Development of Sclerotia and Apothecia of *Sclerotinia sclerotiorum* from Infected Soybean Seed and Its Control by Fungicide Seed Treatment

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ABSTRACT

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Field and laboratory studies were done to evaluate the development of sclerotia and apothecia of *Sclerotinia sclerotiorum* from soybeans and its control with fungicide seed treatment. Soybean seed infected with *S. sclerotiorum* produced mycelia on both seed coats and cotyledons after 48 h on potato dextrose agar (PDA). Obviously infected soybean seed also were placed in aluminum pans containing field soil and placed in soybean fields near Urbana, Illinois and Clinton, Wisconsin. In 1997, a total of 553 sclerotia, 20 stipes, and 10 apothecia were produced from 500 infected seeds. In 1998, 201 sclerotia and 22 stipes were produced, but no apothecia were observed from the 500 infected seeds. Fludioxonil was the most effective fungicide for reducing radial growth of *S. sclerotiorum* on PDA plates and suppressed 99% of the radial growth at 0.1 µg a.i./ml. *S. sclerotiorum* was recovered from 2% of soybean seed lots containing infected seed. When this seed lot was treated with several fungicides, captan + pentachloronitrobenzene + thiabendazole and fludioxonil completely inhibited mycelial growth from infected seed; thiram and thiabendazole each reduced recovery of *S. sclerotiorum* by 90%. In the field, 754 sclerotia and 10 apothecia were produced from 1,000 infected seeds over a two-year period. When evaluating fungicide control in the field, thiram, fludioxonil, and captan + pentachloronitrobenzene + thiabendazole reduced sclerotia formation from infected seed by more than 98%.

Sclerotinia stem rot (SSR) of soybeans (*Glycine max* (L.) Merr.) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is now recognized as a major disease in the North Central region of the United States. There have been severe outbreaks in 1992, 1994, and 1996 in this region (6,7). In 1994, SSR was ranked the most severe disease in Argentina and the second most important disease in the United States (20).

Sclerotinia spp. have a broad host range that includes many important crops, such as alfalfa, dry bean, cabbage, canola, lettuce, peanut, soybean, and sunflower (4,5). Seed infection has been reported on some of these hosts, including dry beans (3,11,16), peanuts (2), soybeans (7,9,13,17), and sunflowers (14).

Over long distances, the greatest potential of *Sclerotinia* spp. dissemination is probably by seed infected with mycelia, or sclerotia mixed with the seed (1,3). Infected seeds appear as smaller, lighter, white, cottony seeds (18). Occasionally, normal-appearing seed is infected with *S. sclerotiorum* but at a very low percentage (7,9). Seed from various hosts, infected with *S. sclerotiorum*, can produce abundant mycelia and eventually sclerotia in either a moist chamber or on artificial media (3,7,12,17). Both the seed coat and the cotyledons have been shown to be colonized by *S. sclerotiorum* (17).

S. sclerotiorum uses the seed of dry beans (19) and soybeans (21) as a nutrient base and may produce sclerotia in soil, thus allowing the fungus an opportunity to establish itself in new fields. For dry beans, under the right environmental conditions, some sclerotia formed apothecia in the same season (11). There are no similar reports for soybean.

The objectives of this study were to (i) confirm that *S. sclerotiorum* was present in the seed coat and cotyledons in the soybean seed, (ii) determine if sclerotia produced from infected soybean seed in the soil could germinate to form apothecia in the same season, and (iii) determine if fungicide seed treatments can control sclerotia formation from infected seed.

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MATERIAL AND METHODS

Seed infection. Seed of Pioneer Brand P9342 was harvested from a soybean field with a high incidence and severity of SSR (9). Based on visible symptoms, healthy and infected soybean seed was separated. Seed coats were removed from the cotyledons and both were surface disinfested in NaOCl (0.53%) for 5 min, rinsed with sterile distilled water, and placed on potato dextrose agar (PDA, Difco Laboratories, Detroit), incubated for 2 to 5 days at 25°C, and observed daily to determine if mycelial growth occurred on the cotyledons, seed coats, or both.

Sclerotia and apothecia formation from infected seed in field. Seed with symptoms caused by *S. sclerotiorum* was separated from seed without symptoms. A total of 50 symptomatic seeds were planted in field soil (1.25 liters) in aluminum pans (20 by 20 cm). The soil type was Plano silt loam and Drummer silt loam at Clinton, Wisconsin and Urbana, Illinois, respectively. The soil was taken from fields with no prior report of infestation of *S. sclerotiorum*. The aluminum pans had several holes punctured in the bottom for water drainage. Each pan was regarded as one replication and a total of five replications per location were used. The experiment was repeated in 1998. The pans were placed in the field at both locations on 3 June and 1 June in 1997 and 1998, respectively, and left under the soybean canopy for 3 months. The soil in the pans dried more quickly than soil in the fields; therefore, approximately 600 ml of water was added on a daily basis to the pans to maintain similar moisture levels. From each pan, soil was collected on 28 August 1997 and 4 September 1998. The number of apothecia formed was observed each day (starting on 1 July in both years) until the soil was collected. The soil was washed through a #18 (1-mm) sieve. The sclerotia were separated from the debris and the number of stipes formed was counted. An analysis of variance was used with year and location combined to form an environment variable which was designated as a random variable (15).

Mycelial growth on fungicide-amended agar. PDA was autoclaved and allowed to cool to 55°C. Several seed treatment fungicides, including captan (Captan 400, Gustafson, Plano, TX), fludioxonil (Maxim, Novartis, Greensboro, NC), metalaxyl (Apron FL, Gustafson), pentachloronitrobenzene (PCNB, Gustafson), thiabendazole (LSP, Gustafson), and

thiram (Thiram 42S, Gustafson) were evaluated in this study. The fungicides were diluted to 0.1, 1, 10, 50, 100, and 500 µg a.i./ml in the PDA. Non-amended agar was used as a control. *S. sclerotiorum* (isolate SSR-113) was grown on non-amended PDA. Plugs (6 mm in diameter) were taken from the margins of the colony and transferred to the center of the five replicated petri plates (9 cm diameter) of each treatment. Plates were then placed in a completely randomized design in a growth chamber at 25°C with a fluorescent light near 100 µmol $m^{-2}s^{-1}$. The length and width of the radial growth was measured 1, 2, 3, and 4 days after inoculation. The area under mycelia growth curve (AUMGC) was calculated by using colony size as the dependent variable and the four dates as the independent variable (15). The entire experiment was repeated.

Control of sclerotia formation from infected seed in a seed germinator. A total of 10 fungicidal seed treatments were tested to control the sclerotia formation from seed infected with *S. sclerotiorum* under controlled laboratory conditions. Healthy (Pioneer Brand 9342) and symptomatic seed was mixed in a 95:5 ratio and 100 g of seed was treated with thiabendazole + captan (Agrosol F, WILFARM, Gladstone, MO), thiabendazole + thiram (Agrosol T), metalaxyl, captan, fludioxonil, pentachloronitrobenzene, captan + pentachloronitrobenzene + thiabendazole (Rival, Gustafson), thiram, and carboxin + thiram (Vitavax 200, Gustafson). A total of 200 seeds from each treatment were placed on saturated KimPac (Unisource-Decatur, Jacksonville, FL) blotters (42 by 62 cm, 30-ply sheets) and placed in a seed germinator (Stults Scientific Engineering Corporation) to determine percent germination and soybean seed infection. The seed was considered germinated if the radicle was longer than the seed after 5 days, and considered infected if white mycelial growth developed and formed sclerotia. The experiment was repeated three times with two replications per trial. Analysis of variance was used with replications designated as a random effect and fungicides as a fixed effect (15).

Control of sclerotia formation from infected seed in the field. In 1997, seed lots with an 80:20 blend of healthy and infected seed (Pioneer Brand P9342) were treated with fludioxonil, thiram, and captan + pentachloronitrobenzene + thiabendazole. In 1998, the study was repeated with the addition of a thiabendazole treatment alone. At Urbana, Illinois and Clinton, Wisconsin, 100 seeds of each seed treatment and 100 non-treated seeds were placed into aluminum pans as previously described, each containing 1.25 liters of soil. The pans were placed in a soybean field on 3 June and 1 June in 1997 and 1998, respectively, and left under the soybean canopy for two months. Soybeans that emerged from the soil were clipped off, except for three to four plants that helped provide shade while the rows closed. The soil was collected from the pans and washed through a 1-mm sieve on 6 August and 22 August in 1997 and 1998, respectively. The debris was allowed to dry and the sclerotia were separated from the debris. Analysis of variance was used with year and locations combined into an environment variable which was a random effect, while fungicide was a fixed effect (15).

Table 1. Area under mycelial growth curve (AUMGC) for fungicides amended in potato dextrose agar (PDA) at six concentrations[a]

	Concentration (µg.a.i./ml)					
Seed treatment	**0.1**	**1**	**10**	**50**	**100**	**500**
Captan	205	205	204	186	53	0.3
Fludioxonil	2.3	0.03	0	0	0	0
Metalaxyl	204	205	195	203	200	188
PCNB	204	199	118	117	114	42
Thiabendazole	195	67	2	2	2	1
Thiram	208	208	185	12	1	0.1

[a] AUMGC value for control (non-amended agar) is 237. AUMGC calculated by: AUMGC = $\Sigma_{i=1}^{n-1}[(X_{i+1} + X_i)/2][t_{i+1} - t_i]$, in which X_i = lesion diameter, expressed in mm at the *i*th observation, t_i = time (days after inoculation) at the *i*th observation, and n = total number of observations. Least significant difference (0.05) = 25.

Table 2. Evaluation of 10 seed treatments for percent seed germination and control of *Sclerotinia sclerotiorum* sclerotia formation from infected seeds at a 5% infection level in a seed germinator

Seed treatment	**Rates (ml a.i./kg)**	**Seed germination (%)**	**Infected seeds/replication[a]**
Control	…	92.9	4.0
Captan	0.50	91.8	0.7
Fludioxonil	0.02	91.9	0.0
Metalaxyl	0.28	90.1	2.3
PCNB	1.58	93.3	3.0
Thiabendazole	0.79	93.5	0.4
Thiram	0.55	92.6	0.4
Captan + PCNB + thiabendazole	0.78	93.5	0.0
Carboxin + thiram	0.90	79.0	0.0
Thiabendazole + captan	0.49	91.8	0.3
Thiabendazole + thiram	0.57	93.5	0.0
LSD (0.05)[b]	…	4.5	1.2

[a] A seed was considered infected if it formed white mycelia with eventually formed sclerotia after five days. Each replication consisted of 200 seeds placed on a saturated KimPac blotter and put in a seed germinator at 25°C.
[b] LSD = least significant difference.

Table 3. Evaluation of four seed treatments for control of *Sclerotinia sclerotiorum* sclerotia formation from infected seeds in the field

		Number of sclerotia formed[a]			
		Clinton		Urbana	
Seed treatment	**Rates (ml a.i./kg)**	**1997**	**1998**	**1997**	**1998**
Control	…	175	89	149	61
Fludioxonil	0.02	0	6	1	1
Thiram	0.55	0	4	1	3
Captan + pcnb + thiabendazole	0.78	0	2	0	1
Thiabendazole	0.79	…	24	…	3

[a] Year and location were combined to form an environment variable. Environment × fungicide interaction was significant ($P < 0.01$). Thiabendazole was not evaluated in 1997. Least significant difference (0.05) = 44.

RESULTS AND DISCUSSION

Seed infection. No mycelial growth was observed after 24 h from any seed. After 48 h, mycelia were observed from both seed coats and the cotyledon from soybean seed with obvious symptoms, as well as from a few seeds with no obvious symptoms. This agrees with earlier reports that indicate seed infection is in the cotyledons (17). Several seeds that did not show visible signs of infection germinated after 48 h, but mycelium was present on the cotyledons that colonized the radicals within 72 h. Most seed that was obviously infected did not germinate. This is important because *S. sclerotiorum* uses the soybean cotyledons as an energy source, which eventually leads to the formation of sclerotia in the soil (21).

Sclerotia and apothecia formation from infected seed in field. From the 500

infected seeds (two locations, five replications, 50 seeds/replication) placed in pans in 1997, 553 sclerotia formed. Of these 553 sclerotia, 20 formed stipes and 10 formed apothecia. Observations for apothecia formation started on 1 July 1997, but no apothecia were formed until 14 to 22 August. In 1998, 201 sclerotia were produced from the 500 infested seeds, which is less ($P < 0.05$) than in the previous year. A total of 22 sclerotia formed stipes, but no apothecia were observed. The potential of SSR to be introduced into uninfested areas through infected seed or sclerotia physically mixed with the seed has already been reported (19). However, the ability of the sclerotia produced from infected seed to germinate and form apothecia in the same season clearly indicates a greater chance of infection of soybeans. With the right environmental conditions, the fungus will infect plants and increase the amount of sclerotia present in the soil for the following seasons. We have not demonstrated infection of soybean plants from infected seed in the same season.

This data indicates the importance of planting clean seed in fields not infested with *S. sclerotiorum*. Fields already having a SSR problem will not be greatly affected by additional sclerotia formed from infected seed. However, planting infected seed in fields with no previous introduction of the fungus is an efficient way for the pathogen to spread over long distances (1).

Mycelial growth on fungicide-amended agar. Radial growth of *S. sclerotiorum* was significantly less on agar amended with the six fungicides compared to growth on the control agar (Table 1). Fludioxonil inhibited mycelial growth at 0.1 µg a.i./ml and thiabendazole inhibited mycelial growth at 10 µg a.i./ml. Thiram and captan inhibited mycelial growth at 100 and 500 µg a.i./ml, respectively. Metalaxyl and pentachloronitrobenzene fungicide reduced mycelial growth at all concentrations (Table 1), but neither was as effective as the other four fungicides.

Control of sclerotia formation from infected seed in a seed germinator. Germination ranged from 90 to 94% for all seed lots, except seed treated with carboxin + thiram, which had 79% germination ($P < 0.05$; Table 2). All of the fungicide seed treatments reduced infection frequency, except for pentachloronitrobenzene. Fludioxonil, captan + pentachloronitrobenzene + thiabendazole, carboxin + thiram, and thiabendazole + thiram completely inhibited mycelial growth from infected seed, while thiabendazole, thiram, and thiabendazole + thiram reduced mycelial growth from seed by 90% (Table 2). Although metalaxyl controls oomycetes, fewer sclerotia were formed with the metalaxyl treatment than the control. We cannot explain this apparent control of seed infection with metalaxyl. All of the seed treatments containing thiabendazole controlled most, if not all, sclerotia formation. Combinations of active ingredients such as captan + pentachloronitrobenzene + thiabendazole and carboxin + thiram also showed excellent control.

Control of sclerotia formation from infected seed in field. In 1997, seed not treated with fungicides formed 324 sclerotia or approximately 2.1 sclerotia per infected seed. Seed treated with fludioxonil and thiram produced only one sclerotium, while seed treated with captan + pentachloronitrobenzene + thiabendazole formed no sclerotia (Table 3). In 1998, the total number of sclerotia recovered from the soil was less than 1997. For non-treated seed, 150 sclerotia were formed, which is approximately 0.9 sclerotia per infected seed. Seed treated with fludioxonil and thiram produced seven sclerotia. Seed treated with captan + pentachloronitrobenzene + thiabendazole formed 3 sclerotia, while seed treated with thiabendazole alone formed 27 sclerotia (Table 3). The application of fungicide seed treatments is an efficient method of reducing sclerotia formation from infected seed (Table 3). Studies conducted in Brazil showed that seed treatments (benomyl, captan, thiabendazole, and thiram) could control 100% of the sclerotia formation from infected seed (10,22). Also, fungicide treatment of sunflower seed reduced seed-borne *S. sclerotiorum* (8). In this study, fludioxonil, thiram, and captan + pentachloronitrobenzene + thiabendazole controlled over 98% of sclerotia formation from infected seed over a 2-year study.

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