Fungi Associated with Soybean Seedling Disease in Iowa

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ABSTRACT

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Surveys were conducted over a 2-year period to identify fungi associated with soybean seedling disease in Iowa. Fungi were isolated from diseased soybean seedlings collected from 52 and 66 locations in 1993 and 1994, respectively. The percentages of major fungal taxa isolated from soybean seedlings in the 2 years were *Rhizoctonia solani*, 27.5% in 1993 and 27.3% in 1994; *Fusarium* spp., 11.9% in 1993 and 13.7% in 1994; and *Pythium* spp. and *Phytophthora sojae*, cumulatively 60.5% in 1993, and 31.7% and 24.3% in 1994 respectively. Other isolated fungi were the seed decay pathogen *Phomopsis longicolla*, and the nonpathogenic *Rhizopus stolonifer* and *Trichoderma viride*. Species of *Fusarium* and *Pythium* were identified as *F. acuminatum*, *F. equiseti*, *F. oxysporum*, *P. aphanidermatum*, *P. irregulare*, *P. myriotylum*, *P. sylvaticum*, *P. ultimum* var. *sporangiiferum*, and *P. ultimum* var. *ultimum*. Repeated tests of pathogenicity confirmed that *Pythium* spp., *Phytophthora sojae*, and *R. solani* were the major causal fungi associated with the seedling disease complex of soybeans in Iowa.

Seedling diseases are among the most widely distributed diseases of soybean (*Glycine max* (L.) Merr.) (6,19). Under field conditions favorable for seedling disease development, yield losses can be significant. For instance, in 1971 Tachibana et al. (23) reported yield losses as great as 60% caused by *Rhizoctonia solani* Kühn in Iowa.

Several microorganisms have been associated with seedling disease problems in soybean. For example, in Mississippi, Killebrew et al. reported on the occurrence of 38 fungal taxa in infected soybean seedlings (7). Similarly, 12 fungal taxa were isolated from diseased soybean seedlings in Florida (16). In Iowa, no comprehensive survey on the causal agents of seedling disease has been conducted. Tachibana et al. first recorded Phytophthora root rot in soybean and reported seedling blight caused by *Phytophthora sojae* M.J. Kaufmann & J.W. Gerdemann in Iowa in 1966 (22). A Fusarium blight of soybeans was reported in Iowa by Dunleavy in 1953 (4). Currently, seed treatment is the major method to control soybean seedling diseases, and effectiveness of the treatment relies on up-to-date information on the major causal agents. The objective of this study was to conduct statewide surveys to determine the major causal agents associated with the post-emergence seedling disease complex in Iowa.

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MATERIALS AND METHODS

Isolation from diseased seedlings. The study was conducted in 1993 and 1994 in Iowa. Commercial soybean fields under all types of tillage were surveyed. In 1993, 52 arbitrarily selected field samples were collected from 31 counties out of 60 counties surveyed. In 1994, 66 samples were collected from 57 counties out of 99 counties surveyed. These samples represented a wide geographic area. Fifteen to 20 soybean seedlings with seedling disease symptoms were sampled from each site by digging whole plants. Plants in each sample were placed in a cooler and transported to the laboratory. Death or injury of soybean seedlings due to herbicides was identified by spray patterns that were normally uniform, and such seedlings were excluded. In each field, sampling date, soybean growth stage (5), size of the disease patches, and visual stand reduction in the patches (percent dead seedlings, as assessed by comparison of diseased areas versus healthy areas) were recorded. The patch sizes were designated small (less than 10 m^2), medium (11 to 100 m^2), and large (greater than 100 m^2), respectively.

In 1993, isolations from diseased soybean seedlings were made on water agar. In 1994, water agar was again used for isolating fungi, as well as $P_{10}VP$ for *Pythium* spp. and *Phytophthora sojae* (24), potato-dextrose agar (PDA) amended with 100 mg of streptomycin sulfate per liter for *R. solani* (20), and Nash-Snyder for *Fusarium* spp. (13). Tissues of seedlings were rinsed in tap water for 1 h, surface disinfested for 1 min in 0.53% sodium hypochlorite, and rinsed with sterile water. Three or four diseased root–hypocotyl segments per plant, each measuring 0.5 cm, were plated per plate. The plates were incubated in the dark at 20°C. Hyphae from the edges of emerging fungal colonies were transferred 24 to 96 h later and stored for further identification.

The identification of *Fusarium*, *Pythium*, *Phytophthora sojae*, *R. solani*, and other fungi was based on colony and spore morphology by the recommended keys and texts (11,19,20,26). No effort was made to separate *Pythium* from *Phytophthora* in 1993. In 1994, *Phytophthora sojae* was separated from *Pythium* using taxonomic keys. ELISA kits for *Phytophthora sojae* and *Pythium* (Neogen Corp., Lansing, MI 48912) also were used when necessary. The proportions of seedlings with the fungus were calculated for individual fields and then summarized into climatological zones.

To determine anastomosis groups for *R. solani* isolates, a total of 20 isolates of *R. solani* in 1993 and 28 in 1994, respectively, were arbitrarily selected and tested. Cultural characteristics such as the presence of dolipore septa, multinucleate hyphae, and complete or incomplete sites of fusion in anastomosing hyphae, provided proof of identifications. The isolates were paired with tester strains of AG-1, AG 2-2, and AG-4 for anastomosis using previously described techniques (2,8,12). Anastomosis groups AG-1 and AG-4 were furnished by C. Martinson of Iowa State University, and AG-2-2 was furnished by R. Jones and C. E. Windels of the University of Minnesota.

Since the species of *Pythium* or *Fusarium* isolated from seedlings could also be nonpathogenic, a total of 82 arbitrarily selected *Pythium* speciated isolates (4 of *P. aphanidermatum* (Edson) Fitzp., 46 of *P. irregulare* Buisman, 17 of *P. sylvaticum* W.A. Campbell & J.W. Hendrix, and 15 of *P. ultimum* Trow.) and 22 arbitrarily selected *Fusarium* isolates were tested for pathogenicity. Seedlings of susceptible soybean cultivar IA 2007 at V1 growth stage were hypocotyl wound-inoculated with 7-day-old fungal cultures (21,27). The plants were incubated at 100% relative humidity in the dark at 16 to 18°C for 72 h. Hypocotyl-wounded seedlings that were not inoculated served as controls. There were five seedlings per pot, four pots per isolate. Isolates were consid-

ered pathogenic if more than 60 to 70% of the inoculated seedlings were lodged, stunted, or dead as a result of lesion development on the hypocotyl. Tests were also conducted to determine the pathogenicity of 32 *R. solani* isolates on soybean seedlings in pots filled with sterilized soil mixed with inoculum (1-week-old cultures in PDA). Pre- and postemergence damping-off were recorded for each isolate. Pathogenicity assays for *Phytophthora sojae* were conducted on cultivar Williams by the hypocotyl wound-inoculation method. However, races were not determined (18). Pathogenicity tests for all groups of fungi were repeated at least once.

Weather data for state average air temperature, soil temperature, and precipitation from May to July were analyzed. The data were obtained from the state climatology office, Iowa Department of Agriculture and Land Stewardship, Des Moines.

RESULTS

Average state temperatures in 1993 for May to July ranged from 15.4 to 20.4°C (0.7 to 1.6°C below normal), and the precipitation range for the same period was 141.7 to 266.7 mm (40.6 to 161.5 mm above normal). Soybean planting was generally delayed 2 weeks in 1993. The cool and wet weather pattern of 1993 favored the development of postemergence damping-off seedling disease of soybeans. In contrast, average state temperatures in 1994 for May to July were 16.2 to 21.5°C (0.1 to 0.6°C above normal), and the precipitation range for the same period was 53.3 to 99.3 mm (0.23 to 1.88 mm below normal).

Diseased soybean seedlings were collected from 52 and 66 commercial soybean fields in the spring of 1993 and 1994, respectively. The areas sampled covered 31 counties for 1993 and 57 counties for 1994, across all nine climatological zones of Iowa (Fig. 1).

In 1993, 52 fields were sampled. Less than 10% visual stand reduction was recorded in 30 fields with small patches of diseased seedlings (Table 1). A range of 10 to 60% stand reduction was observed in six fields with medium patches of diseased seedlings. Fifteen commercial soybean fields were observed with large patches of diseased seedlings having a stand reduction ranging from 20 to 90%. Fields with large patches of seedling disease were mainly observed in central and northwestern Iowa. In 1994, areas of diseased seedlings were usually small, except in five fields where large patches of diseased seedlings were observed. Twelve fungal taxa were identified among the 1993 isolates: *Fusarium acuminatum* Ellis & Everh., *F. equiseti* (Corda) Sacc., *F. oxysporum* Schlechtend.:Fr., *Phomopsis longicolla* T.W. Hobbs, *Phytophthora sojae*, *Pythium aphanidermatum*, *P. irregulare*, *P. myriotylum* Drechs., *P. sylvaticum*, *P. ultimum* var. *sporangiiferum* Drechs., *P. ultimum* var. *ultimum* Trow., and *R. solani*. In 1994, all of the above taxa plus, *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. and *Trichoderma viride* Pers.:Fr. were isolated.

R. solani was isolated from 27.5% of the seedlings in 1993 (Table 2) and 27.3% of the seedlings in 1994 (Table 3). *Fusarium* spp. were isolated from 11.9% of the seedlings in 1993 and 13.7% of the seedlings in 1994. Species of *Pythium* and *Phytophthora sojae* were cumulatively isolated from 60.6% of the seedlings in 1993. In 1994, *Pythium* spp. and *Phytophthora sojae* were isolated from 31.7% and 24.3% of the seedlings for 1994, respectively. *R. solani* isolates included anastomosis groups of AG-2-2 and AG-4, with the latter as the predominant type. *Phomopsis longicolla*, *Rhizopus stolonifer*, and *T. viride* were infrequently isolated from less than 2% of the seedlings in 1994. Pathogenicity tests with *R. stolonifer* and *T. viride* were not run, as these soil fungi are commonly considered not pathogenic. Pathogenicity tests with 82 isolates of *Pythium* spp., 22 isolates of *Fusarium* spp., and 32 isolates of *R. solani* indicated

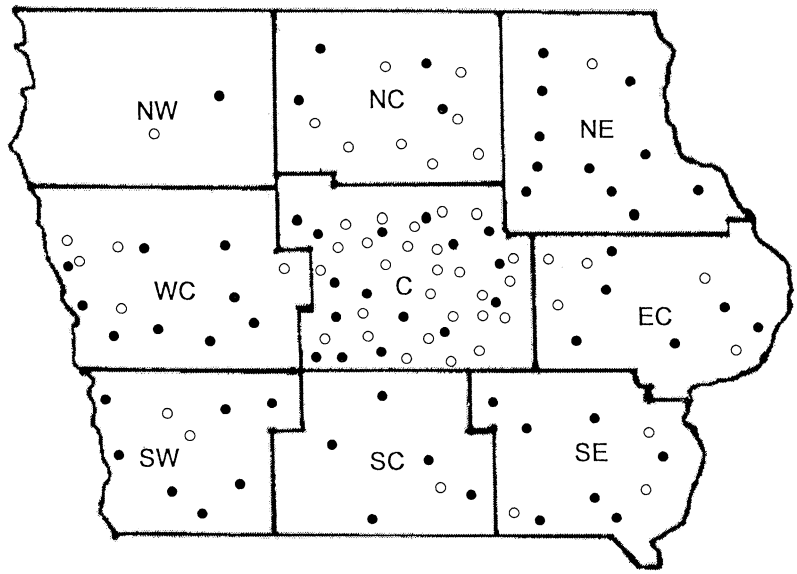

Fig. 1. Sampling map for surveying seedling disease pathogens in 1993 (open circles) and 1994 (closed circles) in Iowa climatological zones: NW = northwest, WC = west central, SW = southwest, NC = north central, EC = east central, C = central, SC = south central, NE = northeast, SE = southeast.

Table 1. Fields surveyed in Iowa in 1993 and 1994 and percent stand reduction in areas with diseased soybean seedlings

	Fields sampled			Stand reduction (%)		
Zone[a]	Small[b]	Medium	Large	Small	Medium	Large
1993	At soybean growth stages V1-V2 (5)					
NW	3	0	0	<1	0	0
NC	4	0	3	<1	0	20-75
NE	1	0	0	<1	0	0
WC	3	0	1	<1	0	40
C	14	4	9	<10	10-60	30-90
EC	5	0	0	<1	0	0
SW	0	0	1	0	0	60
SC	0	0	1	<0	0	40
SE	1	2	0	<1	20-30	0
Total	31	6	15	–	–	–
1994	At soybean growth stages V1-V2 (5)					
NW	1	0	0	<1	0	0
NC	0	2	2	0	30	30-50
NE	11	0	0	<1	0	0
WC	9	0	0	<1	0	40
C	14	1	1	<1	10	30
EC	6	0	0	<1	0	0
SW	5	0	2	<1	0	90
SC	5	0	0	<1	0	0
SE	7	0	0	<1	0	0
Total	58	3	5	–	–	–

[a] Climatological zones: NW = northwest, NC = north central, NE = northeast, WC = west central, C = central, EC = east central, SW = southwest, SC = south central, SE = southeast.

[b] Patch size: small < 10 m^2, medium = 10 to 100 m^2, large > 100 m^2.

that 94% of *Pythium*, 21% of *Fusarium* isolates, and 75% of *R. solani* were pathogenic.

In both years, *Pythium/Phytophthora*, *R. solani*, and *Fusarium* were isolated from diseased seedlings in fields where only small patches of diseased seedlings were observed. For fields with medium or large patches, usually there was one dominant agent. In 1993, of the six medium-sized patches of diseased seedlings, three of these areas were caused mainly by *Pythium/Phytophthora*, one by *Fusarium* spp., and two by *R. solani* and *Pythium/Phytophthora*. Of the 15 fields with large patches of diseased seedlings in 1993, seven were caused by *Pythium/Phytophthora*, one was caused mainly by *R. solani*, and disease for the remaining sites was caused by a combination of these three fungal taxa. In 1994, *Phytophthora sojae* was isolated from two sites with large patches (90% stand reduction on 21 ha).

DISCUSSION

Many fungi have been reported as causal agents of soybean seedling diseases in North America. Our study provides the first documentation on the distribution and frequency of these causal agents in Iowa. The results showed species of *Pythium*, *Phytophthora sojae*, and *R. solani* as the major causes of seedling disease in Iowa. The seed-decaying pathogen *Phomopsis longicolla*, the nonpathogenic fungi *Rhizopus stolonifer*, and *T. viride* also were infrequently isolated from diseased soybeans. Of the three major groups of fungi, *Pythium* and *Phytophthora* seem to be the most important components of the seedling disease complex based on their frequency of isolation and the isolation of these fungi from medium and large patches of diseased soybeans. The two pathogens were isolated from over 56% of seedlings each year. *Fusarium* spp. were isolated in relatively low frequency. Many *Fusarium* spp. may be secondary colonizers, as indicated by their low level of pathogenicity in hypocotyl assays. This study showed that *Fusarium* species are less frequently isolated from diseased seedlings than are *Pythium/Phytophthora* or *R. solani* in Iowa, a conclusion different from that reported in 1961 (4). Differences may be due to the changes in cultural practices that have occurred in the last 35 years.

In most of the fields surveyed, two or more pathogens were isolated from diseased seedlings, although there was often a dominant agent. Previous studies (3,17) showed associations of *Fusarium* infections with *Pythium* or *R. solani*. *Pythium* and *Fusarium* spp. were regularly isolated together in our samples. Schlub and Lockwood also reported a regular association of *Pythium* spp. and *Fusarium* spp. in soybean seedlings afflicted with preemergence seedling rot in Michigan (17). *Fusarium* spp. and *R. solani* also were often isolated from the same seedlings. Datnoff and Sinclair reported the association of *R. solani* and *F. oxysporum* in causing a root rot of soybean in Illinois (3). Both pathogens have been reported previously in Iowa (4,9,23), Minnesota (25), and Mississippi (7).

Results of 2 years of isolations also showed the existence of a *Pythium* complex on soybean in Iowa. This complex consisted of *P. aphanidermatum*, *P. irregulare*, *P. myriotylum*, *P. sylvaticum*, *P. ultimum* var. *sporangiiferum*, and *P. ultimum* var. *ultimum*. Our preliminary study (15) suggested that *P. irregulare* and *P. ultimum* may be the major agents. Rayside et al. (14) found in Florida that *P. irregulare* was the predominant *Pythium* species, with populations increasing at the beginning of soybean growing season and declining at the end of the season. However, Griffin found that *P. ultimum* was the main component of a fungal complex on soybeans in Virginia (6). In Minnesota, *P. ultimum* and *P. debaryanum* were reported as the most prevalent preemergence damping-off pathogens in soybean (1). McCarter and Littrell first reported susceptibility of soybean to *P. myriotylum* (10). We also isolated *P. myriotylum* from diseased soybean seedlings. Damping-off caused by *P. ultimum* and *P. sylvaticum* is favored by cool and wet soil (19), but *P. aphanidermatum* appears in middle to late summer when temperatures are high (20). Future study of the importance of each species will be useful for disease management.

The objectives of this study were to survey and determine the major causal agents of the seedling disease complex in Iowa and to note the prevalence of soilborne pathogens. Results of our study, however, can be utilized in future research in Iowa on the prevalence of soilborne pathogens in different tillage practices. *Pythium/Phytophthora* and *Rhizoctonia* accounted for 75 to 90% of total isolations and were major causal agents in damping-off found in large patches. Results from this study may provide guidance for timely seed treatment. In Iowa, the selection of chemicals for seed treatment should include compounds targeted at these three pathogens if information of causal agents in a specific field is not available.

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Table 2. Percent fungi from selected genera isolated from soybean seedlings in 1993

Zone[a]	Number of fields[b]	*Phytophthora/ Pythium* spp.	*Rhizoctonia solani*	*Fusarium* spp.
NW	1	100	0	0
NC	8	53	30	17
NE	1	43	44	13
WC	5	57	28	15
C	26	64	25	11
EC	5	42	46	12
SW	2	57	30	13
SC	1	71	25	4
SE	3	79	14	7
Mean[c]		60.6	27.5	11.9

[a] Climatological zones: NW = northwest, NC = north central, NE = northeast, WC = west central, C = central, EC = east central, SW = southwest, SC = south central, SE = southeast.
[b] Soybean growth stages V1-V2. V1 = completely unrolled leaf at unifoliolate stage; V2 = completely unrolled leaf at first node above the unifoliolate node (5).
[c] Means given by sample number per region as weight factor.

Table 3. Percent fungi isolated from soybean seedlings in 1994

Zone[a]	Fields (no.)	*Pythium* spp.	*Phytophthora sojae*	*Rhizoctonia solani*	*Fusarium* spp.	*Trichoderma viride*	*Phomopsis longicolla*	*Rhizopus stolonifer*
NW	1	0.0	0.0	0.0	100.0	0.0	0.0	0.0
NC	4	13.8	59.8	13.7	5.7	1.4	4.2	1.4
NE	11	31.3	23.0	26.2	18.7	0.8	0.0	0.0
WC	9	31.0	18.1	37.5	13.4	0.0	0.0	0.0
C	16	24.9	29.8	29.6	12.1	1.2	1.2	1.2
EC	6	52.4	11.7	21.6	10.0	4.3	0.0	0.0
SW	7	25.4	21.9	34.5	10.9	0.0	7.3	0.0
SC	5	40.6	30.0	17.0	9.8	2.6	0.0	0.0
SE	7	46.3	13.0	26.9	11.0	0.0	2.8	0.0
Mean[b]		31.7	24.3	27.3	13.7	1.1	1.6	0.4

[a] Climatological zones: NW = northwest, NC = north central, NE = northeast, WC = west central, C = central, EC = east central, SW = southwest, SC = south central, SE = southeast.
[b] Means computed by the number of samples per region as a factor.

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