 0.04$ and $P = 0.01$, respectively.
[v] Isolates were taken from various hosts and differ in aggressiveness (Table 1).
[w] Data for the cotyledon inoculation method are area under disease progress curve (20).
[x] Means with a common letter within a screening method and isolate do not differ ($P \leq 0.05$).
[y] Data for the cut stem inoculation method are lesion length in cm.
[z] Data for the detached leaf inoculation method are lesion area in cm^2.

Table 3. Disease ratings for six *Sclerotinia sclerotiorum* isolates using mycelial plug inoculation of cotyledons, cut stems, and detached leaves for three soybean and three dry bean cultivars

Cotyledon		Cut stem		Detached leaf	
Isolate	AUDPC[v]	Isolate	Lesion length[w]	Isolate	Lesion area[x]
Soybean cultivars[y]					
105	272.8 a[z]	105	11.8 a	105	4.2 ab
279	171.2 b	279	11.0 ab	279	5.0 a
143	194.6 b	143	10.3 bc	143	2.5 c
3	32.1 c	3	9.4 c	3	3.2 bc
110	55.9 c	110	7.9 d	110	3.0 bc
277	3.2 c	277	6.4 e	277	2.0 c
Dry bean cultivars[y]					
105	243.5 a	143	4.6 a	3	8.7 bc
279	183.7 b	105	4.5 a	279	11.2 a
143	178.5 b	279	4.2 ab	143	5.2 d
3	49.4 c	110	3.4 bc	105	8.3 c
110	23.3 c	3	3.2 cd	110	9.8 b
277	14.6 c	277	2.5 d	277	4.9 d

[v] Data for the cotyledon inoculation method are AUDPC (20).
[w] Data for the cut stem inoculation method are lesion length in cm.
[x] Data for the detached leaf inoculation method are lesion area in cm^2.
[y] Soybean (NKS19-90, Bell, and Williams 82) and dry bean (Beryl, B7354, and PC-50) cultivar means combined for each isolate.
[z] Letters indicate mean separation based on least significant differences ($P = 0.05$).

DISCUSSION

The usefulness of resistance screening techniques for greenhouse and laboratory is determined by the efficacy of the technique to distinguish differences in disease susceptibility among host cultivars in accordance with field performance. The results of this study indicate that three screening methods varied in correct identification of partially resistant and susceptible host cultivars, in detection of different levels of pathogen aggressiveness, and in sensitivity to detect cultivar-by-isolate interactions. The choice of method(s) of statistical analysis impacts the conclusions that can be drawn regarding cultivar disease susceptibility.

Separation of NKS19-90 and Williams 82 by the cotyledon inoculation method was isolate-dependent, with two of six isolates identifying resistant and susceptible cultivars. The cut stem method was the most effective test with which to separate resistant and susceptible soybean cultivars, and the results were consistent with five of six isolates. Although the least aggressive isolate caused disease symptoms, it failed to separate partially resistant and susceptible cultivars. With the detached leaf method, dry bean cultivars were separated by four of the six isolates, but Beryl appeared more resistant, and PC-50 appeared more susceptible, which were inversely related to the reported field screening results (22). The cut stem method most accurately identified resistant and susceptible dry bean cultivars, and results were consistent with all six isolates. These results suggest that correct identification of resistant and susceptible soybean and dry bean cultivars using controlled environment methods is dependent on the screening method employed and the selection of *S. sclerotiorum* isolates.

Variation in isolate aggressiveness may influence the success of controlled-environment resistance screening. Pathogen aggressiveness is defined as the relative ability to colonize the host and cause damage (20). Agricultural populations of *S. sclerotiorum* on numerous hosts are a mosaic of genotypes (15), and isolates within single soybean fields vary widely in level of aggressiveness (16). For this research, isolates were selected based on relative aggressiveness levels previously determined by each screening method and on each respective host (Table 1). It is notable that irrespective of host or inoculation method, the six *S. sclerotiorum* isolates performed as previously described and represented a wide range of aggressiveness on both soybean and dry bean. Additionally, isolates varied in ability to separate resistant and susceptible soybean and dry bean cultivars. The range in aggressiveness of *S. sclerotiorum* isolates in agricultural populations may impact cultivar performance.

The five statistical methods used in the analysis varied in how the screening methods were ranked and in their capacity to detect cultivar and isolate effects and interactions. The cut stem method had the smallest root mean square errors and smallest coefficient of variation and was able to detect interaction. The sensitivity ratio of both the detached leaf and the cotyledon inoculation methods relative to the cut stem method showed the cut stem method to be better than both other methods for both soybean and dry bean. Of these five statistical measures, the sensitivity ratio is less dependent on the disease rating scale of the screening method (17). The coefficient of variation (CV) is often used for comparing methods; however, the value of the CV depends on the scale of measurement, and thus it is of questionable value for making comparisons of methodologies. The sensitivity ratio is not affected by any type of scale transformation and is considered the preferred method of comparison (17).

Another important consideration in using the sensitivity ratios is that they are based on the assumption of a near perfect regression relationship which is not observed with these data. Often a poor regression relationship will result in biased estimates of the sensitivity ratio (17). Using a method to correct for bias as suggested in Snedecor and Cochran (21), we estimated that the slopes were biased downward by approximately 10%. Even after adjusting the sensitivity ratios upward using this 10% bias of the slopes, the cut stem test remained better than the other two methods. The cut stem method had the smallest coefficient of variation, the ability to detect interaction, and correlated with the other methods for both hosts. We conclude that based on these data, the cut stem method was in general statistically better than the cotyledon and detached leaf methods for evaluating resistance in soybean and dry bean cultivars.

Notably, only the cut stem method consistently identified isolate-by-cultivar interactions. The sensitivity of the cut stem method to detect interactions is an important consideration. Effective selection of a screening method that can detect interactions allows for identification of specific resistance/susceptibility for each cultivar to isolates relative to other cultivars. Based on the capacity to detect interaction, the cut stem test would appear best. Overall, the ability to detect isolate-by-cultivar interactions was based not only on the type of statistical analysis, but also on the screening method. The analyses utilized in this report could serve as a model approach to compare the technical merit of resistance screening methods for other host–pathogen systems.

Reliable and accurate screening methods are important to classical as well as molecular programs to identify differences in

Table 5. Slopes and sensitivity ratios for comparing the detached leaf and cotyledon inoculation methods relative to the cut stem method for evaluating resistance to *Sclerotinia sclerotiorum* on soybeans and dry beans

	Slopes		Sensitivity ratios[y]	
Method	**Soybeans**	**Dry beans**	**Soybeans**	**Dry beans**
Detached leaf	0.29	0.08	0.15	0.04
Cotyledon inoculation	37.43**[z]	43.56**	0.75	0.88

[y] Sensitivity ratio (SR) = the ratio for two methods (M and N) is: $SR(M/N) = |dM/dN|/(\sigma_M / \sigma_N)$, where the relationship between the methods is dM/dN.
[z] Significance at $\alpha = 0.01$ is indicated by **.

Table 4. Root mean square error (RMSE), coefficient of variation (CV), isolate by cultivar interaction, and rank correlations of isolate and cultivar least significant means for three methods of evaluating resistance to *Sclerotinia sclerotiorum* on dry beans and soybeans

				Rank correlation[w]					
				Soybeans			Dry beans		
Method	**RMSE[x]**	**CV[x]**	**Iso. × Cv.**	**DL[y]**	**CI**	**CS**	**DL**	**CI**	**CS**
Detached leaf	2.3	41.1	NS	…	0.55*[z]	0.76**	…	0.37	–0.03
Cotyledon inoculation	59.7	50.3	NS	0.55*	…	0.71**	0.37	…	0.54*
Cut stem	1.2	16.7	**	0.76**	0.71**	…	–0.03	0.54*	…

[w] Spearman's rank correlation.
[x] Root mean square error (RMSE) and coefficient of variation (CV) are based on residual error variance.
[y] DL = detached leaf, CI = cotyledon inoculation, CS = cut stem.
[z] Significance at $\alpha = 0.05$ is indicated by *, $\alpha = 0.01$ by **.

levels of resistance to *S. sclerotiorum*. In order to implement effective programs to identify resistant germplasm, both field and controlled-environment experiments should be compared and utilized. Prior to adapting a standard greenhouse and laboratory screening method, it should be tested in different controlled environments that include multiple isolates that vary in aggressiveness on the host and represent the range in aggressiveness found in field environments. Although a significant cultivar and isolate interaction may not be detected depending on type of analysis, our data showed that both weakly and highly aggressive isolates may result in resistant and susceptible cultivars not differing in disease assessment ratings. Finally, several screening methods should be initially considered and compared by a number of statistical procedures to determine which of the screening methods provides the best information on separating host genotypes, isolates, and their interactions.

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