Comparison of Seeds and Crop Residues as Sources of Inoculum for Pod and Stem Blight of Soybeans

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ABSTRACT

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Soybean seed lots of three cultivars (in 1979) and of one cultivar (in 1980) infected with different amounts of *Phomopsis* sp. and *Diaporthe phaseolorum* var. *sojae* (collectively referred to as *Phomopsis*), the causal organisms of pod and stem blight, were planted in fields near Ames, IA, with cropping histories of continuous soybeans, corn rotated with soybeans, or continuous corn. No relationship was found between the amount of seedborne inoculum and severity of *Phomopsis* infection on seedlings or on mature plants. Marked differences, however, occurred among cropping practices, with the most severe infection in the continuous-soybean field, less in the corn-soybean-rotation field, and least in the continuous-corn field. Soil potassium content and plant lodging were eliminated as possible explanations for this disease pattern. Transmission of *Phomopsis* from viable artificially inoculated seeds to soybean seedlings was detected in a continuous-corn field, but inoculum from that source could not be distinguished from that from other sources in a continuous-soybean field. Transmission of *Phomopsis* from nonviable artificially inoculated seeds to adjacent viable seedlings was not demonstrated.

Both soybean (*Glycine max* (L.) Merr.) seeds and crop residues are recognized as sources of inoculum for *Phomopsis* sp. and *Diaporthe phaseolorum* var. *sojae* (Cke. & Ell.) (collectively referred to as *Phomopsis* in this paper), the causal organisms of pod and stem blight and soybean seed decay (7,8). These fungi overwinter on soybean crop residues and subsequently sporulate on these tissues (7,8), producing primary inoculum that can infect soybean seedlings (7). Pod and stem blight is more severe in fields previously cropped to soybeans than in those cropped to corn (6). Evidence for seed as an inoculum source is limited to laboratory germination tests where *Phomopsis* may be observed growing out of viable infected seeds (9,11). It has not been demonstrated, however, that the pathogens can be transmitted in this way to seedlings in the field.

It also is not known whether dead *Phomopsis*-infested seeds can act as an inoculum source for adjacent seedlings. This study compared the importance of seedborne and soilborne sources of inoculum for pod and stem blight.

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MATERIALS AND METHODS

Detection of *Phomopsis* on plant parts. The level of *Phomopsis* infection in soybean seed lots planted in all experiments was estimated with a blotter test (9). *Phomopsis* infection of soybean hypocotyls and stems (3.2 cm long), pods, and seeds sampled in experiments was determined by surface-sterilizing plant parts in 1.3% sodium hypochlorite (Clorox) for 1 min, rinsing in sterile distilled water for 30 sec, and plating on potato-dextrose agar adjusted to pH 4.5 with lactic acid. After incubation for 5–7 days in the dark at 28 C, plates were examined for development of *Phomopsis* colonies. No attempt was made to distinguish between *Phomopsis* sp. and *D. phaseolorum* var. *sojae*. *D. phaseolorum* var. *caulivora* Athow & Caldwell was isolated occasionally but not counted. Stem infection at harvest maturity (R8) (3) was estimated by counting stems on which *Phomopsis* pycnidia could be seen. A dissecting microscope was used to distinguish these fruiting bodies from acervuli of *Colletotrichum* spp.

Field tests to compare seedborne and soilborne sources of *Phomopsis* inoculum. In 1979, two seed lots each of three soybean cultivars, Amsoy 71, Wells, and Beeson, were selected with low (2, 0, 1%) or moderate (10, 6, 8%) levels of seedborne *Phomopsis* infection, respectively. Different cultivars were used because of difficulties in finding *Phomopsis*-infected seed lots. All three cultivars, however, were susceptible to pod and stem blight. Each seed lot was planted on 17 May 1979 in fields with previous cropping histories of continuous soybeans for 10 yr, corn rotated with soybeans for 5 yr, or continuous corn for 10 yr. The fields were within 11 km of each other. Soil types were all of the Clarion-Nicollet-Webster association. Individual plots consisted of four 4.8-m rows 0.8 m apart with 100 seeds per row. *Phomopsis* infection was measured on samples of 10 plants collected from the middle two rows of each plot, of stems at the V4, R3, and R8 growth stages, of pods at R3, R7, and R8, and of seeds at R7 and R8. At all growth stages except V4, respective plant parts were sampled from the lower, middle, and upper sections of each plant and then bulked by plant. At V4, only a lower stem section was sampled. Within fields, treatments consisting of factorial combinations of the main effects (cultivar, level of seedborne *Phomopsis*, and growth stage) were planted in a split-split plot arrangement of a randomized complete-block design with four growth stages as whole plots, three cultivars as subplots, and two levels of seedborne *Phomopsis* as sub-subplots. Treatments were replicated four times and individual plots were sampled only once. A two-way analysis of variance (ANOVA) was calculated by cultivar for each cropping practice, growth stage, and plant-part combination.

The experiment was repeated in 1980 using six seed lots of the cultivar Wells with seedborne *Phomopsis* infection levels of 0, 4, 26, 37, 46, and 77%, respectively. Experimental sites were located in different parts of the continuous-soybean and continuous-corn fields used in 1979 and a corn-soybean rotation field 0.2 km from the corresponding 1979 field. Treatments were replicated four times in a split-plot arrangement of a randomized complete-block design, with four growth stages at the whole plots and six levels of seedborne *Phomopsis* infection at the subplots. For each cropping practice, growth stage, and plant-part combination, a two-way ANOVA was calculated that included a regression analysis of *Phomopsis* plant-part infection on level of seedborne *Phomopsis* infection.

Because of the possibility that *Phomopsis* seed infection might be increased by potassium deficiency (1,5,10), soil potassium content was tested in June 1980 by taking eight core samples (2 × 15.2 cm) from each experimental site used in 1979 and 1980. These were analyzed for available

Table 1. *Phomopsis* infection of plant parts of soybeans[a] at the V4 and harvest maturity (R8) growth stages in relation to inoculum of *Phomopsis* on planted seed and cropping history[b] of the field

	Planted seed		Harvest maturity										
		Phomopsis infection	V4 stem[c]			Stem[d]			Pod[c]			Seed[c]	
Year	Cultivar	(%)	S	CS	C	S	CS	C	S	CS	C	S	CS	C
1979[e]	Amsoy 71	2	85.0	10.0	2.5	87.5	95.0	72.5	80.8	92.5	64.4	10.3	7.3	0.0
		10	77.5	22.5	0.0	90.0	92.5	67.5	80.9	95.7	64.2	3.0	4.4	1.3
	Wells	0	82.5	17.5	0.0	97.5	97.5	77.5	83.3	96.7	46.7	14.3	15.7	0.7
		6	87.5	10.0	0.0	97.5	97.5	82.5	82.5	95.8	40.0	15.7	12.0	1.0
	Beeson	1	87.5	2.5	0.0	90.0	100.0	67.5	77.5	92.4	66.8	0.7	5.4	0.7
		8	80.0	2.5	0.0	80.0	97.5	72.5	79.2	95.0	55.2	0.3	1.7	0.4
1980[f]	Wells	0	72.5	37.5	0.0	97.5	56.1	62.0	44.2	12.5	3.3	4.7	2.3	0.0
		4	70.0	27.5	2.5	95.0	62.5	77.2	41.7	23.3	0.8	8.0	3.1	0.3
		26	60.0	22.5	5.0	100.0	77.5	67.5	40.0	23.3	7.5	8.7	3.7	0.0
		37	62.5	27.5	5.0	100.0	70.0	77.5	55.8	17.5	6.7	8.7	0.7	0.3
		46	67.5	42.5	7.5	97.5	80.0	75.0	46.7	30.8	5.0	16.0	6.0	0.7
		77	72.5	52.5	10.0	100.0	92.5	76.4	53.4	31.7	4.2	7.0	2.7	0.3

[a] Percent infected of total number tested.
[b] Cropping histories: S = continuous soybeans for 10 yr, CS = corn rotated with soybeans for 5 yr, and C = continuous corn for 10 yr.
[c] Estimated by plating tissues on acidified potato-dextrose agar.
[d] Estimated by presence of *Phomopsis* pycnidia.
[e] Within cultivars, for each growth stage, plant part, and cropping history combination, no significant differences ($P = 0.05$) in *Phomopsis* plant part infection were detected regardless of initial level of seedborne *Phomopsis*.
[f] No significant relationships were detected ($P = 0.05$) between *Phomopsis* plant part infection and initial level of seedborne *Phomopsis* regardless of growth stage, plant part, or cropping history, except for pod infection at harvest maturity in the CS field, where linear regression was significant at $P = 0.05$.

potassium (4) at the Iowa State University Soil Testing Laboratory at Ames. Potassium concentration in leaves was also measured in 1980 on plants grown from seed with 0 and 77% seedborne *Phomopsis* infection, respectively. Leaves from upper and lower parts of 10 plants per plot at the R3 growth stage were dried, ground in a Stein mill, and analyzed for potassium concentration (2) at the Research-Extension Analytical Laboratory, Ohio Agricultural Research and Development Center at Wooster.

Lodging, another factor that might affect plant infection (12), was rated on the center two rows of each plot at harvest maturity in both years by using a scale of 1–5, where 1 = all plants erect and 5 = all plants horizontal.

Tests with inoculated seeds. Seeds of the cultivar Wells, which were free of *Phomopsis* infection, were inoculated with a *Phomopsis* isolate by spraying a conidial suspension onto seeds, incubating for 2 days at 70% relative humidity and 28 C, and then drying at 22 C. Dead *Phomopsis*-infested seeds also were prepared from the same seed lot by adding the conidial suspension to autoclaved seeds. After 7 days of incubation, infested seeds were removed, separated, and dried at 22 C. The following seed lots then were prepared: 1) original uninoculated viable seeds, 2) inoculated viable seeds, 3) inoculated viable seeds treated with Captan 30DD at the rate of 1.2 ml/kg seed, 4) uninoculated viable seeds mixed at a 1:1 ratio with dead *Phomopsis*-infested seeds, and 5) uninoculated viable seeds mixed at a 1:1 ratio with dead uninfested seeds. Each seed lot was planted on 21 May 1980 in the continuous-soybean and continuous-corn fields described previously. Individual plots consisted of a single 4.8-m row of 100 seeds. Plots were 0.8 m apart. Treatments were replicated four times and arranged in a randomized complete-block design. Repeated measurements of *Phomopsis* infection were made 5, 12, 19, and 26 days after emergence on hypocotyl sections of 10 plants harvested from each plot. For each cropping practice, a split-plot ANOVA was calculated with the five seed lot treatments as the whole plots and the four sampling times the subplots. Regression analysis of hypocotyl infection on days after emergence also was performed.

RESULTS AND DISCUSSION

No significant relationships were detected between the amount of seedborne inoculum and disease severity either on seedlings or on stems, pods, or seeds of mature plants regardless of year, cropping history, or cultivar, except for 1980 pod infection at R8 in the corn-soybean-rotation field (Table 1). Marked differences, however, did occur in *Phomopsis* infection among cropping histories, with the most severe disease occurring in the continuous-soybean field, less in the corn-soybean-rotation field, and least in the continuous-corn field. Data are not shown, but similar results were found for samplings at the R3 and R7 growth stages. Lodging and potassium deficiencies were eliminated as possible explanations for this disease pattern. In both years, lodging was most severe in the continuous-corn field, where the least amount of *Phomopsis* infection occurred, with average ratings in 1979 and 1980 of 3.5 and 2.2, respectively, compared with 2.5 and 1.6 in the corn-soybean-rotation field and 1.7 and 1.5 in the continuous-soybean field. Although soil potassium levels were lowest in the continuous-soybean field, these values (188 and 165 kg/ha in 1979 and 1980, respectively) and those on leaves were above the deficiency range that might affect *Phomopsis* infection of seeds (5). The most probable explanation for the disease pattern is that it reflected the amounts of *Phomopsis*-infested soybean crop residues in the field, thus confirming previous evidence that these are a major inoculum source for pod and stem blight (6,7).

The finding that seedborne inoculum, even on 77% of the seeds, could not be detected either on seedlings or mature plants in fields with continuous-soybean or corn-soybean-rotation cropping histories indicates that seeds were a minor inoculum source compared with soybean crop residues. This conclusion also seemed to hold for the continuous-corn field despite the fact that little soybean crop residue could have been left after 10 yr of continuous corn. The uniform pattern of disease development across treatments throughout the growing season was not what would be expected had seed been an important source of inoculum. It is more likely that *Phomopsis*, carried in by equipment or blown in from adjacent fields, was the major inoculum source. The experiment with inoculated seeds, however, provided evidence that seedborne *Phomopsis* may be transmitted to soybean seedlings because *Phomopsis* infection was significantly greater ($P = 0.01$) on seedlings grown in the continuous-corn field from inoculated seeds than on those grown from inoculated seeds treated with captan or from uninoculated seeds (Fig. 1). This effect was not seen in the continuous-soybean field, where seedling infection was significantly greater than that found in the continuous-corn field,

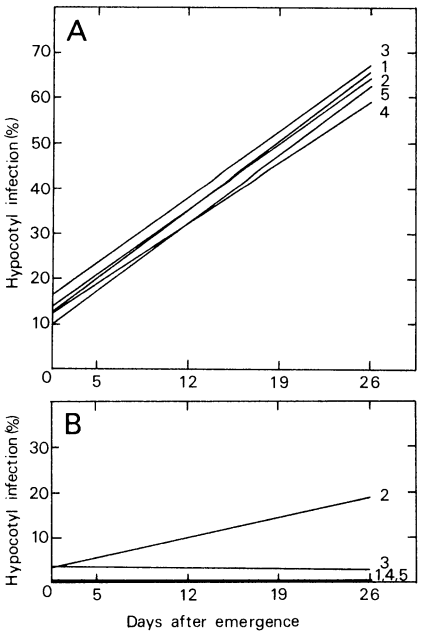

Fig. 1. Linear regression of *Phomopsis* hypocotyl infection of soybean seedlings grown from seeds subjected to five *Phomopsis* inoculation treatments on days after emergence in **(A)** a continuous-soybean and **(B)** a continuous-corn field. Inoculation treatments: (1) viable uninoculated seeds, (2) viable inoculated seeds, (3) viable inoculated seeds treated with captan, (4) viable uninoculated seeds mixed with nonviable inoculated seeds, and (5) viable uninoculated seeds mixed with nonviable uninoculated seeds.

presumably because of the presence of large amounts of soybean-crop residue-borne inoculum. Planting of dead *Phomopsis*-infested seeds with uninoculated seeds had no effect on seedling infection in either field.

Although it seems that little control of pod and stem blight can be achieved by planting *Phomopsis*-free seed in established soybean-growing areas where pod and stem blight is endemic, *Phomopsis*-infected seeds may be of practical significance as a means of introducing *Phomopsis* into new areas. Viable infected seeds would seem to be more important than dead infested seeds in this respect. This is an important point because dead infested seeds usually are visible, shrunken, and easily removed by seed-conditioning equipment. On the other hand, viable infected seeds rarely show disease symptoms and are normal in size. Seed health tests for plant quarantine purposes would therefore need to be sensitive enough to detect these kinds of seeds.

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