Methods for Evaluating Soybean Cultivars for Resistance to *Sclerotinia sclerotiorum*

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ABSTRACT

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In several areas of Illinois, stem rot or white mold caused by *Sclerotinia sclerotiorum* has become a problem in soybean (*Glycine max*) fields previously cropped to snap beans (*Phaseolus vulgaris*). The disease reaction of soybean cultivars from maturity groups II–IV was evaluated by three inoculation methods. Plants in full bloom (R2) were inoculated by spraying an ascospore suspension ($1–5 \times 10^5$ ascospores per milliliter) to runoff. Four-week-old prebloom plants (V4-V5) were inoculated by placing pieces of autoclaved carrot colonized by the fungus directly onto the leaf surface. After inoculation with either ascospores or colonized carrot pieces, plants were placed in a mist chamber for 7–12 days at 20–25 C with a 12-hr photoperiod. Reactions of all cultivars tested by these two inoculation methods were highly susceptible. Ascospores readily infected plants in the blossom stage and only infected 4-wk-old plants when an exogenous nutrient source was added or when tissues were injured. Limited-term inoculation, the third method, was accomplished by attaching autoclaved celery pieces colonized by the fungus to the nodes of 4-wk-old plants (V4-V5) for 24 hr. Differences in susceptibility were detected among 10 soybean cultivars evaluated by this method.

Sclerotinia sclerotiorum (Lib.) de Bary stem rot has been considered a disease of minor importance on soybean (*Glycine max* (L.) Merr.). Several recent reports, however, indicate that incidence of Sclerotinia stem rot of soybean is increasing and is caused by rotation of soybean with other susceptible crops such as snap bean (*Phaseolus vulgaris* L.), cabbage (*Brassica oleracea* L.), sunflower (*Helianthus annus* L.) (6), and peanut (*Arachis hypogea* L.) (10). Sclerotinia stem rot of soybean has become a more frequent problem in Illinois since 1978, although the disease was first reported in the state by Chamberlain in 1951 (3).

Pathogenicity of *S. sclerotiorum* on soybean has been demonstrated by artificial stem inoculations of immature plants with mycelium of the fungus (3,5). Ascospores are the primary inoculum for white mold on *Phaseolus* spp., first colonizing senescing flower parts and then progressing into the stem tissues (1,2). Although ascospores also are thought to be the primary inoculum for soybean (6), their pathogenicity has not been reported. This paper describes the results of ascospore inoculations of flowering soybean. In addition, we report on the use of limited-term inoculation (LTI), developed as a rapid method of screening *Phaseolus* spp. for resistance to white mold under greenhouse and growth chamber conditions (7,8), as applicable to soybean.

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MATERIALS AND METHODS

Three or four soybean plants representing cultivars from groups II–IV commonly grown in Illinois were grown in 15-cm clay pots in the greenhouse with supplemental lighting. Plants were hardened off in a growth chamber with a 14-hr photoperiod for 1 wk before inoculation. Growth stages of plants at inoculation are reported (4).

The *S. sclerotiorum* isolate used in this study was recovered from sclerotia formed on naturally infected soybean plants collected in Hoopsten, IL, in 1978 and maintained on potato-dextrose agar. Either ascospores or autoclaved carrot or celery pieces colonized by the fungus were used to inoculate soybean plants. In some tests, inoculated plants were placed in a mist chamber at 20–25 C with a 12-hr photoperiod and relative humidity maintained near 100% by a mist system that sprayed plants for 15 min every hour for the first 3 days and 15 min every 2 hr for the remaining incubation period. In other tests, plants were placed in premoistened polyethylene bags in a growth chamber at 21 C with a 12-hr photoperiod. Plants were removed each day, sprayed with distilled water until runoff, and rebagged.

After specified incubation periods in either the mist chamber or the growth chamber, plants were rated for disease severity according to a scale of 0–5 where 0 = no symptoms, 1 = water-soaking of flowers and/or arrested small lesions in the axils or on the main stem, 2 = water-soaking of petioles and leaves only and/or leaf drop, 3 = lesions on the main stem resulting in stem collapse and/or mycelial growth covering up to 25% of foliage, 4 = mycelial growth covering up to 50% of foliage, and 5 = dead plant.

Ascospore inoculations. Apothecia were produced using the technique described by Kohn (9) except sclerotia were incubated in sterile preparation dishes containing vermiculite rather than glass wool. Ascospores were collected (11) and the concentration adjusted to $1–5 \times 10^5$ spores per milliliter and the suspension was atomized onto each plant. Nine plants in full bloom (R2) from each of 17 soybean cultivars were inoculated and placed in the mist chamber and rated for disease development after 3, 7, and 14 days. In a separate experiment, 16 plants in full bloom from each of 23 soybean cultivars were inoculated, bagged, and placed in the growth chamber for 7 days. Because of the variability in the results of the ascospore inoculations of flowering plants, we compared this method, where 60 plants were inoculated, with one in which detached fully opened flowers were sprayed with an ascospore suspension and placed in leaf axils (8). Sixty-five plants were inoculated by the flower-leaf axil method, then bagged and placed in the growth chamber for 7 days. Controls were plants misted with sterile distilled water and placed either in the mist chamber or in polyethylene bags in the growth chamber.

Colonized-carrot inoculations. Five-millimeter mycelial agar plugs containing hyphal tips from the advancing margins of 3- to 4-day-old colonies were transferred to autoclaved carrot-root pieces in 9-cm glass petri dishes. After incubation at 21 C for 24 hr, a 5-mm plug of the colonized carrot piece was placed on the center leaflet of the oldest trifoliolate leaf of four plants in the V4-V5 growth stage (4 wk old) from each of 16 soybean cultivars. Plants were placed in the mist chamber and rated for disease development after 7 and 14 days. Controls were plants inoculated with uncolonized autoclaved carrot pieces.

Limited-term inoculations. Celery-petiole pieces (4 × 8 mm) that had been colonized by the fungus for 24 hr (8) were placed in either the second or third node

of 4-wk-old soybean plants (V4-V5). The stems were then wrapped with a piece of moistened cotton, bagged, and placed in the growth chamber. The inoculum and cotton were removed after 24 hr and the plants were wetted, rebagged, and placed back in the growth chamber and rated for disease development after 7 days. Two replicates of 16 plants each from 10 soybean cultivars were inoculated by this method. Controls were plants inoculated with uncolonized autoclaved celery-petiole pieces.

RESULTS

Ascospore inoculations. The pathogenicity of *S. sclerotiorum* ascospores was established on 27 soybean cultivars. Water-soaking of the flowers was the first symptom to develop. Stem lesions originated on leaf axils at the point of flower attachment and extended up and down the stem. Petiole and leaf infection was also observed at the nodes near flower attachments. Additional infections occurred when infected flowers dropped onto underlying green stem or leaf tissue. Multiple stem and/or petiole and leaf infection occurred on single plants and infections occurred more frequently on upper nodes than on lower nodes even though they all contained fully mature flowers.

No significant differences among severity ratings were determined for the 17 cultivars incubated in the mist chamber after inoculation. There was 100% disease incidence for all cultivars and nearly 100% plant death after 14 days for all cultivars except Union and Corsoy, which had 56 and 33% death, respectively. Elf, a determinate cultivar, was the only cultivar on which the fungus colonized and caused collapse of the main stem after 3 days.

Severity ratings of the 23 soybean cultivars inoculated, bagged, and placed in the growth chamber were variable. Disease incidence was not 100% by this method and there was greater variability in severity ratings for plants of individual cultivars compared with the mist chamber incubation. Mean severity ratings for the ascospore (flowers cast naturally) and the flower-leaf axil inoculation methods were significantly different (Table 1).

Colonized-carrot inoculations. Colonized carrot-root pieces were an effective exogenous nutrient source for *S. sclerotiorum* to infect green tissues of 4-wk-old soybean plants (V4-V5). Disease incidence was 100% for all 16 cultivars tested. No significant differences occurred among severity ratings by this inoculation method. It is notable that Corsoy had a 34% mortality compared with 100% of the plants for all other cultivars tested after 14 days.

Limited-term inoculations. By the LTI method, statistical differences were detected among severity ratings of 4-wk-old soybean plants (V4-V5) from 10 cultivars tested (Table 2). The cultivars Elf and Evans that ranked high in severity ratings also produced large numbers of sclerotia. Corsoy, Williams, and Union consistently developed restricted reddish brown lesions at the nodes or on the main stem at the inoculation point.

DISCUSSION

These investigations establish the pathogenicity of *S. sclerotiorum* ascospores on flowering soybean. This substantiates the observation of Grau et al (6) that stem lesions and lateral branch infections originate at the leaf axils where the flowers are positioned, indicating that this is the primary infection site. We were unable to obtain infection of prebloom soybean when inoculated with ascospores alone. Infection did occur, however, if an exogenous nutrient source such as sucrose was added to the inoculum or if the leaves were mechanically injured with Carborundum before inoculation as reported for *Phaseolus* spp. (1,2). This indicates that some infection in the field could occur on injured tissue.

Limited-term inoculation was the only successful inoculation method of the three tested in distinguishing differences in disease susceptibility among the soybean cultivars evaluated. Results of ascospore inoculations were variable. Infected flowers often aborted, preventing the spread of infection to the main stem at the point of attachment in the leaf axil. Mean severity ratings for almost all cultivars where flowering plants were sprayed with an ascospore suspension were lower compared with those where ascospore-inoculated flowers were placed directly in the leaf axils (Table 1). Hunter et al (8) also reported inconsistencies in the results of ascospore inoculations of flowering plants. In addition, we found it difficult to maintain a continuous film of moisture on ascospore-inoculated plants placed in polyethylene bags, even with frequent misting. Free moisture must be present for extended periods in order for infection to occur (1,2).

Mortality of all cultivars occurred when ascospore- or colonized carrot-inoculated plants were left under continuous mist and high humidity for extended periods in the mist chamber or if the colonized celery pieces were not removed from the plants incubated in

Table 1. Disease severity of *Sclerotinia sclerotiorum* stem rot of flowering soybean (R2) inoculated with ascospores by two methods

Cultivar	Flowers cast naturally	Flowers placed in leaf axils
Amsoy 71	2.8[a]	3.1
Bonus	1.2	2.2
Calland	3.9	3.3
Clark 63	2.5	2.7
Cutler 71	2.4	4.1
Elf	3.0	3.5
Union	3.4	4.1
Williams	2.0	3.5
Woodworth	3.0	2.4
Elf (uninoculated)	0.0	0.0
Mean	2.7*[b]	3.3

[a] Severity ratings based on a scale of 0–5; 0 = no symptoms; 1 = water soaking of flowers and/or arrested small lesions in the axils or on the main stem; 2 = water soaking of petioles and leaves only and/or leaf drop; 3 = lesions on the main stem resulting in stem collapse and/or mycelial growth covering up to 25% of foliage, 4 = mycelial growth covering up to 50% of foliage; and 5 = dead plant. n = 60 (flowers cast naturally) and n = 65 (flowers placed in leaf axils).

[b] Asterisk indicates a significant difference (P = 0.05) based on a t test between the mean severity values of the two inoculation methods.

Table 2. Severity of *Sclerotinia sclerotiorum* stem rot after 7 days in 4-wk-old greenhouse-grown soybean plants (V4-V5) of selected cultivars inoculated with colonized celery-petiole pieces removed 24 hr after inoculation

Cultivar and flower color[a]		Severity rating[b]	Plants with sclerotial production (%)	Plants with restricted lesions (%)
Elf	(P)	4.8	80	0
Evans	(W)	4.2	44	0
Bonus	(P)	4.0	38	0
Wells II	(P)	3.5	63	0
Gnome	(P)	3.1	38	0
Beeson	(P)	2.6	25	0
Wayne	(W)	2.4	25	0
Corsoy	(P)	2.3	0	23
Williams	(W)	1.8	0	75
Union	(W)	1.2	0	63
Elf (uninoculated)		0.0	0	0
FLSD (P = 0.05)		0.9	...	...

[a] Flower color: P = purple and W = white.

[b] Severity ratings based on a scale of 0–5: 0 = no symptoms, 1 = water-soaking of flowers and/or arrested small lesions in the axils or on the main stem, 2 = water-soaking of petioles and leaves only and/or leaf drop, 3 = lesions on the main stem resulting in stem collapse and/or mycelial growth covering up to 25% of foliage, 4 = mycelial growth covering up to 50% of foliage, and 5 = dead plant. Means from a total of 32 plants in two replicates.

polyethylene bags in the growth chamber. Resistant lines of *Phaseolus* spp. could be killed if the inoculum were left in contact with the plant in a humid chamber for long periods (7,8).

Disease severity ratings of plants inoculated by the LTI method can be affected, however, by the age of the tissue and the light intensity under which plants are grown. Etiolated plants were made more susceptible to *S. sclerotiorum* than nonetiolated plants, as were tissues above the second internode (M. N. Cline, *unpublished*). This has also been reported for *Phaseolus* spp. (8).

Corsoy, Williams, and Union, determined moderately resistant by the LTI method, consistently produced reddish brown lesions at the inoculation site. Reddish brown lesions have been reported on naturally infected Corsoy plants (5). Preliminary studies indicate that glyceollin production may play a role in disease resistance of soybean to *S. sclerotiorum* (M. N. Cline and J. D. Paxton, *unpublished*). It has been suggested that resistance to stem invasion may be related to factor(s) associated with purple-flowered cultivars (6); however, Williams and Union are white-flowered moderately resistant cultivars as determined by LTI.

Limited-term inoculation is a feasible method for evaluating soybean cultivars for resistance to Sclerotinia stem rot because the disease severity ratings obtained by this method parallel observations made on field-infected plants. Corsoy resistance is detected by the LTI method and has also been reported in field-evaluated plants (6). Elf is very susceptible when evaluated by any of the three inoculation methods tested in the greenhouse. Both of these cultivars should be included as susceptible and resistant lines in future cultivar evaluations. More studies are needed to correlate soybean resistance of many different cultivars in greenhouse and field trails. As Sclerotinia stem rot becomes more prevalent on soybean, identification of less susceptible lines as possible sources of resistance will be of great value in a breeding program.

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