Effects of *Cercospora kikuchii* on Soybean Seed Germination and Quality

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ABSTRACT

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Soybean (*Glycine max*) seeds of 13 cultivars were harvested from plants field-inoculated with one of four isolates of *Cercospora kikuchii*. There was an inverse correlation between purple-stained seeds and percentage of germination on potato-dextrose agar and cellulose pads. Recovery of *C. kikuchii* was significantly greater for all isolates from seeds of cultivars Amsoy 71, Bragg, Davis, Hood 75, and Williams than from seeds of Bedford, Dare, Forrest, Lee 74, Mack, PI 80837, and Pickett 71. The correlation coefficient between the recovery of *C. kikuchii* and *Phomopsis longicolla* was $R^2 = -0.59$ and for the occurrence of *Alternaria*, *Aspergillus*, and *Fusarium* spp. it was $R^2 = -0.37$. Soybean seed lots from the cultivars Amsoy 71, Bragg, Davis, Hood 75, Tracy, and Williams uninoculated or inoculated in the field with one of four isolates of *C. kikuchii* and a seed lot from naturally infected Sieban brand plants also were studied. An analysis of combined data over all cultivars showed that seeds from plants inoculated with isolate PR had significantly reduced seed density and weight, increased free fatty acid content and protein, and reduced oil content compared with those inoculated with all other isolates.

Cercospora kikuchii (T. Matsu. & Tomoyasu) Gardner causes Cercospora leaf spot and blight and purple seed stain of soybeans (*Glycine max* (L.) Merr.) and is found wherever soybeans are grown (19). *C. kikuchii* initially colonizes the soybean seed coat, where it produces the characteristic purple pigment that is restricted to the colonized tissues (9,20). The fungus penetrates embryonic seed tissues and causes necrosis of cotyledonary cells and vascular elements (20). Yeh and Sinclair (24) reported that germination in the laboratory was reduced, and that stunted, low vigor seedlings resulted from soybean seeds when 50% or more of the seed coat was stained purple. Their studies and those on the effect of seed infection by *C. kikuchii* on physical and chemical properties of soybean seeds used only single isolates (22,23). Soybean seeds often are colonized by more than one fungus (19). Other fungi that reduce soybean seed germination and quality and that cause seed decay are *Alternaria*, *Aspergillus*, and *Fusarium* spp., and *Phomopsis longicolla* Hobbs (2,7,15, 18,19). Hepperly and Sinclair (7) reported that with increased percentage of occurrence of *C. kikuchii* in soybean seeds there was a concomitant decrease in the occurrence of *Phomopsis* sp.

The objectives of this study were to compare 1) the effect of four isolates of *C. kikuchii* on soybean seed germination, 2) the occurrence of *C. kikuchii* and other fungi in the seeds of 13 soybean cultivars inoculated in the field with the four isolates, and 3) the effect of the four isolates and a naturally occurring isolate on various soybean yield components and seed quality parameters.

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MATERIALS AND METHODS

Thirteen soybean cultivars were planted on the Agronomy-Plant Pathology South Farm at Urbana in 1986 and 1987. The experiment was replicated four times in a split-plot arrangement of a randomized complete block design in which four isolates of *C. kikuchii* were main plots and the 13 cultivars were subplots. The 2.5-m rows of each cultivar were planted on 0.5-m centers within each replicate. There were 3 m between subplots and 2 m between replicates. The 3 m between subplots was planted with sweet corn (*Zea mays* L.) to prevent cross-contamination by the fungal isolates.

The plants in each subplot were either spray-inoculated separately with a conidial suspension from 3-day-old agar cultures of one of the isolates of *C. kikuchii* or with water (control). Cultures were prepared by transferring a 4-mm agar disk from a stock culture to freshly prepared V-8 juice agar plates and were incubated for 12 hr under alternating light and dark at 25 C. The light source was two 40-W, cool-white fluorescent tubes (30 $\mu E \cdot m^{-2} \cdot s^{-1}$) mounted 30 cm above the plates. After 3 days, 10 ml of sterile, deionized distilled water was added to a plate and agitated to make a spore suspension (25). The plants were inoculated separately with each isolate or sprayed with water four times during the growing season at approximately the R_2, R_3, R_5, and R_8 growth stages. Inoculum (2.7×10^5 conidia/ml of water) always was applied with a Root Lowell garden sprayer (Root Lowell Mfg. Co., Lowell, MO) until runoff after 1600 hr when there was little or no air movement and humidity was increasing.

The four isolates used were ATCC-36864 (American Type Culture Collection, Rockville, MD, obtained in 1985); IL-ATCC (T. S. Abney, Purdue University, West Lafayette, IN, originally obtained in 1975 as ATCC-36864); IN-C4 (T. S. Abney); and PR, isolated from a soybean seed with purple seed stain (P. R. Hepperly, USDA-ARS, SAS, TARS, Mayaguez, PR). Although IL-ATCC was originally obtained as ATCC-36864, it did not compare with ATCC-36864 received in 1985. All isolates were maintained on V-8 juice agar. Each isolate varied in culture characteristics on Difco potato-dextrose agar (PDA): ATCC-36864, IL-ATCC, IN-C4, and PR produced, respectively, a typical purple pigment, a light pink-purple pigment, a light gray to whitish pink-purple pigment, and a dark gray-purple pigment that became darker with age.

Seeds of each cultivar/treatment combination were harvested separately by hand at maturity and were cleaned and stored in cloth bags at 5 C. For germination studies, seed lots from inoculated and uninoculated plants of Amsoy 71, Bragg, Davis, Hood 75, Tracy, and Williams were separated into five groups based on the percent of the seed surface area with purple discoloration: 1 = no stain, 2 = 1–25%, 3 = 26–50%, 4 = 51–85%, and 5 = 86–100% discoloration. Six 100-seed lots from each group were surface-sterilized in 0.05% NaOCl (10% sodium hypochlorite) for 4 min. They were then were washed twice in deionized distilled water for 3 min. The percent of germination was recorded after 7 days under two conditions: 1) on 9-cm PDA culture plates with three randomized replicates of 20 plates with five seeds per plate or 2) on cellulose pads (Kimpac) moistened with sterile water with three randomized

replications of 100 seeds each. Seeds for both studies were incubated in a seed germinator for 12 hr under alternating light and dark, approaching 100% relative humidity at 25 C. A seed was considered germinated when the radicle was 2.5 times the length of the cotyledons. Each experiment was done twice.

To determine the occurrence of seedborne fungi, seed lots from each cultivar/inoculation were combined. Three separate 100-seed lots of each were selected at random and surface-sterilized as described previously. Seeds from each cultivar were plated separately on 9-cm PDA culture plates with three randomized replicates of 20 plates with five seeds per plate and were incubated under 12 hr light and dark at 25 C. The occurrence of *C. kikuchii*, *P. longicolla*, and species of *Alternaria*, *Aspergillus*, and *Fusarium* was recorded after 7 days.

Seeds of six of the 13 cultivars with symptoms of purple seed stain (symptomatic) from plants spray-inoculated with one of the four isolates of *C. kikuchii* or asymptomatic from water-sprayed plants were each kept separate. Each seed lot consisted of the combined seeds harvested from the four replicates of the following cultivars: Amsoy 71, Bragg, Davis, Hood 75, Tracy, and Williams. In addition, symptomatic seeds from a naturally infected seed lot of Sieben brand (a private cultivar) grown in Illinois were used and referred to as being infected with naturally occurring isolate *C. kikuchii*. The following yield components were evaluated on each seed lot: seed density and volume and 300-seed weight.

To determine seed density, 10 subsamples of 15 seeds of each seed lot were randomly selected and placed in a gradient density column. The density was recorded in grams per cubic centimeter. Volume was determined with six replicates of a 100-seed lot using a Multi-Volume Gas Pycnometer, model 1305 (Micrometrics, Inc.) The volume of test samples was calculated using the following formula: $V_{sample} = V_{cup} - V_{ex.co.}/(1-P_1/P_2)$, where V_{cup} and $V_{ex.co.}$ are the cup volume and expansion coefficients, respectively, and P_1 and P_2 are the initial and final readings of the sample, respectively, recorded in cubic centimeters. Seed weights in grams were based on three replicates of 300 seeds each from each seed lot.

The seed quality components of free fatty acid (FFA), oil, and protein content of each seed lot was measured. Oil and protein content were read directly using a Dickey-John Infrared GAC-II model 600 (8) grain analysis computer and recorded in percent of seed dry weight. Three replicates of 50-g seed samples of each seed lot were assayed. All seed samples were dried in an oven for 1 hr at 130 C. They were then ground to a fine flour and placed in glass jars with screw caps for analysis. The FFA content of crude oil was determined by placing 15 g of flour from each seed lot, as described previously, into extraction thimbles that were then placed into Soxlet extractors. The oil was extracted with petroleum ether for 6 hr at 65 C. The solvent was flash-evaporated. Finally, the oil samples were placed in a vacuum oven overnight at 60 C. FFA was determined by the method of the Association of Official Analytical Chemists (method 28.030) for soybeans (13). Three determinations (replicates) were made for each seed lot. FFA was expressed as a percent of oleic acid.

Data analysis. Analysis of all data was done using the Statistical Analysis System (SAS) (17). Fisher's least significant difference (FLSD), at $P = 0.05$, was used to separate treatment means.

RESULTS

Germination and assay studies. Percent of germination of soybean seeds with purple seed staining varied among seed lots from plants spray-inoculated with one of the four isolates of *C. kikuchii* (Table 1). Seeds from plants inoculated with isolate IL-ATCC had a significantly higher germination and those from plants inoculated with isolate PR had a significantly lower germination than those inoculated with the other isolates, whether on PDA or cellulose pads. The germination of seeds from plants inoculated with the other two isolates was intermediate.

There was an inverse correlation between purple-stained seeds and germination on both media (Table 2). The percentage of purple-stained seeds from water-sprayed plants ranged from 0 to 2% (average 0.7%) with a germination of 90.4% on PDA and 81.9% on cellulose pads. The germination of seeds with over 75% purple stain was 63% less than control seeds on both media.

The combined mean percent of occurrence of *C. kikuchii* from seeds from plants spray-inoculated with the four isolates among the 13 cultivars fell into three groups: those with close to 20% or more (Amsoy 71, Bragg, Davis, Hood 75, Tracy, Williams), those with 1.3% or less (Bedford, Lee 74, Mack, Pickett 71), and those with none (Dare, Forrest, PI 80837) (Table 3). The occurrence of *C. kikuchii* influenced the establishment of the other seedborne fungi recorded. The correlation coefficient between the occurrence of *C. kikuchii* and *P. longicolla* was $R^2 = -0.59$, and the correlation coefficient for the other fungi combined was $R^2 = -0.37$.

Seed quality studies. Seed density and weight of all seed lots from plants inoculated with *C. kikuchii* were significantly lower than those of uninoculated (control) plants. Seed density and weight of seed lots from plants inoculated with isolate PR were significantly lower than those of seed lots from plants inoculated with the other isolates (Table 4). The volume of all symptomatic seed lots, except those from plants inoculated with isolate IL-ATCC, was not significantly different from that of the control.

FFA, oil, and protein. Seeds from plants inoculated with isolate PR had significantly higher FFA and protein and significantly lower oil content than seeds inoculated with any of the other isolates and the control, whereas those from plants inoculated with isolate IL-ATCC had the lowest FFA content, and those with isolate IN-C4 had the highest oil and lowest protein content, exluding the control (Table 4).

DISCUSSION

The effect of *C. kikuchii* on the

Table 1. Mean percent of germination of soybean seeds on two media from plants not sprayed (control) or spray-inoculated four times in the field with one of four isolates of *Cercospora kikuchii*

Isolate[w]	Germination (%)	
	Potato-dextrose agar[x]	Cellulose pads[y]
IN-C4	75.9 c[z]	65.5 c
IL-ATCC	79.6 b	69.4 b
PR	72 8 e	62.1 d
ATCC-36864	74.7 d	65.0 c
Control	90.4 a	81.9 a
FLSD ($P=0.05$)	0.8	1.9

[w] IN-C4 and IL-ATCC from T. S. Abney, Purdue University; ATCC-36864 from American Type Culture Collection; and PR from P. R. Hepperly, USDA-ARS, Mayaguez, Puerto Rico.
[x] Based on three replicates of 20 9-cm culture plates with five seeds per plate.
[y] Based on three replicates of 100 seeds each.
[z] Fisher's least significant difference. Means followed by the same letter are not significantly different at $P = 0.05$.

Table 2. Mean percent of germination of soybean seeds on two media with various percent of the seed coat showing purple staining caused by *Cercospora kikuchii*

Purple stain (%)	Germination (%)	
	Potato-dextrose agar[x]	Cellulose pads[y]
0	90.4 a[z]	81.9 a
1–25	83.9 b	71.2 b
26–50	76.5 c	65.9 c
51–75	71.1 d	56.5 d
76–100	56.9 e	51.9 e
FLSD ($P = 0.05$)	0.9	0.7

[x] Based on three replicates of 20 9-cm culture plates with five seeds per plate.
[y] Based on three replicates of 100 seeds per replicate.
[z] Fisher's least significant difference. Means followed by the same letter are not significantly different at $P = 0.05$.

Table 3. Combined mean percent of occurrence of seedborne fungi from soybean seeds of various cultivars inoculated in the field with four isolates of *Cercospora kikuchii*

Cultivar	Percent of occurrence[y]				
	C. kikuchii	*Phomopsis longicolla*	*Alternaria* spp.	*Aspergillus* spp.	*Fusarium* spp.
Amsoy 71	22.5 b[z]	10.8 c	15.3 ab	7.9 e	13.1 abc
Bedford	1.3 d	24.3 a	14.6 ab	10.3 cde	···
Bragg	23.1 b	9.9 c	16.1 ab	10.8 bcde	12.5 abcd
Dare	0.0 d	22.3 ab	16.3 ab	10.9 bcd	···
Davis	23.2 b	9.4 c	17.4 a	8.7 de	11.9 bcd
Forrest	0.0 d	19.6 b	15.7 ab	14.7 a	13.1 abc
Hood 75	26.1 a	9.3 c	14.7 ab	8.5 de	10.5 d
Lee 74	0.2 d	20.2 b	14.8 ab	10.1 ab	12.1 bcd
Mack	0.7 d	20.1 b	17.7 a	11.2 bcd	13.5 abc
PI 80837	0.0 d	19.7 b	16.8 ab	13.3 ab	13.8 ab
Pickett 71	0.3 d	19.7 b	17.2 ab	12.9 abc	13.5 abc
Tracy	23.3 b	10.8 c	13.8 b	10.7 bcde	10.6 d
Williams	19.9 c	10.9 c	17.3 ab	10.9 bcd	11.5 bcd
FLSD ($P = 0.05$)	1.7	2.9	3.5	2.9	2.3

[y] Based on three replicates of 100 seeds each on 20 9-cm potato-dextrose agar culture plates with five seeds per plate.
[z] Fisher's least significant difference. Means followed by the same letter are not significantly different at $P = 0.05$.

Table 4. Effect on yield components and seed quality parameters of soybean seeds from plants uninoculated (control) or inoculated in the field with one of five isolates of *Cercospora kikuchii*

Isolate[t]	Yield component			Quality component		
	Density[u] (g/cm^3)	Weight[v] (g)	Volume[w] (cm^3)	Free fatty acid[x] (% oleic acid)	Oil[y] (% dry wt)	Protein[y] (% dry wt)
IN-C4	1.165 b[z]	42.7 c	0.137 ab	0.5225 b	22.3 ab	38.9 c
IL-ATCC	1.160 bc	44.1 b	0.114 b	0.4502 c	21.8 bc	39.9 bc
PR	1.147 d	39.5 e	0.151 a	0.5404 a	21.2 d	41.4 a
ATCC-36864	1.158 c	40.8 d	0.140 a	0.5250 b	21.7 cd	40.8 ab
NO	1.157 c	40.8 d	0.142 a	0.4325 d	21.4 cd	40.5 ab
Control	1.182 a	52.9 a	0.149 a	0.2500 e	22.9 a	37.3 d
FLSD ($P = 0.05$)	0.006	1.1	0.024	0.0075	0.5	1.1

[t] IN-C4 and IL-ATCC from T. S. Abney, Purdue University; ATCC-36864 from American Type Culture Collection; PR from P. R. Hepperly, USDA-ARS, Mayaguez, Puerto Rico; and NO from naturally infected soybean seeds.
[u] Based on 10 replicates of 15 seeds each.
[v] Based on three replicates of 300 seeds each.
[w] Based on six replicates of 100 seeds each.
[x] Based on three replicates of 50 seed samples each.
[y] Based on six determinations.
[z] Fisher's least significant difference. Means followed by the same letter are not significantly different at $P = 0.05$.

germination of infected soybean seeds has been controversial. Han (6) and Lehman (11) reported no effect on germination, whereas Wilcox and Abney (22) and Yeh and Sinclair (24) claimed that *C. kikuchii* reduced germination. All of these foregoing studies used single isolates of *C. kikuchii* and few cultivars. Our studies using four isolates and 13 cultivars showed that as the percent of purple stain increased the percent of germination decreased. These results agreed with those of Yeh and Sinclair (24). The effect of *C. kikuchii* on germination differed among isolates and cultivars. This may explain, in part, the difference reported by Han (6) and Lehman (11) from our results and those of others (22,24). The differences among cultivars suggested that there may be genetically influenced resistance to *C. kikuchii* among them.

An interaction between *C. kikuchii* and fungi of the *Diaporthe/Phomopsis* complex has been reported (7,18). Hepperly and Sinclair (7) reported that an isolate of *C. kikuchii* from a soybean seed grown in Puerto Rico was antagonistic to an unidentified *Phomopsis* sp. Roy and Abney (18) reported antagonism between an isolate of *C. kikuchii* and *D. phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* (Lehman) Wehm. We confirm those reports and, in addition, show that this antagonism also may affect the occurrence of species of *Alternaria*, *Aspergillus*, and *Fusarium* in soybean seeds. This may be explained in part by competition for nutrients or space. However, *C. kikuchii* and other *Cercospora* spp. produce a nonspecific toxic compound, cercosporin, which was reported to be antagonistic to seedborne fungi of soybeans (1,3–5,12,14). One explanation for the antagonism of cercosporin produced by *C. kikuchii* also may explain the effect of the fungus on seed germination.

Katsube (10) showed that *C. kikuchii* reduced crop growth and yield. Our five isolates of *C. kikuchii* varied in their reduction of the yield components of seed density and weight, but not volume. Previous studies on the effect of *C. kikuchii* on seed quality components were limited to a single isolate and one or two parameters (10,16,21). Our results showed that the extent of the effect of *C. kikuchii* on seed quality parameters depends upon the isolate of the fungus involved. Katsube (10) and Park et al (16) reported that soybean seeds severely infected with *C. kikuchii* did not differ in protein content, whereas Taira et al (21) reported a higher protein content in diseased seeds. Variations in the isolates of the fungus used in their studies would explain these differences. Taira et al (21) also reported a lower oil content for seeds with symptoms compared with asymptomatic seeds.

Soybean buyers generally discount seed lots containing purple-stained seeds regardless of the number of seeds stained or the extent of discoloration on seeds. This may be justified, in part, if the seeds are to be used for processing when a high level of FFA is critical. The effect of *C. kikuchii* on oil and protein content may be due in part to seed infection, but also may be due to plant infection, because the seeds from the experimental field plots came from inoculated plants.

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