Antagonism Between Cercospora kikuchii and Other Seedborne Fungi of Soybeans

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ABSTRACT

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Incidence of several fungi, primarily *Diaporthe phaseolorum* var. *sojae*, was reduced in pods and seeds of soybean plants inoculated at various growth stages with *Cercospora kikuchii*. Both pods and seeds were implicated as sites of interaction between *C. kikuchii* and *D. phaseolorum* var. *sojae*, but pods were the most likely site. Reductions in percentage pod infection and pod colonization by *D. phaseolorum* var. *sojae* suggest that establishment of infection and growth within pods both were inhibited. Variability in frequency and magnitude of reductions in seed infection by *Diaporthe* was caused primarily by the effect of weather on establishment of *C. kikuchii*. Increased seed germination from inoculation treatments was an indirect result of the reduction in seed infection by *D. phaseolorum* var. *sojae*. In investigations involving *C. kikuchii*-inoculated plants the effect(s) on other fungi and the consequences should be considered.

Additional key words: *Diaporthe phaseolorum* var. *caulivora*, *Alternaria* spp., imperfect seed coats.

The most frequently cited seedborne fungi of soybeans [*Glycine max* (L.) Merr.] are *Diaporthe phaseolorum* (Cke. & Ell.) Sacc.' var. *sojae* (Lehman) Whem., *D. phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell, *Cercospora kikuchii* (Mat. & Tomoy.) Gardner, and *Alternaria* spp. (3, 5, 7, 11). Usually these fungi are associated with poor seed quality and reduced seed germination (3, 7, 8), but little or no adverse effect on germination has been reported for *C. kikuchii* (10). Both *D. phaseolorum* var. *sojae* and *C. kikuchii* are frequently isolated from seeds that have imperfectly developed seed coats (7, 9).

Under field conditions, *C. kikuchii* causes high levels of seed infection in plants inoculated during the flowering period (6, 9) but lower levels in plants inoculated at later growth stages. Previously, we reported the effects of inoculating soybeans with *C. kikuchii* on its incidence in seeds, on seed germination, and on the incidence of imperfect seed coats (9). Reductions in incidence of seed infection by *Diaporthe* spp. and *Alternaria* spp. were associated with the *C. kikuchii* inoculations. This study presents evidence of an antagonistic relationship between *C. kikuchii* and other fungi.

MATERIALS AND METHODS

Soybean lines were planted at the Purdue University Agronomy Farm near Lafayette, Indiana, in a randomized block design. The seeds that were used originally were grown in Lafayette and were pathogen-free. Soybean lines were planted in rows 2.9 m long, and were replicated four times in 1970 and 1971, and five times in 1972. In 1975, Amsoy 71 was planted in rows 1 m long and was replicated three times. Amsoy 71, Wayne, Cutler, and P. I. 80837 which represented maturity groups II, III, IV, and IV, respectively, were planted simultaneously at four dates in 1970 and 1971. In 1970, the planting dates were 8 May, 27 May, 17 June, and 6 July; in 1971, they were 24 May, 7 June, 23 June, and 7 July. These lines were inoculated with *C. kikuchii* when they were in full bloom. In addition, the same lines were planted 8 May, 1970 and 24 May, 1971, and were inoculated at five stages of growth (4) referred to as R2 (full bloom), R3, R5, R6 (intermediate stages of pod and seed development), and R8 (95% of pods brown with seeds at harvest maturity). Lindarin 63 (maturity group II) was planted 20 May 1972 and inoculated at four stages of growth: V6 (pre-bloom stage), R3, R5, and R8. Amsoy 71 was planted 15 May 1975, and was inoculated in stage R2.

Three-day-old cultures of *C. kikuchii*, grown in petri plates on both V-8 juice agar and potato-dextrose agar (PDA), were suspended in water by fragmentation in a Waring Blendor. The final inoculum suspension applied to the above-ground portions of each soybean line consisted of 1.7×10^5 conidia of *C. kikuchii* per ml H_2O. Inoculum was applied in the afternoon with a compressed air sprayer.

Seed and pod samples were harvested at plant maturity and maintained at 14% moisture until assayed for fungi. In 1970, 1971, and 1972, 100 seeds were assayed per sample. In 1975, a random sample of pods was selected

from the main stems of 10 Amsoy 71 plants per replication. One 5-mm-diameter disk of tissue from the peduncle (P) and stylar (S) end, and middle (M), of the pod was removed with a "paper-punch" from each of 80 three-seeded pods, and these and the seeds from the pods were assayed per sample. The pod disks and seeds were surface-sterilized with 1% sodium hypochlorite (NaOCl) and plated on PDA. During 8 days of incubation at 22 C, all fungi growing out from seeds and pod disks were recorded. *Percentage pod infection* was taken as the highest incidence of a given fungus in either the P, M, or S position. *Percentage pod colonization* was taken as the mean of total P, M, and S pod infections. *Percentage seed infection* also was taken as the mean of total P, M, and S seed infections. Seed germination in vitro was recorded at the end of the incubation period. In 1970 and 1971, the incidence of imperfect seed coats also was recorded.

Owing to the large number of seed samples examined and difficulty in distinguishing *D. phaseolorum* var. *sojae* from var. *caulivora* without subculturing, these two fungi were recorded as *Diaporthe* spp. in 1970 and 1971. However, a subsample of *Diaporthe* isolates was used to estimate relative frequencies of the two fungi in 1971 and they were recorded separately in 1972 and 1975.

The data were statistically analyzed using Duncan's multiple range test.

RESULTS

Inoculation of soybean lines in stage R2 (full bloom) with *C. kikuchii* nearly always reduced seed infection by *Diaporthe* spp. below that of the noninoculated lines (Tables 1 and 2). In 1971, *D. phaseolorum* var. *sojae* was more frequent in seeds than var. *caulivora* (91.3% vs. 8.7%, respectively). Significant reductions in *Diaporthe* spp. occurred frequently in Amsoy 71 and Wayne, the two soybean lines most susceptible to seed infection by both *C. kikuchii* and *Diaporthe* spp. There were no significant reductions in seed infection by *Diaporthe* spp. in Cutler either year, but there was a significant reduction in *Diaporthe* spp. in seeds of P. I. 80837 harvested from the first 1971 planting date. The soybean line P. I. 80837, which is less susceptible to *C. kikuchii* than the other three lines, consistently had the least *C. kikuchii* seed infection.

TABLE 1. Effect of inoculating soybean lines (planted at different times) at full bloom with *Cercospora kikuchii* (abbreviated Ck.) on the incidence of infection by *C. kikuchii* and *Diaporthe* spp. (abbreviated Dp.) infection in seeds harvested at maturity in 1970

		Percentage seed infection by the respective fungi per date of inoculation[a]							
	Seed source	Date 1		Date 2		Date 3		Date 4	
Soybean line	plant	Dp.	Ck.	Dp.	Ck.	Dp.	Ck.	Dp.	Ck.
Amsoy 71	I[b]	4.2*[d]	70.8*	3.8*	71.2*	8.0*	55.0*	5.8*	67.2*
	NI[c]	24.0	13.8	10.0	32.0	30.5	12.0	24.5	14.0
Wayne	I	2.0	51.8*	0.2	45.5*	2.8*	32.2*	1.8	46.0*
	NI	6.0	19.0	1.0	14.2	8.5	7.5	6.0	4.2
Cutler	I	0.0	26.5*	0.8	31.2*	1.0	29.8*	3.0	22.0*
	NI	1.8	14.8	0.8	4.5	4.2	8.2	4.8	0.0
P.I. 80837	I	1.0	14.2	2.5	2.2	2.0	26.2*	1.2	10.0
	NI	2.5	6.8	6.0	5.0	6.0	3.8	1.8	1.0

[a]Inoculation dates 1-4: Amsoy 71 (7/9, 7/23, 8/8, 8/23); Wayne (7/15, 8/1, 8/12, 8/30); Cutler (7/19, 8/3, 8/15, 8/30); and P.I. 80837 (8/1, 8/10, 8/18, 8/30), respectively.
[b]I = Seeds harvested from plants inoculated with *C. kikuchii*.
[c]NI = Seeds harvested from noninoculated plants.
[d]Significantly different from control ($P = 0.05$).

TABLE 2. Effect of inoculating soybean lines (planted at different times) at full bloom with *Cercospora kikuchii* (abbreviated Ck.) on the incidence of *C. kikuchii* and *Diaporthe* spp. (abbreviated Dp.) infection in seeds harvested at maturity in 1971

		Percentage seed infection by the respective fungi per date of inoculation[a]							
	Seed source	Date 1		Date 2		Date 3		Date 4	
Soybean line	plant	Dp.	Ck.	Dp.	Ck.	Dp.	Ck.	Dp.	Ck.
Amsoy 71	I[b]	2.3*[d]	85.5*	8.0*	74.8*	28.8*	66.8*	32.0	29.5
	NI[c]	7.8	28.8	25.3	26.5	43.5	7.3	30.5	25.5
Wayne	I	4.5*	71.5*	5.0	74.8*	14.0*	47.0*	10.0	21.8
	NI	12.3	29.3	10.3	16.8	21.8	11.5	15.5	13.0
Cutler	I	3.0	57.3*	1.3	53.3*	18.0	13.8*	5.5	34.0*
	NI	7.3	11.8	5.8	12.3	22.0	3.5	1.5	10.5
P.I. 80837	I	4.0*	31.3*	7.3	10.5	3.0	2.5	0.3	8.5
	NI	15.5	5.5	9.3	8.8	3.0	0.5	2.0	5.3

[a]Inoculation dates 1-4: Amsoy 71 (7/6, 7/20, 8/8, 8/25); Wayne (7/12, 7/31, 8/19, 8/25); Cutler (7/23, 8/4, 8/21, 8/27); and P.I. 80837 (7/28, 8/10, 8/24, 8/27), respectively.
[b]I = Seeds harvested from plants inoculated with *C. kikuchii*.
[c]NI = Seeds harvested from noninoculated plants.
[d]Significantly different from control ($P = 0.05$).

Also, it had the most seed infection by *Diaporthe* spp. in the first planting date of 1971. Although the stage of host development at the time of inoculation with *C. kikuchii* significantly influenced seed infection by *C. kikuchii* and *Diaporthe* spp. (Fig. 1), variability in frequency and magnitude of reductions in seed infection by *Diaporthe* was caused primarily by the effect of weather on the establishment of *C. kikuchii*. This was particularly evident in comparing 1971 results of R3, R5, and R6 inoculations in the first planting date (Fig. 1) with the R2 inoculations in planting dates 2, 3, and 4 (Table 2); differences in seed infection due to *C. kikuchii* inoculation from R2 to R6 in the same environment were not significant.

The reductions in seed infection by *Diaporthe* spp., particularly *D. phaseolorum* var. *sojae*, which occurred in plants inoculated with *C. kikuchii*, indicate that *C. kikuchii* infection is closely related to the incidence of seed infection by *D. phaseolorum* var. *sojae* (Fig. 1). Correlation coefficients were computed between the incidences of seed infection by *C. kikuchii* and *Diaporthe* spp. and between the incidence of seed infection by *C. kikuchii* and *D. phaseolorum* var. *sojae* in lines inoculated at different growth stages. The correlation values for the *C. kikuchii* - *Diaporthe* spp. interaction were: in 1970, Amsoy 71, - 0.97, significant at $P = 0.01$; in 1971, Amsoy 71, - 0.99, significant at $P = 0.01$; in 1971, Wayne, - 0.93, significant at $P = 0.05$; and in 1971, P. I. 80837, - 0.94, significant at $P = 0.05$. The correlation value for the *C. kikuchii* - *D. phaseolorum* var. *sojae* interaction for Lindarin 63 was - 0.99, significant at $P = 0.01$.

In 1975, seed infection by *D. phaseolorum* var. *sojae* and var. *caulivora* was significantly reduced in plants inoculated with *C. kikuchii* (Table 3) as compared to noninoculated plants. Pod infection and colonization by *D. phaseolorum* var. *sojae* also were reduced by the inoculations. Although the levels of pod infection and colonization by *D. phaseolorum* var. *caulivora* were lower in inoculated than in noninoculated plants, the differences were not statistically significant. Significant

TABLE 3. Effect of inoculating Amsoy 71 soybean plants at full bloom with *Cercospora kikuchii* (abbreviated Ck.) on infection and colonization of pods and infection of seed by *Diaporthe phaseolorum* var. *sojae* (abbreviated Dps.), *D. phaseolorum* var. *caulivora* (abbreviated Dpc.) and *C. kikuchii* in 1975

	Pod infection			Pod colonization			Seed infection		
Inoculated with *C. kikuchii*	Dps. (%)	Dpc. (%)	Ck. (%)	Dps. (%)	Dpc. (%)	Ck. (%)	Dps. (%)	Dpc. (%)	Ck. (%)
Yes	13.8 A[a]	3.1 A	92.1 A	9.9 A	1.2 A	84.6 A	1.8 A	0.1 A	91.5 A
No	20.8 B	5.4 A	42.3 B	15.9 B	2.9 A	34.7 B	5.3 B	2.9 B	25.7 B

[a]Means not followed by the same letters within a column are significantly different ($P = 0.05$).

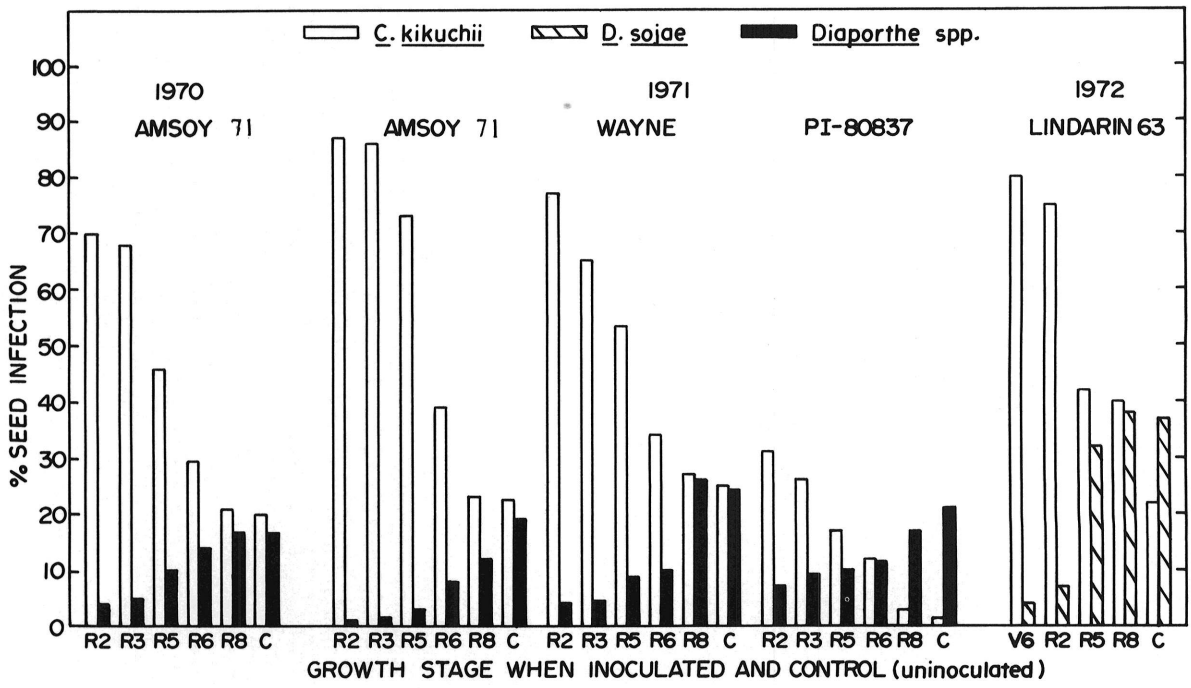

Fig. 1. Effect of inoculating soybean lines at different growth stages with *Cercospora kikuchii* on the incidence of *Diaporthe phaseolorum* var. *sojae* and *Diaporthe* spp. seed infection at plant maturity. Growth stages: V6 = pre-bloom; R2 = full bloom; R3, R5, and R6 = intermediate stages of pod and seed development; and R8 = 95% of pods brown (harvest maturity).

TABLE 4. Effect of inoculating Amsoy 71 and Wayne soybeans at different growth stages with *Cercospora kikuchii* on germination of seeds harvested at maturity in 1971

Soybean line	Germination per growth stage when plants[a] were inoculated with *C. kikuchii*					
	R2	R3	R5	R6	R8	Control
Amsoy 71	94.0 A[b]	92.0 A	91.0 A	91.3 A	89.5 AB	85.8 B
Wayne	95.3 A	94.3 AB	91.0 ABC	92.0 ABC	88.5 BC	89.5 C

[a]Growth stages: R2 = full bloom; R3, R5, and R6 = intermediate stages of pod and seed development; and R8 = 95% of pods brown (harvest maturity).

[b]Means not followed by the same letters within a row are significantly different ($P = 0.05$).

reductions in *Alternaria* spp. and fungi other than *Diaporthe* spp. occurred in 1970 and 1971, but not in 1972 and 1975. The data on *Alternaria* spp. and other fungi were variable, but these and our unpublished data indicate that their incidence in seeds was reduced by the inoculation of soybean plants with *C. kikuchii*.

Inoculations with *C. kikuchii* gave significantly improved seed germination in Amsoy 71 in both 1970 and 1971, and in Wayne in 1971 (Table 4). Correlation coefficients computed between percentage *Diaporthe* seed infection and percentage seed germination in Amsoy 71 and Wayne in those years were −0.94 and −0.96, respectively, both significant at $P = 0.01$. Correlation coefficients computed between percentage *C. kikuchii* seed infection and percentage seed germination were +0.79 and +0.92, respectively, the latter significant at $P = 0.01$.

Significant reductions in imperfect seed coats from the *C. kikuchii* inoculation treatments were frequent. Although these reductions apparently were due to the inoculations, we were unable to relate them to changes in frequency of other fungi, including *Diaporthe* spp., which are reported to be associated with imperfect seed coats (7, 9).

DISCUSSION

The reductions in fungal seed infection in soybean lines inoculated with *C. kikuchii* indicate that *C. kikuchii* is an antagonist of certain naturally-occurring fungi on soybean pods and seeds, especially *D. phaseolorum* var. *sojae*. The influence of time of inoculation with *C. kikuchii* on the incidence of *Diaporthe* spp. (Table 1, 2) suggests that environmental conditions at the time of inoculation or shortly thereafter, as well as stage of plant growth, influenced the interaction. Presumably, the variability in frequency and magnitude of the reductions in seed infection by *Diaporthe* spp. resulted primarily from the effect of weather on establishment of *C. kikuchii* infection, but the effect of weather on infection by *Diaporthe* spp. and on the interaction itself also may have contributed to the variability. Weather conditions during the growing season (especially drought periods with high temperatures) reduce the prevalence and severity of seed infection by *C. kikuchii* (9) and *Diaporthe* spp. (Roy and Abney, *unpublished*). However, at the time of soybean maturity (R8) higher (20-25 C), but not lower (5-10 C), temperatures with moist atmosphere favor seed infection by *D. phaseolorum* var. *sojae* (1).

Cercospora kikuchii may antagonize other fungi indirectly by making the host an unsuitable substrate for penetration and colonization, or by direct competitive and/or chemical inhibition. *Diaporthe phaseolorum* var. *sojae* and var. *caulivora*, the main fungi antagonized, both infect stems, leaves, pods, and seeds (2). Since all above-ground plant parts inoculated with *C. kikuchii* can become infected (8, 9), antagonism between *Diaporthe* and *C. kikuchii* may have occurred on all inoculated plant tissues or only specific ones. The close, inverse relationship between seed infection by *Diaporthe* spp. and by *C. kikuchii* (Fig. 1) suggests that the seed was the site of the antagonism. On the other hand, the level of *C. kikuchii* seed infection in P. I. 80837 was less than half that in Amsoy 71 and Wayne in 1971 (Table 2), yet the reductions in seed infection by *Diaporthe* spp. among these three lines were comparable. Infection and colonization of pods by *D. phaseolorum* var. *sojae* were reduced in 1975 by *C. kikuchii* (Table 3), which suggests that establishment of *D. phaseolorum* var. *sojae* in pods was inhibited. Although the reductions in *D. phaseolorum* var. *caulivora* pod infection and colonization were not statistically significant, the trends are consistent with this interpretation. Since colonization of pods by fungi apparently lasts much longer than colonization of seeds (5), the opportunity for interactions between *C. kikuchii* and *Diaporthe* spp. and *C. kikuchii* and other fungi would exist for a longer period in pods than seeds. Thus, pods seem the most likely site for the antagonism.

The increase in seed germination in Amsoy 71 and Wayne (Table 4) apparently is an indirect result of the antagonism between *C. kikuchii* and *D. phaseolorum* var. *sojae*. Although *C. kikuchii* can lower seed germination (8), the correlation values indicate that the main effect of this fungus was to decrease the incidence of *D. phaseolorum* var. *sojae*, a virulent pathogen which is most often responsible for the low germination of seeds from control plants.

We suggest that in investigations involving plants inoculated with *C. kikuchii*, the incidence of other seedborne fungi, especially *D. phaseolorum* var. *sojae*, should be considered. Seed germination, imperfect seed coats, and perhaps other parameters that were investigated may be indirectly affected by the inoculations. In addition, the consistent reduction by *C. kikuchii* of infection of soybean seeds by *D. phaseolorum* var. *sojae* may indicate that it is potentially valuable for biological control of *D. phaseolorum* var. *sojae* on seeds.

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