Efficacy of Fungicides on *Sclerotinia sclerotiorum* and Their Potential for Control of Sclerotinia Stem Rot on Soybean

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ABSTRACT

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Sclerotinia stem rot of soybean, caused by *Sclerotinia sclerotiorum,* is a major disease in the north central region of the United States. One approach to managing Sclerotinia stem rot on soybean is the use of fungicides. *S. sclerotiorum* was assayed for sensitivity to benomyl, tebuconazole, thiophanate methyl, and vinclozolin in pure cultures on agar medium, inoculated soybean seedlings, detached inoculated leaves, and in experimental field plots. To evaluate the inhibitory effect of four fungicides on growth of *S. sclerotiorum* in vitro, potato dextrose agar (PDA) was amended with the fungicides at six concentrations. Based on measurements of fungal radial growth, vinclozolin was the most effective in inhibiting *S. sclerotiorum* mycelial growth at 1.0 µg a.i./ml of PDA. Ranges of reduction of radial growth of 91 isolates of *S. sclerotiorum* on PDA amended with thiophanate methyl and vinclozolin were 18 to 93% and 93 to 99%, respectively, when compared with the nonamended agar control. Benomyl, thiophanate methyl, and vinclozolin applied to greenhouse-grown seedlings prevented *S. sclerotiorum* from expressing symptoms or signs on leaf tissue. Detached leaves sprayed with thiophanate methyl and then inoculated with mycelial plugs of *S. sclerotiorum* did not express symptoms or signs. Of 13 different environments in Illinois, Indiana, Ohio, and Wisconsin from 1995 through 2000, six had low Sclerotinia stem rot incidence (<1%), three environments had low to moderate Sclerotinia stem rot incidence (5 to 25%), and four environments had high Sclerotinia stem rot incidence (>25%). When disease incidence was high, no consistent control of Sclerotinia stem rot was observed with benomyl or thiophanate methyl using different application systems. However, under low disease incidence, spray systems that were able to penetrate the canopy reduced the incidence of Sclerotinia stem rot an average of 50%.

Sclerotinia stem rot of soybean (*Glycine max* (L.) Merr.), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is a major disease in the north central region of the United States (9). There were severe outbreaks of this disease in 1992, 1994, and 1996 in this region (9,10,12). In 1994, Sclerotinia stem rot was ranked the most severe soybean disease in Argentina and the second most important disease in the United States (27).

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Trade and manufacturers' names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Crop management practices and cultivar selection contribute to the control of Sclerotinia stem rot, but the effectiveness of these tactics can be modified by inoculum potential. Furthermore, other pests and pathogens, government regulations, economics, and intended use of soybean crop may be obstacles to the use of agronomic practices and partially resistant cultivars to control Sclerotinia stem rot. Management plans for control of Sclerotinia stem rot would benefit from a tactic that could be implemented on a need basis during the growing season. Fungicides would offer this flexibility and also augment preventive measures.

Blossoms serve as an energy source to support ascospore infection of healthy plants (1,3). Because flower petals are the sites of primary colonization, the application of fungicides must be directed at flower petals, especially in the lower portions of the crop canopy. Once established, infections can spread to leaves, petioles, internodes, and also to adjacent plants through contact with diseased plants.

The use of fungicides to control *S. sclerotiorum* has been evaluated on snap bean (*Phaseolus vulgaris* L.). However, control has been inconsistent (14,24), primarily due to difficulties in achieving good coverage with fungicides and timing of application in relationship to ascospore release. The level of control is directly related to the number of blossoms within the canopy that are treated with fungicide (19). For example, the lower half of the dry bean canopy received little or no fungicide with an aerial application (26).

Many of the same problems that limit the use of foliar fungicides on snap bean may also exist in soybean. For example, indeterminate soybean cultivars flower over a 1- to 5-week period, which provides many opportunities for infection to occur, even with two fungicide applications. Moreover, the canopy of soybean is similar to the snap bean's canopy, where it is difficult for the fungicides to penetrate the canopy and cover the blossoms. This is especially true in soybean fields with row spacing less than 76 cm.

The objectives of this study were to (i) investigate the effect of different concentrations of several fungicides on mycelial growth of *S. sclerotiorum*; (ii) determine the effect of two fungicides on the growth of various isolates of *S. sclerotiorum*; (iii) evaluate several fungicides for control of *S. sclerotiorum* on soybean in the greenhouse and field; and (iv) assess different spray application techniques for fungicidal control of *S. sclerotiorum* in soybean.

MATERIALS AND METHODS

In vitro growth of *S. sclerotiorum* on fungicide-amended agar. Potato dextrose agar (PDA; Difco, Detroit, MI) was autoclaved at 122°C for 15 min and cooled to 45 to 50°C. Filter sterilized benomyl (Benlate 50 WP, DuPont, Wilmington, DE), tebuconazole (Folicur 3.6 F; Bayer Corporation, Kansas City, MO), thiophanate methyl (Topsin M 70WP; Elf Atochem, Philadelphia, PA), and vinclozolin (Ronilan 50 WP; BASF Corp., Research Triangle Park, NC) were diluted in distilled water and added to PDA to yield 0.1, 1, 10, 50, 100, and 500 µg a.i./ml of PDA of each fungicide. Benomyl was diluted in 95% EtOH before being added to the tempered

agar, and nonamended PDA was used as a control. Twenty milliliters of amended or nonamended PDA was poured into 9-cm-diameter petri dishes. Initial cultures of *S. sclerotiorum* (SSR-113; Henry Co., Geneseo, IL) were grown on nonamended PDA. Plugs (6-mm-diameter) were taken from the actively growing margins of the colony, and one plug was transferred to the center of five replicated plates of each treatment. Plates then were placed in a growth chamber at 25°C under constant fluorescent light at 100-$\mu E \cdot m^{-2} \cdot s^{-1}$. The diameter of the radial growth was measured daily for 3 days after the transfer. Area under mycelia growth curve (AUMGC) was calculated using colony diameter as the dependent variable and the three dates as the independent variable (20). The experiment was arranged in a completely randomized design and was done three times.

Another in vitro assay was done to determine the effective concentration of vinclozolin and thiophanate methyl for a 50% reduction in growth (EC_{50}) of *S. sclerotiorum* isolate SSR-113. Vinclozolin was diluted to 0.1, 0.3, 0.7, 1.0, 3.0, 5.0, 7.0, and 10 μg a.i./ml of PDA, and thiophanate methyl was diluted to 0.1, 0.3, 0.7, 1.0, 3.0, 7.0, 10, 20, 30, 40, and 50 μg a.i./ml of PDA. Nonamended PDA was used as a control. The diameter of the radial growth was measured daily for 3 days after the transfer. AUMGC was calculated using colony diameter as the dependent variable and the three dates as the independent variable (20). The experiment was arranged in a completely randomized design, there were five replications (plates) per treatment, and the experiment was done twice.

Vinclozolin and thiophanate methyl both were diluted to 1.0 μg a.i./ml of PDA to evaluate the sensitivity of 91 isolates of *S. sclerotiorum*. Isolates grown on nonamended PDA were included as a control. The same experimental procedure was used as previously described with five replications per treatment, and the experiment was repeated. These isolates are part of a soybean pathogen collection housed at the National Soybean Research Center at the University of Illinois. The isolates originated from four countries, eight states, 19 counties within Illinois, and nine different hosts. A complete list of isolates used can be obtained from the corresponding author.

Greenhouse experiment. Four fungicides were evaluated for their control of *S. sclerotiorum* on two soybean cultivars, NK S19-90 (Syngenta Seeds, Golden Valley, MN) and BSR101, which are partially resistant and susceptible to Sclerotinia stem rot, respectively (16).

A highly aggressive isolate of *S. sclerotiorum* (SSR-113) from soybean was used for this study (17). Inoculum was prepared by mixing 500 ml of wheat seed (*Triticum aestivum* L.), 500 ml of deionized water, 10 μg of sucrose, and 2 ml of 1 N HCl in a 3.8-liter plastic container. The mixture was autoclaved for 1 h at 121°C on two consecutive days. Three-day-old cultures growing on PDA were cut into 1 cm^2 plugs. Approximately 10 plugs were transferred into each plastic jug and incubated at 23°C for 7 days, being shaken vigorously twice a day. The colonized wheat grain was air-dried for 2 days and ground through a 3-mm stainless steel screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). The inoculum was stored at 4°C.

A mist chamber, 4.3 × 1.4 × 0.9 m, was constructed on a greenhouse bench. Sand was evenly spread over a plastic sheet covering the bench to a depth of 4 cm. Another transparent plastic sheet was draped over the frame to enclose the chamber. Two humidifiers (Herrmidifer Co., Lancaster, PA) were placed at each end of the chamber to maintain the relative humidity at 100%. A humidostat was placed in the center of the chamber, but not directly in the path of the humidified air. Temperature was maintained at 21 ± 4°C.

Approximately 800 ml of a soil/sand mixture (1:1) was added to 947-ml polypropylene containers. Three 3-mm-diameter holes were drilled into the bottom of the containers for drainage. Six seeds of each cultivar were planted in each container, and the containers were arranged in a randomized complete block design with four replications. The fungicides benomyl, tebuconazole, thiophanate methyl, and vinclozolin were applied at recommended rates. The plants were uniformly sprayed at growth stage V2 (7) using a greenhouse sprayer calibrated to apply the equivalent of 238 liters/ha using XR8003VS flat spray tips (Spraying Systems Co., Wheaton, IL). After 24 h, these plants were lightly misted with water, and approximately 0.1 g of ground inoculum was applied evenly over every leaf. Plants were then placed in a humidity chamber and after 3 days were individually rated on a scale of 0 to 5, where 0 = no disease, 1 = lesion <5 mm on leaf, 2 = lesion >5 mm on leaf, 3 = lesion >5 mm on leaf and start of stem colonization, 4 = severe stem colonization, and 5 = plant completely dead. The experiment was done three times.

Soybean plants, grown in the greenhouse similarly to the previous study, were sprayed with the same four fungicides at growth stage V2 (7) at the same rates and allowed to dry for 1 day. Trifoliolate leaflets from each treatment were detached and placed on filter paper saturated with water in a 9-cm-diameter petri plate. Agar plugs, 6-mm-diameter, were transferred from actively growing margins of isolate SSR-113 of *S. sclerotiorum* to the center of each leaflet. The detached leaves were incubated in a growth chamber at 25°C under constant fluorescent light at 100-$\mu E \cdot m^{-2} \cdot s^{-1}$ light. Water was added as needed to keep the filter paper saturated for 4 days. The length and width of the lesion was determined daily for 4 days after inoculation, and area under disease progress curve (AUDPC) values were calculated using lesion length and width as the dependent variable and the four dates as the independent variable (25). The experiment was

Table 1. Field location, soybean cultivar(s), plant population, row width, and previous crop of fields used for evaluation of efficacy of fungicide for management of Sclerotinia stem rot under field conditions in three states from 1995 to 2000

Environment and year	Location	Cultivar(s)[a]	Seed population (seeds/ha)	Row width (cm)	Previous crop
IL-98	Watseka, IL	Pioneer 9363	556,000	19	Soybean
IL-99	Woodstock, IL	Pioneer 9163	556,000	19	Corn
IN-00	Kentland, IN	Dairyland DSR272RR	445,000	76	Corn
OH-98	Mt. Vernon, OH	Croton 3.9	445,000	19	Soybean
OH-99	Wooster, OH	Croton 3.9	494,000	38	Corn
OH-00	Shreve, OH	Asgrow/Monsanto AG3003	445,000	19	Corn
WI-95	Waunakee, WI	NK Brand S19-90 BSR101 Sturdy	544,000	19	Soybean
WI-96	Waunakee, WI	NK Brand S19-90 BSR101 Sturdy	544,000	19	Corn
WI-97	Waunakee, WI	NK Brand S19-90	544,000	19	Soybean
WI-98-1	Waunakee, WI	Kaltenberg KB256RR Asgrow/Monsanto AG2501	593,000	19	Corn
WI-98-2	Waunakee, WI	Kaltenberg KB256RR	519,000	38	Corn
WI-99	Arlington, WI	Kaltenberg KB256RR Asgrow/Monsanto AG2501	519,000	38	Small grains

[a] Cultivars NK Brand S19-90, Asgrow/Monsanto AG 2501 are considered moderately resistant, while Asgrow/Monsanto AG 3003, BSR 101, Croton 3.9, Dairyland DSR272RR, Kaltenberg KB256RR, and Pioneer 9163 and 9363 are considered susceptible to Sclerotinia stem rot.

arranged in a randomized complete block design with four replications, and the experiment was done twice.

Field experiments. In fields naturally infested with *S. sclerotiorum*, different methods were used to evaluate benomyl and/or thiophanate methyl between 1995 and 2000 in Illinois, Indiana, Ohio, and Wisconsin for efficacy against *S. sclerotiorum* on soybean. Field location, cultivar selection, seeding rate, row width, and previous crop are presented in Table 1.

Illinois and Indiana. In 1998, 1999, and 2000, field studies were conducted at Watseka and Woodstock, IL, and Kentland, IN, respectively, in infested fields to evaluate thiophanate methyl. The Watseka and Kentland locations had silt loam type soil, while the Woodstock location had sandy loam type soil. All three fields were tilled in the fall and spring prior to planting. Soybean cultivar Pioneer 9363 was planted in 6-row plots spaced 19 cm apart and 6.6 m long at 494,000 seeds per hectare. Thiophanate methyl was applied with a CO_2 pressurized sprayer calibrated to deliver 238 liters/ha at 173 kPa pressure using XR8003VS flat nozzles (Spraying Systems Co.). All applications were made at approximately 5.0 km/h at the R1 or R3 growth stage over the entire plot. Treatments included thiophanate methyl applied at the R1 growth stage, thiophanate methyl applied at both the R1 and R3 growth stages, and a nontreated control. The treatments were arranged in a randomized block with five replications in 1998 and four replications in 1999 and 2000. Sclerotinia stem rot incidence was estimated by assessing 50 consecutive plants in each plot at the R7 growth stage (7). Plots were harvested with a Hege small plot combine (Waldenburg, Germany), and grain yields were adjusted to 13.5% moisture.

In a commercial field, water-sensitive paper cards for monitoring spray distribution (Syngenta, Basle, Switzerland) were placed on top of the canopy, 25 cm into the canopy, and on the soil surface inside the canopy. Pioneer 93B01 was planted with a row spacing of 19 cm at 494,000 seeds per hectare. Distilled water was applied at R2 growth stage using the same system as previously described. Percent coverage was determined using a computer base area scanner (Root Analysis System Version 0.95, Mark Belding, Illinois State Water Survey, 1998).

Ohio. Two different broadcast nozzles on a conventional sprayer and one nozzle on a Myers Mity Mist air assist sprayer (Ashland, OH) were studied in infested fields using thiophanate methyl (1.12 kg a.i./ha) in Ohio during the 1998, 1999, and 2000 growing seasons. All field locations were silt loam, and only the 1999 location was fall tilled prior to planting. The 1998 and 2000 fields were no-till. Fields were planted with a drill in 1998 and 2000 and a planter in 1999. The conventional broadcast sprayer had a boom length of 3.7 m with nozzles spaced 51 cm on center. Fungicide was applied at 187 liters/ha with two nozzles, XR8002 and D2-23 (Spray Systems Co., Wheaton, IL), at 296 and 1,654 kPa pressure, respectively, at the R2 growth stage. The Myers Mity Mist sprayer used XR110015 nozzles at 193 kPa pressure spaced 30 cm on center. The average air outlet speed was 20 m/s. The air and spray discharge was directed approximately 30° back from vertical. All applications were made at 5.0 km/h. The six treatments consisted of a fungicide application with a conventional broadcast sprayer with XR8002 nozzles, conventional broadcast with D2-23 nozzles, and Myers air assist with XR110015 nozzles; the three controls were water applied with a conventional boom with XR8002 flat-fan nozzles or Myers air assist and nontreated. The treatments were applied to plots 3.7 m wide by 16.8 m long in a randomized block design with five replications in 1998 and 1999 and four replications in 2000. When the leaves were beginning to turn yellow (growth stage R7), the incidence of Sclerotinia stem rot from 200 consecutive plants in each plot was recorded. Plots, 3 m wide by 15 m long, were harvested with a Kincaid plot combine (Kincaid, KS), and yields were adjusted to 13.5% moisture.

Wisconsin. Evaluations of benomyl and thiophanate methyl were done in naturally infested fields located near Arlington and Waunakee, WI, from 1995 through 1999. A silt loam type soil was present at both locations, and fields were tilled in the fall and spring prior to planting. Seeds were planted in 3 × 7.6 m plots at a density of 556,000 seeds per hectare in 19-cm row spacing in all years of the study. Benomyl was applied at 0.56 and 1.12 kg a.i./ha and thiophanate methyl was applied at 0.84 and 1.12 kg a.i./ha at either the R1 or R2 growth stage using a multiboom plot sprayer mounted on an IH Cub (Case International, Racine, WI). In 1995 and 1996, the system was calibrated to deliver 183 liters/ha at 228 kPa pressure using SS730154 nozzles (Spraying Systems Co.). In 1997, 1998, and 1999, the system was calibrated to deliver 275 liters/ha at 241 kPa pressure using XR80015VS flat-fan nozzles (Spraying Systems Co.). All field experiments were designed as a randomized complete block with four to five replications. Sclerotinia stem rot incidence was recorded at growth stage R7 by a visual percent estimate of plants killed by *S. sclerotiorum* from the center of the plots. Plots were harvested with an Almaco plot combine (Allen Machine Co., Nevada, IA), and yields were adjusted to 13.5% moisture.

Data analysis. An analysis of variance of the AUMGC values was completed using the general linear models procedure (PROC GLM) in SAS (SAS Institute, Cary, NC) with trials treated as a random effect and fungicides and fungicide concentrations as fixed effects. Means were compared with Fisher's protected least significant difference test at $P \leq 0.05$. EC_{50} values for vinclozolin and thiophanate methyl were calculated from the regression line using the average diameter for each concentration. For the 91 isolates, an analysis of variance of the AUMGC values was completed using PROC GLM with replications treated as a random effect and isolates as a fixed effect for each treatment (nontreated control, thiophanate methyl-amended PDA, and vinclozolin-amended PDA). Means were compared with Fisher's protected least significant difference test at $P \leq 0.05$. For the greenhouse study, foliar ratings were analyzed with PROC GLM using replications and trial as random effects and plants, fungicide, and cultivar as fixed effects. For the detached leaf study, the AUDPC value was calculated and analyzed with PROC GLM using replications and trial as random effects and fungicide and cultivar as fixed effects. The interaction between fungicide and cultivar was also analyzed. For the field studies, each location–year combination was analyzed separately. An analysis of variance of the percent Sclerotinia stem rot and yield was completed using PROC GLM in SAS with replications designated as a random effect and application method with or without fungicide as a fixed effect. Means were separated with the Fisher's protected least significant difference test at $P \leq 0.05$, except for the means from the Wisconsin study, which were at $P \leq 0.10$.

Table 2. Area under mycelial growth curve (AUMGC) of *Sclerotinia sclerotiorum* grown on potato dextrose agar (PDA) amended with four fungicides at six concentrations[a]

	Concentration (µg a.i./ml of agar)					
Fungicide	**0.1**	**1**	**10**	**50**	**100**	**500**
Benomyl	240	73	5	4	2	0
Tebuconazole	211	24	0	0	0	0
Thiophanate methyl	236	82	52	7	6	4
Vinclozolin	67	8	0	0	0	0

[a] AUMGC value for control (nonamended PDA) is 237. AUMGC calculated by: AUMGC = $\sum^{n-1}_{i=1}$ $[(X_{i+1} + X_i)/2][t_{i+1} - t_i]$, in which X_i = colony diameter, expressed in mm at the ith observation, t_i = time (days after inoculation) at the ith observation, and n = total number of observations. Least significant difference ($P \leq 0.05$) = 8.3.

RESULTS AND DISCUSSION

In vitro growth of *S. sclerotiorum* on fungicide-amended agar. Agar amended with each of the four fungicides reduced

mycelial growth of *S. sclerotiorum* compared with the growth on nonamended PDA (Table 2). Vinclozolin inhibited mycelial growth at 1 µg a.i./ml of agar; benomyl and tebuconazole at 10 µg a.i./ml of agar; thiophanate methyl at 50 µg a.i./ml of agar. Our findings are similar to a previous report where vinclozolin completely inhibited mycelial growth at 1 µg a.i./ml of agar (23). In addition, benomyl and thiophanate methyl were reported to reduce mycelial growth 80 to 90% at 5 µg a.i./ml of agar (11). The EC_{50} values in our study for vinclozolin and thiophanate methyl were 0.6 and 2.2 µg a.i./ml of PDA, respectively.

In vitro studies have been used to identify specific fungicides and rates for fungicidal activity against *S. sclerotiorum* (11). In addition, many isolates of *S. sclerotiorum* must be evaluated to detect the presence of strains that express resistance to fungicides. Screening fungicides at different concentrations against many isolates can potentially identify fungicides that may have limited efficacy against *S. sclerotiorum*. In our study, there were differences ($P < 0.01$) in rate of mycelial growth among the 91 isolates in the nonamended PDA, thiophanate methyl- and vinclozolin-amended PDA. All the isolates completely colonized the agar in the petri dishes within 3 days on the nonamended PDA. The AUMGC value for the nonamended PDA ranged from 127 to 224. Mean mycelial growth of all isolates in the thiophanate methyl- and vinclozolin-amended agar was reduced 18 to 93% and 93 to 99%, respectively, when compared with the nonamended control (Table 3).

In a previous study, no resistance to benomyl was detected within 100 *S. sclerotiorum* isolates collected from a snap bean field treated with benomyl (14). However, field isolates with resistance to fungicides have been reported in other *Sclerotinia* spp. affecting peanut (*Arachis hypogaea* L.) in Virginia (5), lettuce (*Lactuca sativa* L.) in California (13), and creeping bentgrass (*Agrostis palustris* Huds.) in Michigan (6). Hubbard et al. (13) attributed the failure to control *S. minor* with fungicides in the field to fungicide-resistant isolates. The variation in sensitivity to thiophanate methyl observed in 91 isolates indicates that the potential for fungicide resistance in *S. sclerotiorum* exists. However, there was very little variation in sensitivity to vinclozolin. With the variation in *S. sclerotiorum* to thiophanate methyl, there is a need to identify fungicides with different modes of action to control Sclerotinia stem rot. These fungicides with different modes of action could be used in a rotation to help manage further development of fungicide-resistant isolates of *S. sclerotiorum*.

Greenhouse experiments. There was a significant ($P < 0.01$) effect among the four fungicides tested on disease severity, but not a significant effect of cultivar, plant, or any interactions ($P \leq 0.05$). Plants treated with benomyl, thiophanate methyl, and vinclozolin did not express symptoms or signs of Sclerotinia stem rot (Table 4). Plants treated with tebuconazole had both restricted and expanded lesions on leaves, but no stem symptoms or signs were observed. All the plants treated with fungicides survived the inoculation and added new leaves. Plants not sprayed with a fungicide had expanded foliar lesions that caused defoliation, fungal colonized stems, and some dead plants (Table 4). These results agree with previous studies showing that spraying the entire plant with effective fungicides will provide excellent control of *S. sclerotiorum* (14).

Ascospore colonization of blossoms causes the primary infection (1), while secondary infection may also occur on leaves, petioles, and stems from direct contact with infected blossoms and diseased adjacent plants. The inoculation procedure used in this study is similar to natural infection resulting from direct contact. Therefore, with the control exhibited in the greenhouse using this inoculation technique, there is a potential to control secondary spread of Sclerotinia stem rot.

Table 3. Host, location, area under mycelial growth curve (AUMGC), and percent reduction of mycelial growth from thiophanate methyl and vinclozolin for isolates[a] of *Sclerotinia sclerotiorum*

Isolate[a]	Host	County, state, or country	Nonamended AUMGC[b]	Vinclozolin[c] (% inhibition)[d]	Thiophanate methyl[c] (% inhibition)[d]
3	Unknown	North Carolina	203	97.2	17.7
151	Soybean	LaSalle Co., IL	204	94.1	27.3
28	Soybean	Iroquois Co., IL	215	96.5	27.6
103	Soybean	Macon Co., IL	220	94.8	29.8
160	Soybean	Argentina	210	97.1	37.7
34	Rape	Canada	210	94.3	40.7
110	Pear	Oregon	188	95.8	46.0
113	Soybean	Henry Co., IL	221	98.6	66.2
105	Soybean	Iowa	214	98.4	68.4
7	Dry bean	Scotts Bluff, NE	206	95.4	69.0
144	Sunflower	Switzerland	206	94.7	69.3
16	Soybean	Rock Co., WI	212	96.8	78.8
152	Soybean	Champaign Co., IL	209	96.9	84.5
87	Soybean	Indiana	202	97.8	88.6
149	Soybean	Ingham Co., MI	189	98.3	93.2
Mean			208	96.8	59.5
Range			127 to 224	93 to 99	18 to 93
LSD ($P < 0.01$)[e]			10.2	2.0	16.1

[a] A total of 91 isolates of *Sclerotinia sclerotiorum* from the soybean pathogen collection housed at the National Soybean Research Center at the University of Illinois were screened for sensitivity to thiophanate methyl and vinclozolin. The 15 isolates presented here represent different host, locations, and reaction to thiophanate methyl.

[b] AUMGC calculated by: AUMGC = $\sum^{n-1}_{i=1}[(X_{i+1} + X_i)/2][t_{i+1} - t_i]$, in which X_i = colony diameter, expressed in mm at the ith observation, t_i = time (days after inoculation) at the ith observation, and n = total number of observations.

[c] Vinclozolin and thiophanate methyl both were used at 1 µg a.i./ml in potato dextrose agar (PDA).

[d] Percent inhibition of mycelial growth for each fungicide was calculated by dividing the AUMGC of the fungicide amended PDA by the AUMGC of the nonamended PDA for each replication.

[e] LSD = Fisher's protected least significant difference.

Table 4. Disease severity ratings for fungicide treated and untreated soybean seedlings and detached leaves that were inoculated with *Sclerotinia sclerotiorum* in the greenhouse

Fungicide	Rates (kg a.i./ha)	Disease severity[a] (seedlings)	AUDPC[b] (detached leaves)
Nontreated control	–	2.7	48.7
Benomyl	0.56	0.0	1.7
Tebuconazole	0.13	1.1	0.8
Thiophanate methyl	0.78	0.0	0.0
Vinclozolin	1.12	0.0	0.0
LSD ($P < 0.01$)[c]		0.3[d]	4.7[e]

[a] Seedlings inoculated in greenhouse and rated on a scale of 0 to 5, where 0 = no disease, 1 = restricted lesion on leaf, 2 = lesion expanded on leaf, 3 = leaf with expanded lesion and start of stem colonization, 4 = severe stem colonization, 5 = plant completely dead.

[b] Detached leaflets sprayed with fungicides and inoculated with an inverted PDA plug with *S. sclerotiorum*. AUDPC calculated by: AUDPC = $\sum^{n-1}_{i=1}[(X_{i+1} + X_i)/2][t_{i+1} - t_i]$ in which X_i = lesion diameter, expressed in mm at the ith observation, t_i = time (days after inoculation) at the ith observation, and n = total number of observations.

[c] LSD = Fisher's protected least significant difference.

[d] Benomyl, thiophanate methyl, and vinclozolin not included in analysis of variance.

[e] Thiophanate methyl and vinclozolin not included in analysis of variance.

The detached leaf assay used in our experiments to evaluate fungicide efficacy is similar to a method previously used to screen alfalfa (*Medicago sativa* L.) and soybean for resistance to *S. sclerotiorum* (16,22). Thiophanate methyl and vinclozolin prevented *S. sclerotiorum* from colonizing the detached leaf beyond the initial PDA plug. AUDPC was greater ($P < 0.01$) for the no fungicide control (Table 4) compared with the fungicide treatments. There was no significant difference between NK S19-90 and BSR 101 with any fungicide or the inoculated control.

Field experiments. Of the 12 different environments in Illinois, Ohio, and Wisconsin, 11 included at least one cultivar susceptible to Sclerotinia stem rot, while five included a cultivar partially resistant to Sclerotinia stem rot. Five of the 12 environments had low Sclerotinia stem rot incidence (<1%), three environments had low to moderate Sclerotinia stem rot incidence (5 to 25%), and four environments had high Sclerotinia stem rot incidence (>25%). For the Illinois studies, high disease incidence (trial mean of 60%) was achieved only in 1998 (Table 5), but no differences in Sclerotinia stem rot incidence or yield occurred between the fungicide treated and untreated plots. In 1999 and 2000, there was less than 1% disease incidence.

For the Ohio studies, thiophanate methyl, when applied conventionally with both nozzles and the air assist sprayer, significantly ($P \leq 0.05$) reduced the incidence of Sclerotinia stem rot compared with air assist water and nontreated controls in 1998 (Table 6). Interestingly, water applied with the conventional sprayer also reduced Sclerotinia stem rot. Applications of water to soybean plants have reduced the incidence of Sclerotinia stem rot in other trials (A. Dorrance, B. Diers, J. Kurle, C. Grau, B. Potter, and X. B. Yang, *unpublished data*). However, the question of water having a direct effect on the infection process or diluting ascospore inoculum present at the time of application is an area that warrants further research. All of the application techniques with thiophanate methyl significantly increased yields ($P \leq 0.05$) in 1998 compared with the nontreated control. Using the same two spray systems in 1999 and 2000, there was no disease, or no control of Sclerotinia stem rot, respectively. There was also no effect of fungicide on yield in 1999 and 2000. For the Wisconsin study in 1995, there were moderate levels of disease (range 20 to 23% incidence). The fungicide applications had no effect on disease incidence; however, there was a significant ($P = 0.10$) increase in yield for thiophanate methyl at 1.12 kg a.i./ha compared with the control and the low rates of thiophanate methyl and benomyl (Table 7). This yield increase was apparently not related to control of Sclerotinia stem rot. In 1996, disease incidence was higher than in 1995 (range 35 to 52% incidence); however, there was no significant effect on yield for any treatments (Table 7). In 1997, although there were varying levels of Sclerotinia stem rot, there were no statistical differences in disease incidence or yield between the treated and untreated plots. In 1998 and 1999, there was very low disease incidence.

Previous reports have demonstrated inconsistent control of *S. sclerotiorum* in the field with fungicides in different bean species: dry bean (*P. vulgaris*) (26), lima bean (*Phaseolus lunatus* L.) (21), snap bean (2,8,14,15,18,21) and white bean (*P. vulgaris*) (19). From our greenhouse and detached leaf assays, fungicides were able to control Sclerotinia stem rot on soybean in the greenhouse. However, we were not able to obtain consistent, adequate levels of control of Sclerotinia stem rot in the field in a number of field locations and environments. Several reasons could contribute to the lack of control. First, there could be isolates of *S. sclerotiorum* with resistance to thiophanate methyl and benomyl present in these field environments. Although we found isolates with varying levels of resistance to thiophanate methyl, this only demonstrates that there is potential for the fungus to be insensitive. The greenhouse study with four fungicides showed excellent control with complete coverage. Thus, another possible explanation would be

Table 5. Sclerotinia stem rot incidence and soybean seed yield for Pioneer 9363 treated with thiophanate methyl at Watseka, IL, in 1998

Treatment	Rate (kg a.i./ha)	Disease incidence (%)[a]	Yield (kg/ha)
Dry control	–	57.0	3,287
Thiophanate methyl	1.12 (R1)[b]	61.0	3,001
Thiophanate methyl	1.12 (R1) + 1.12 (R3)	63.0	3,154
LSD ($P \leq 0.05$)		n.s.	n.s.

[a] Disease incidence was determined by the number of diseased plants out of 50 in each plot.
[b] Thiophanate methyl applied at growth stages R1 and R3 using a CO_2 pressurized sprayer calibrated to deliver 238 liters/ha at 173 kPa pressure using XR8003VS flat spray tips.

Table 6. Sclerotinia stem rot incidence and yield from a comparison of two spray application techniques with thiophanate methyl for control of *Sclerotinia sclerotiorum* of soybean in Ohio in 1998 and 2000

			Year			
			1998		2000	
Treatment	Sprayer	Nozzle	SSR[a] (%)	Yield (kg/ha)	SSR (%)	Yield (kg/ha)
Nontreated[b]	–	–	22.0	2,949	57.1	2,848
Wet control[b]	Conventional broadcast	XR8002	13.5	3,367	66.6	2,815
	Myers Mity Mist	XR110015	22.0	3,219	–	–
Thiophanate methyl[c]	Conventional broadcast	XR8002	9.5	3,852	62.7	2,902
	Conventional broadcast	D2-23	10.5	3,818	53.5	3,037
	Myers Mity Mist	XR110015	11.0	4,263	42.3	3,731
LSD ($P \leq 0.05$)			8.9	646	n.s.	n.s.

[a] SSR is Sclerotinia stem rot; SSR incidence determined by the number of plants with Sclerotinia stem rot out of 200 in each plot.
[b] Nothing was applied on the soybean plants for the nontreated; wet control water was applied.
[c] Thiophanate methyl was applied at 1.12 kg a.i./ha at the R2 growth stage.

Table 7. Comparison of fungicides and rate of application at the R1 growth stage for the control of Sclerotinia stem rot of soybean in Wisconsin in 1995 and 1996

		1995		1996	
Treatment	Rate (kg a.i./ha)	SSR[a,b] (%)	Yield[b] (kg/ha)	SSR[a,b] (%)	Yield[b] (kg/ha)
Dry control	–	23	3,881	47	2,606
Benomyl	0.56	21	3,749	52	2,703
	1.12	20	4,026	35	2,758
Thiophanate methyl	0.84	21	3,701	49	2,703
	1.12	20	4,123	38	2,779
LSD ($P \leq 0.10$)		n.s.	201	7	n.s.

[a] SSR is Sclerotinia stem rot; SSR incidence was recorded at growth stage R7 by a visual percent estimate of plants killed by *Sclerotinia sclerotiorum* from the center of the plots.
[b] Averaged from three cultivars NK Brand S19-90, BSR101, and Sturdy.

poor coverage of the fungicide on the existing infection sites as well as additional blossoms developing after fungicide has been applied. High yield management practices such as early planting, narrow row spacings, and high seeding rates were utilized in all experiments. While these practices contributed to an environment conducive for Sclerotinia stem rot development, the ability of fungicides to penetrate the soybean canopy was limited. The inability of fungicides to penetrate the soybean canopy resulted in poor coverage of soybean blossoms. The water-sensitive paper indicated that there was <1% coverage at the soil surface under the soybean canopy, and <2% coverage 25 cm below the top of the canopy, compared with about 80% coverage on the top of the canopy using the CO_2 pressurized sprayer system in the Illinois field study. The importance of covering blossoms with fungicides was reported on snap bean (14,19). With complete coverage, fungicides may control Sclerotinia stem rot (4,14,19), but a more efficient method of penetrating the canopy with fungicide in the field, especially in the 19-cm rows, needs to be identified. In 76-cm rows, using three flat-fan nozzles (1 nozzle horizontal above the row and 1 on each side of row spraying downward) for applying benomyl suppressed disease development and increased seed yield of white bean, compared with using only one flat-fan nozzle per row (19). Additional advantages of penetrating the soybean canopy would be covering the soil surface with the fungicide. This may result in reducing sclerotial germination, apothecial production by germinating sclerotia, rate of apothecial production, and delay in apothecial emergence (28).

Further studies are needed on application techniques such as drop nozzles or with fungicides that will systemically move down the plant to protect the stems. In these field evaluations, the studies were established in fields with a history of Sclerotinia stem rot and managed to provide an environment conducive to Sclerotinia stem rot development. However, over the different years and environments, disease developed 40% of the time. Even if soybean blossoms were protected with fungicide, there would be no advantage to applying fungicides in years with no disease. Although further research and technology are needed to achieve greater efficacy of fungicides, research to more accurately forecast Sclerotinia stem rot epidemics is equally important in order to manage Sclerotinia stem rot in a profitable manner.

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