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Reaction of Soybean Cultivars to Sclerotinia Stem Rot in Field, Greenhouse, and Laboratory Evaluations

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ABSTRACT

Sclerotinia stem rot of soybean [*Glycine max* (L.) Merr.], caused by the fungal pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary, recently has increased in importance in the northern U.S. soybean production area. The objective of our study was to determine the effectiveness of three different inoculation techniques in predicting the field reactions of cultivars to sclerotinia stem rot. Eighteen soybean cultivars were field tested in six Michigan environments from 1994 to 1996 and tested in the greenhouse or laboratory with three inoculation methods. The cultivars were inoculated by placing infested oat (*Avena sativa* L.) seed or mycelial plugs on cotyledons or by placing mycelial plugs on detached leaves. There were significant ($P < 0.05$) differences in resistance to sclerotinia stem rot among cultivars at all but one field environment and for all inoculation methods. The disease severity ratings based on the inoculations were significantly correlated with the field results, with the exception of one method. Disease severity ratings for the three inoculation methods were significantly correlated with only two exceptions. Cultivars such as Novartis S19-90 and Corsoy 79 consistently had the lowest disease severity ratings in the field tests and for the inoculation methods. Similarly, a number of cultivars were rated as susceptible in all tests. Ratings for cultivars with intermediate reactions were not consistent across tests. The inoculation methods tested can provide some useful information on the resistance of soybean genotypes to sclerotinia stem rot. However, resistance identified by inoculation methods should be confirmed with field tests, since these methods can misclassify the resistance of some cultivars.

SCLEROTINIA STEM ROT (syn. white mold) of soybean is caused by the fungal pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary (Grau and Hartman, 1999). This disease has recently increased in importance in the northern USA, and breeding for resistance has become an objective for many soybean cultivar development

programs. Soybean cultivars have been evaluated for resistance to sclerotinia stem rot under field conditions and some with partial resistance to the disease have been identified (Grau et al., 1982; Boland and Hall, 1987; Nelson et al., 1991; Kim et al., 1999). Although researchers have been successful in identifying partial resistance using field evaluations, these evaluations are difficult because of the need for a cool, wet environment for disease development and the high spatial variability of disease foci across fields. For these reasons, researchers would benefit from having a controlled-environment screening method that accurately predicts the reaction of soybean germplasm in field environments.

Both physiological resistance and escape mechanisms contribute to differences in the reaction of cultivars to sclerotinia stem rot in field trials. Escape mechanisms include early flowering and maturity, less lodging, and an upright, open canopy. One or more of these mechanisms have been shown to be significantly associated with reduced levels of sclerotinia stem rot in several studies (Boland and Hall, 1987; Nelson et al., 1991; Kim et al., 1999; Kim and Diers, 2000). Kim and Diers (2000) found genetic evidence of both escape mechanisms and physiological resistance. In a population derived from a cross between Novartis S19-90 by 'Williams 82', they mapped three quantitative trait loci (QTL) controlling sclerotinia stem rot resistance. Two of these loci were significantly associated with flowering date or plant height and lodging, indicating these loci contribute to resistance through disease escape. The third QTL was not associated with escape mechanisms, indicating it may be a gene contributing to physiological resistance to the disease.

Several research groups have developed inoculation techniques for evaluating soybeans for resistance to sclerotinia stem rot. Chun et al. (1987) and Nelson et al. (1991) inoculated excised stems with *S. sclerotiorum* mycelium and measured the length of the lesions that developed. Although both groups observed significant differences among cultivars for lesion length, these re-

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Abbreviations: AUDPC, area under the disease progress curve; DSI, disease severity index; MSU, Michigan State University; PDA, potato-dextrose agar; QTL, quantitative trait loci; UIUC, University of Illinois at Urbana-Champaign.

sults were not consistently correlated with field ratings. Cline and Jacobsen (1983) inoculated flowering plants by spraying them with *S. sclerotiorum* ascospores. They observed a high disease incidence but no significant differences among cultivars. Both Cline and Jacobsen (1983) and Boland and Hall (1986) found the limited-term inoculation technique resulted in significant differences in disease ratings among genotypes. However, Boland and Hall (1986) did not find significant correlation between the limited-term inoculation and field results. Wegulu et al. (1998) tested a number of greenhouse inoculation methods, including mycelial inoculation of foliage, stems, and detached leaves, and the response of detached stems to oxalic acid. They found that the detached leaf inoculation method had the greatest correlation with field disease ratings, although the repeatability across experiments was low. They concluded that the most reliable results came from immersing stem cuttings into an oxalic acid solution and measuring lesion lengths on stems or pink pigment levels in the oxalic acid solution.

No widely accepted method for the evaluation of sclerotinia stem rot reactions in greenhouse or laboratory settings is available. Until there is agreement, new methods should be investigated. We have addressed deficiencies in the detached leaf method discussed by Wegulu et al. (1998) and investigated the inoculation of soybean seedlings at the cotyledon stage as a screening method for sclerotinia stem rot. Thus, our objective was to compare the effectiveness of three inoculation methods in predicting the field reactions of cultivars to sclerotinia stem rot.

MATERIALS AND METHODS

Field Tests

Eighteen soybean cultivars (Table 1) were evaluated in field tests from 1994 to 1996 in East Lansing, MI and near Zilwaukee, MI. The details of the field testing are described in Kim et al. (1999). Briefly, the soybean lines were sown in plots seven rows wide with an 18-cm row spacing and a length of 6.7 m. The 1994 trial was arranged in a randomized complete block design, while the 1995 and 1996 trials were arranged in an alpha-lattice design. All trials consisted of three replicates, although results were taken from only two replicates at the 1994 Zilwaukee site because flooding damaged the third replicate. The East Lansing fields were infested with *S. sclerotiorum* sclerotia obtained from screenings of harvested dry bean (*Phaseolus vulgaris* L.). This location was sprinkler irrigated with ≈ 2.5 mm of water nightly during flowering. The Zilwaukee location was naturally infested with *S. sclerotiorum* and was not irrigated. The soil at the East Lansing location is a Capac loam (fine-loamy, mixed, mesic Aeric Endoaqualf), and the soil at the Zilwaukee location is a Sloan loam (fine-loamy, mixed, superactive, mesic Fluvaquentic Endoaquoll).

Plots were evaluated for sclerotinia stem rot severity according to the system of Grau et al. (1982) at approximately R7 (Fehr et al., 1971), when pods were yellowing and 50% of the leaves were yellow. Thirty plants from the center rows of the plots were individually rated for disease severity on a scale of 0 to 3, with 0 = no symptoms, 1 = lesions on lateral branches only, 2 = lesions on the main stem but little or no effect on pod fill, and 3 = lesions on main stem resulting in

plant death and poor pod fill. A disease severity index (DSI) was calculated for each plot by the following formula:

$$DSI = \left(\frac{\sum(\text{rating of each plant})}{3 \times \text{number of plants rated}} \right) 100 \quad [1]$$

The DSI ranges from 0 for no plants rated as diseased to 100 for a uniform mortality of rated plants.

Infested Oat Seed Inoculations

Sclerotinia sclerotiorum isolated from the stem of a diseased soybean plant from a field in Michigan was maintained by routine subculture on potato-dextrose agar (PDA) medium at 23°C (Dann et al., 1998). Mycelium from this culture was used for preparation of oat seed inocula and mycelial plugs.

There were two inoculation trials with three replicates arranged in randomized complete blocks in each trial. Fifteen to 18 seeds of each cultivar were planted in 13-cm clay pots filled with Bacto Professional planting mix (Michigan Peat Co., Houston, TX) and thinned to 10 seedlings per pot after emergence. Plants were grown without fertilizer in the greenhouse at $22 \pm 2^\circ\text{C}$ until they were inoculated at the V1 growth stage (Fehr et al., 1971), which was 10 to 14 d after planting. The oat-seed inoculum was prepared by first soaking oat seed in water for 24 h in a 1-L flask. The water was drained and the seed were autoclaved once. The oat seed were inoculated with plugs of mycelium grown on PDA and the seed were incubated at 20°C for 25 to 30 d. The oat seed were shaken at least once daily during incubation. After sclerotia formed in the flask, the infested oat seed were dried at room temperature and stored in plastic bags in a refrigerator. Plants were inoculated at V1 by first cutting a 2.0-mm-diameter hole on one cotyledon of each plant ≈ 3 mm from the main stems with a cork-borer. One infested oat grain was placed into each hole, and the plants were placed in a mist chamber for 24 h at $27 \pm 2^\circ\text{C}$. The frequency of misting was adjusted to maintain free water on plant surfaces. The pots were then placed in the greenhouse and evaluated daily for the number of dead or severely wilted plants during the next 5 to 7 d.

Mycelial Plug Inoculations at Michigan State University

The procedures described for the infested oat seed inoculations were employed for growing plants in the greenhouse and culturing *S. sclerotiorum* on PDA medium. There were two inoculation trials with three replicates arranged in randomized complete blocks for each trial. Plugs of inoculum were prepared by cutting discs 3 mm in diameter and 2 to 3 mm thick from edges of 2-d-old colonies growing on freshly made PDA. The plugs were placed mycelial side down on one cotyledon of each plant ≈ 3 mm from the stem. The inoculated plants were then placed in a mist chamber for 40 to 44 h and then moved to a greenhouse. Conditions in the mist chamber and greenhouse were the same as for the Michigan State University (MSU) oat seed inoculations. The number of dead or severely wilted plants was recorded daily for 7 d.

Mycelial Plug Inoculations at the University of Illinois at Urbana-Champaign

An isolate of *S. sclerotiorum*, originating from infected soybean plants from Dekalb, IL in 1994, was maintained in the dark at 4°C on PDA. Mycelial plugs were transferred to acidified PDA for 1 d. A 3-mm-diameter cork-borer was used to cut plugs from the margin of the colony, and plugs were transferred to new PDA. After 1 to 2 d, plugs from the colony

Table 1. Sclerotinia stem rot resistance reactions of 18 soybean cultivars grown in field trials in Michigan, inoculated with infested oat seed on cotyledons, and inoculated with mycelial plugs on cotyledons and leaves.

| Cultivars | Field evaluations across 1994–1996 | | Infested oatseed on cotyledons | | Mycelial plugs on cotyledons at MSU† | | Mycelial plugs on cotyledons at UIUC‡ | | Mycelial plugs on leaves | |
|--------------|------------------------------------|------|--------------------------------|------|--------------------------------------|------|---------------------------------------|------|--------------------------|------|
| | DSI§ | Rank | AUDPC¶ | Rank | AUDPC | Rank | AUDPC | Rank | Lesion size# | Rank |
| NK S19-90 | 15.7 | 1 | 34.2 | 1 | 17.4 | 1 | 9.3 | 1 | 2.7 | 2 |
| Asgrow A2506 | 16.7 | 2 | 48.7 | 7 | 45.8 | 14 | 27.3 | 6 | 3.5 | 7 |
| Collfax | 16.7 | 3 | 44.8 | 4 | 33.5 | 6 | 32.9 | 10 | 3.8 | 12 |
| Corsoy 79 | 18.2 | 4 | 44.4 | 3 | 32.8 | 5 | 18.6 | 3 | 2.9 | 3 |
| Olympus | 19.9 | 5 | 53.7 | 9 | 40.5 | 10 | 39.5 | 17 | 4.0 | 15 |
| Hardin 91 | 20.6 | 6 | 53.8 | 11 | 50.6 | 17 | 26.0 | 5 | 2.4 | 1 |
| Vinton 81 | 24.3 | 7 | 46.8 | 6 | 32.2 | 4 | 14.8 | 2 | 3.3 | 5 |
| CIBA 3253 | 24.9 | 8 | 45.0 | 5 | 40.6 | 11 | 37.4 | 15 | 3.7 | 11 |
| Felix | 26.1 | 9 | 59.8 | 16 | 39.4 | 9 | 31.3 | 9 | 4.1 | 16 |
| Jack | 26.1 | 10 | 60.3 | 18 | 35.6 | 7 | 46.9 | 18 | 3.0 | 4 |
| Elgin 87 | 26.6 | 11 | 53.7 | 10 | 23.2 | 2 | 24.1 | 4 | 3.7 | 9 |
| Chapman | 27.8 | 12 | 36.2 | 2 | 31.2 | 3 | 27.7 | 7 | 4.2 | 17 |
| Kenwood 94 | 30.0 | 13 | 55.9 | 13 | 44.5 | 12 | 33.8 | 11 | 4.0 | 14 |
| Conrad 94 | 30.6 | 14 | 59.9 | 17 | 49.0 | 16 | 36.3 | 14 | 3.9 | 13 |
| Dunbar | 31.2 | 15 | 55.3 | 12 | 45.0 | 13 | 39.0 | 16 | 4.9 | 18 |
| BSR 101 | 31.9 | 16 | 56.9 | 14 | 37.2 | 8 | 34.9 | 12 | 3.4 | 6 |
| Resnik | 32.0 | 17 | 57.4 | 15 | 49.0 | 15 | 28.4 | 8 | 3.6 | 8 |
| Fairbault | 37.1 | 18 | 50.7 | 8 | 59.1 | 18 | 35.9 | 13 | 3.7 | 10 |
| Average | 25.4 | | 51.0 | | 39.4 | | 30.2 | | 3.6 | |
| LSD (0.05)†† | 10.6 | | 12.1 | | 16.3 | | 18.0 | | 1.2 | |

† Evaluations done in a greenhouse at Michigan State University.

‡ Evaluations done in a greenhouse at the University of Illinois.

§ Disease severity index.

¶ Area under disease progress curve.

Area of lesions in cm².†† Least significant difference for individual cultivars at $P = 0.05$.

edges were used to inoculate plants at the V1 to V2 growth stage (Fehr et al., 1971). A single plug was placed mycelial side down on a cotyledon ≈ 2 mm from the stem of each seedling. All seedlings were then lightly misted with water by a hand-atomizer to increase humidity and covered with plastic domes that fit over individual flats. The dome-covered flats were placed ≈ 1 m under black mesh shade cloth (80% light reduction) to prevent heat buildup inside the domes. After 2 d, the domes were removed, and after two more days, the shade cloth was removed. The number of seedlings that died was counted daily, usually beginning with the day the dome was removed until plants stopped dying, which was 4 to 5 d after the first rating. There were two inoculation trials with three replicates in each trial arranged in a randomized complete block design.

Excised Leaf Inoculation

Soybean cultivars were grown in single-row plots, 1.2 m long and 0.75 m apart at the University of Nebraska East Campus Agronomy Farm in Lincoln, NE. Seeds were planted at 20 seeds m⁻¹ and plants were not thinned. The cultivars were screened using a modified version of a detached leaf assay reported by Leone and Tonneijck (1990). Leaves were sampled 28 d after planting. The youngest fully expanded trifoliolate leaf (Fehr et al., 1971) of each plant sampled was cut at the juncture of the petiole and main stem, immediately wrapped in moist paper, and placed in a 3.78-L (gallon) plastic bag with the petioles submerged in water for transport to the laboratory. Leaf samples for each cultivar were obtained from random plants in a row. Plants sampled for one replication were tagged so they were not sampled again. One replicate consisted of one leaf from each of the 18 cultivars, arranged in an incomplete block design for the detached leaf assay. Three replicates were evaluated on each of three different days, for a total of nine replicates.

In the laboratory, the petioles were placed in orchid tubes containing tap water. Aluminum roasting pans were lined with

paper towels and four glass petri plates were placed in the bottom of each pan. The petri plates were used to support the central leaflet above the moist paper towel. Leaves from each of the 18 cultivars were randomly assigned to pans for each replication. An 8-mm plug from a culture of *S. sclerotiorum*, isolate 265 from soybean, was centered between veins of the middle leaflet. The inoculum was taken from the advancing margin of a 36- to 48-h culture grown on PDA. New cultures for each replication were initiated from sclerotia rather than subcultures. Tap water (300 mL) was added to the bottom of each roasting pan and the pan was covered tightly with plastic wrap to maintain high humidity and incubated at $22 \pm 1^\circ\text{C}$ with diurnal laboratory lighting supplemented with daylight. After 48 h, the length and width of each lesion was measured, and the lesion area (cm²) was determined by calculating the area of an oval or circle.

Data Analysis

Field data were analyzed with PROC GLM in SAS (SAS Institute, 1985) and a randomized complete block design. Each location-year combination was treated as a separate environment, and environments were considered a random effect and genotypes a fixed effect.

The area under the disease progress curve (AUDPC) was used (Shaner and Finney, 1977) to summarize the progress of disease severity for the infested oat seed inoculation and mycelial plug inoculation assays. The modified standardized AUDPC was calculated according to the formula:

$$\text{Standardized AUDPC} = \sum_{i=1}^n [(x_i + x_{i-1})/2](t_i - t_{i-1}) \quad [2]$$

in which n is the number of evaluation times, x_i is the disease intensity at each evaluation time, and $(t_i - t_{i-1})$ is the time duration. Disease intensity was measured as the proportion of plants that were dead or severely wilted at each rating. The

Table 2. Correlations between the ratings of 18 cultivars for the area under the disease progress curves (AUDPC) from infested oat seed and mycelial plug inoculations, lesion sizes on inoculated excised leaves, and disease severity indexes (DSI) across four Michigan field environments for sclerotinia stem rot resistance tests.

| | Infested oat seed on cotyledons | Mycelial plugs on cotyledons at MSU† | Mycelial plugs on cotyledons at UIUC‡ | Mycelial plugs on leaves |
|---|------------------------------------|---|--|-----------------------------|
| Mycelial plugs on cotyledons at MSU | 0.53* | | | |
| Mycelial plugs on cotyledons at UIUC | 0.62** | 0.52* | | |
| Mycelial plugs on leaves | 0.21NS | 0.22NS | 0.47* | |
| Field evaluations across 1994-1996 | 0.50* | 0.51* | 0.46NS | 0.47* |

* ** Significant at the 0.05 and 0.01 levels of probability; NS is not significant.

† Evaluations done in a greenhouse at Michigan State University.

‡ Evaluations done in a greenhouse at the University of Illinois.

AUDPC data were analyzed across trials with PROC GLM in SAS with trials treated as a random effect and genotypes a fixed effect.

The data for the detached leaf experiment were analyzed using PROC GLM in SAS to obtain LS means for lesion sizes of genotypes. Pearson product-movement correlations were calculated with PROC CORR in SAS to compare the disease ratings from the field and inoculation methods. Because the cultivar \times inoculation trial interactions were not significant for the infested oat seed and mycelial plug inoculations, the correlations were calculated using the means of the cultivars across the two trials for each inoculation method.

RESULTS AND DISCUSSION

The 18 soybean cultivars were evaluated for their reaction to sclerotinia stem rot at two field locations across 3 yrs (Table 1). There were significant ($P < 0.05$) differences among cultivars for the disease ratings across field environments and at each environment, with the exception of Zilwaukee in 1994 and 1996. There was a significant environmental effect and genotype \times environment interaction. Across environments, greater DSI ratings were correlated with less yield ($r = -0.75$) and more plant lodging ($r = 0.48$). The DSI was not significantly correlated with maturity date, R1 date, or plant height across environments. The cultivars Novartis S19-90, Asgrow A2506, Colfax, and Corsoy 79 had the lowest DSI ratings, suggesting that they have partial resistance to sclerotinia stem rot. More detailed discussions of the field test results are provided in Kim et al. (1999).

There were significant differences between the two inoculation trials, among cultivars, and there was a non-significant cultivar \times trial interaction for the infested oat grain inoculations (Table 1). Plants killed by the pathogen were first observed 3 d after inoculation in the first trial and 2 d after inoculation in the second trial. Additional dead plants were observed for two more days for both trials. The mean AUDPC ratings of cultivars across the two oat grain inoculation trials were significantly correlated with the field test DSI ratings (Table 2).

There were significant differences between the two inoculation trials, among cultivars, but the cultivar \times trial interaction was nonsignificant for the mycelial plug inoculations at MSU (Table 1). The symptoms occurred slower with the mycelial plug inoculations than with the infested oat grain inoculations. There was little symptom

development observed until 5 d after inoculation for the first trial and 4 d after inoculation for the second trial. Additional dead plants were observed until 8 d after inoculation for the first trial and 6 d after inoculation for the second trial. The mean AUDPC ratings across trials were significantly correlated with the field DSI ratings (Table 2).

There were significant differences between the two inoculation trials, among cultivars, but there was a non-significant cultivar \times trial interaction for the mycelial plug inoculations at the University of Illinois at Urbana-Champaign (UIUC). Symptomatic plants were first observed 2 d after inoculation for both trials. The AUDPC ratings across trials were not significantly correlated with the field DSI ratings at $P < 0.05$ but were significant at $P = 0.057$.

There were significant differences among cultivars for the size of the lesions that resulted from the excised leaf inoculations. The lesion sizes were significantly correlated with field DSI ratings. The disease ratings for the inoculation methods were significantly correlated, with the exception that the excised leaf inoculation method was not significantly correlated with the oat inoculation and the mycelial plug inoculation methods at MSU (Table 2).

The rating of some cultivars was consistent for the different screening methods. For example, Novartis S19-90 ranked first in the field and first or second for each inoculation method (Table 1). Corsoy 79 also consistently expressed partial resistance in both the field and greenhouse, ranking from third to fifth most resistant. Other cultivars, such as Kenwood 94, Conrad 94, Dunbar, BSR101, Resnik, and Faribault, were consistently ranked susceptible in both the field and inoculation evaluations. In contrast, some cultivars were not consistently ranked in the field and artificial inoculations. For example, Asgrow A2506 was ranked as the second most resistant in the field but ranked from five to 13 with the inoculation methods. Colfax ranked the third most resistant in the field, but ranked from three to 12 with the inoculation methods.

There are a number of reasons why there are inconsistencies in results between field tests and inoculation methods. One reason is the relatively large error variances of the inoculation and field trials (Table 1). An additional reason is that field ratings are probably the result of a combination of both physiological resistance

and escape mechanisms. The inoculation methods we used only assayed physiological resistance; therefore, any effect from escape mechanisms is not included in these ratings. In our field tests, DSI was significantly correlated with plant lodging. Although the correlations between DSI and plant height, date of flowering, and maturity date were not significant over environments in our field tests, two studies have shown these traits to be significantly correlated with resistance to *S. sclerotiorum* (Kim and Diers, 2000; Boland and Hall, 1987). There can also be different types of resistance mechanisms that are expressed in various plant tissues. The majority of field infections are initiated following colonization of flower petals by ascospores (Grau and Hartman, 1999). In the inoculation methods we used, cotyledons or leaves were directly inoculated with mycelium. It is possible that cultivars have different types of resistance providing protection to flowers compared with vegetative tissue. However, flower colonization under natural conditions is followed by stem colonization by mycelium, supporting the relevance of mycelial inoculation.

Another factor that may have contributed to the inconsistencies among evaluation methods is the testing environment. Pennypacker and Risius (1999) observed that light levels influenced resistance responses and these responses varied for the different cultivars tested. We did not measure light levels in our experiments and are uncertain of their influence on our results. The effect of the testing environment deserves further study.

The deficiencies in the excised leaf test discussed by Wegulu et al. (1998) were uniform leaf size (age), inoculum placement, and incubation conditions. These variables were tightly controlled in our experiment, which improved the consistency of this test compared with the results of Wegulu et al. (1998). The excised leaf test is useful for evaluating sclerotinia stem rot resistance as it offers a nondestructive method of screening and can be used on field, greenhouse, or growth chamber-grown plants anytime before the R2 growth stage. The cotyledon tests are also rapid in that young plants can be evaluated within a week from seeding and the total test takes only 15 d.

Other researchers have studied the correlation between field and artificial inoculations for sclerotinia stem rot resistance. Chun et al. (1987) and Nelson et al. (1991) reported that the excised stem inoculation method was not consistently correlated with field ratings. Boland and Hall (1986) found no correlation between a limited-term inoculation method and field resistance. Wegulu et al. (1998) found that their greenhouse

test results were not consistently correlated with field resistance. We observed moderate correlations between our inoculation methods and field results. These correlations were similar for our different inoculation methods (Table 2), and there was no clear indication that one method was superior to the others in predicting field performance. Our findings together with the work of others suggest that inoculation methods can be used to obtain preliminary information on the resistance of genotypes, but these results need to be confirmed with field tests.

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