Predisposition to Seed Infection by *Phomopsis sojae* in Soybean Plants Infected by Soybean Mosaic Virus

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ABSTRACT

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Twenty-five soybean (*Glycine max*) lines were grown in the field and either inoculated at the primary leaf stage with the Illinois severe isolate of soybean mosaic virus (SMV) or not inoculated. In 20 lines, SMV inoculation reduced germination and increased seedborne incidence of *Phomopsis sojae* (*Diaporthe phaseolorum* var. *sojae*). Inoculation of Williams soybeans with SMV before or during flowering reduced germination and increased the incidence of *P. sojae* seed infection. Inoculation with SMV during pod development neither reduced germination nor increased seed infection by *P. sojae*. Regardless of the date of inoculation, Williams soybeans inoculated with SMV always yielded less than noninoculated plants. A. K. Harrow and Mansoy soybean plants grown in controlled-environment chambers were inoculated with SMV or *P. sojae* alone or in combination. The production of seeds with symptoms of *P. sojae* infection (moldy seeds) required both SMV and *P. sojae* inoculation. Soybean mosaic virus alone did not reduce seed germination or lead to production of damaged seed. When plants inoculated with SMV showed increased susceptibility to *P. sojae*, seed germination was reduced and damaged seed production was increased.

Additional key words: Cercospora kikuchii, Colletotrichum dematium var. *truncata*, seedborne pathogens.

Soybean mosaic virus (SMV), which causes a mosaic, and *Phomopsis sojae* Leh. (*Diaporthe phaseolorum* [Eke. & Ell.] var. *sojae* Wehm.), which causes pod and stem blight, are pathogens common on soybean (*Glycine max* [L.] Merr.) throughout the world (1,8,10). Both pathogens are seedborne and are associated with reduced seed quality. *P. sojae* reduces soybean germination, particularly if infection rate exceeds 20% (3,6). During the 1977 growing season, soybean lines screened for resistance to seed transmission of the Illinois severe isolate of SMV produced seed with low germination. We report studies to determine the cause of germination problems in plants infected with SMV.

MATERIALS AND METHODS

Soybean mosaic virus inoculation and indexing. Virus inoculum was prepared from Rampage soybean plants infected with the Illinois severe isolate of SMV. Tissues infected with SMV were homogenized with a sterile mortar and pestle in 0.05 M $NaPO_4$ (pH 7) (4 ml buffer/g tissue). A pinch of Carborundum (600 mesh, Union Carbide Corp., New York, NY 10017) was added, and the homogenate was applied manually with sterile gauze pads to a leaflet of soybean plants in the first trifoliolate leaf stage. Infection was confirmed by local lesion indexing 3–4 wk after inoculation (7).

Seed assay. Four or more lots of 100 seeds each were selected from each treatment. In each lot, the number of soybean seeds apparently infected with *P. sojae* (moldy seeds) was recorded. Seed lots were surface-disinfested by immersion in 0.5% NaOCl (10% Clorox) for 4 min, then aseptically transferred to sterile potato-dextrose agar (PDA, Difco) and incubated for 5 days at 25 C. The percentage germination and incidences of *Cercospora kikuchii* (T. Matsu & Tomoyasu) Chupp, *Colletotrichum dematium* (Pers. ex Fr.) Grove var. *truncata* (Schw.) Arx., and *P. sojae* were recorded. A seed was considered germinated if the radicle was 2.5 times the length of the cotyledons.

Field studies. In late May, 1977, 25 soybean lines (maturity groups II, III, and IV) were planted at the University of Illinois Plant Pathology Research Center, Urbana. Plants were either inoculated at the primary leaf stage with SMV as previously described or not inoculated. Experimental units were arranged in a randomized complete block design with four replications for each treatment in each soybean line. In 1978, Williams soybean plants were inoculated with SMV at weekly intervals, beginning when plants were in the third-node stage (V_3) and continuing until the bean-filling stage (R_5) (4). Experimental units were arranged in a randomized complete block design with five replications per treatment. In both years, 50 seeds were sown by hand in 1-m rows on 0.8-m centers. Seeds were harvested at maturity in October. In 1977, four lots of 100 seeds from each treatment in each soybean line were tested for seedborne fungi as previously described. In 1978, plot yield and 100-seed weights were determined before assay for seedborne fungi.

Selective inoculation studies. Seeds of two soybean cultivars, A. K. Harrow (susceptible to *P. sojae*) and Mansoy (resistant to *P. sojae*) (P. R. Hepperly and G. R. Bowers, Jr., *unpublished*), treated with thiram (Arasan 50 red, E. I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898) at 125 g/100 kg of seeds to prevent seedling infection by *P. sojae*, were germinated on cellulose pads (Kimpac) and examined for symptoms of SMV. Only seedlings free of the two pathogens were transferred to a sterile soil mix of sand, soil, and peat (1:2:1, v/v) in plastic pots. Six plants were transplanted into each pot, and each pot was thinned to a single seedling after 10 days. Sixteen plants from each soybean line were treated in one of the following ways: (i) noninoculated, (ii) inoculated with SMV, (iii) inoculated with *P. sojae*, or (iv) inoculated with SMV and *P. sojae*. *P. sojae* was applied three times when plants were in R_4, R_5, and R_6 growth stages (4), whether inoculated or not inoculated with SMV.

P. sojae inoculum was prepared from the surface mycelium of 1-wk-old cultures on PDA. The mycelium was ground for 1 min with a mortar and pestle, mixed with 10 ml of sterile distilled water, and passed through a 100-mesh screen. Mycelium suspension (100 ml) was atomized on each maturing plant at each inoculation date. Plants were grown in an environmental growth chamber that was programmed for 12 hr of light (800 μEin/m^2/sec) and 28 C day

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and 22 C night temperature. After each *P. sojae* inoculation, night humidity was maintained above 95% for three successive nights. Seeds were harvested at maturity, and the number of moldy seeds was recorded.

RESULTS

Field studies. In 1977, inoculation with SMV significantly reduced germination and incidence of *C. kikuchii* and *C. dematium* var. *truncata* and significantly increased incidence of moldy seeds, seedborne *P. sojae*, and total seedborne fungi (Table 1). For all seed assay variables, the analysis of variance revealed significant differences among soybean lines and significant interaction of soybean lines with SMV inoculation; most of the experimental variation could be explained by SMV inoculation. Five soybean lines (Midwest, PI 360.835, PI 86.146, PI 181.549, and Williams) had a high germination rate and low incidence of *P. sojae* after SMV inoculation (Table 2), thus showing no predisposition to *P. sojae* infection after SMV inoculation. Virginia had a germination rate and incidence of *P. sojae* approaching those of the five lines but was not considered to have the same level of tolerance because of the high incidence of moldy seeds.

In 1978, the yield of Williams soybeans inoculated with SMV was significantly less than that of noninoculated plants, regardless of growth stage at inoculation (Table 3). Only plants inoculated with SMV on or before growth stage R_2 produced seed with lower germination and 100-seed weights and higher incidence of seedborne *P. sojae* than noninoculated plants.

Selective inoculation studies. Inoculation with SMV was an important predisposing factor for *P. sojae* infection, as assessed by isolation of this fungus from seed (Table 4). The analysis of variance of combined means revealed a significant difference between the soybean lines for moldy seed production (A. K. Harrow = 50 and Mansoy = 33), significant effects of *P. sojae* and SMV inoculations on the production of moldy seeds, and no interaction between the various factors. Qualitative differences were found between noninoculated plants and plants inoculated with SMV, both of which were also inoculated with *P. sojae*. Profuse leaf and pod lesions and defoliation were recorded from plants inoculated with both pathogens. Plants inoculated with *P. sojae* alone had few lesions and no premature defoliation.

TABLE 1. Combined mean assay values for seeds of 25 soybean lines either noninoculated or inoculated with the Illinois severe isolate of soybean mosaic virus (SMV) in the field, 1977[a]

Assay criteria	SMV-inoculated (mean %)	Noninoculated (mean %)
Germination	49.2**[b]	70.2
Moldy seeds[c]	13.4**	7.4
Cercospora kikuchii	1.7**	4.0
Colletotrichum dematium f. *truncata*	1.6**	4.6
Phomopsis sojae	64.4**	38.6
Total seedborne fungi	70.0**	48.9

[a]Based on 10,000 seeds from noninoculated and SMV-inoculated plants, 800 seeds from each of 25 soybean lines.
[b]** indicates highly significant difference (a = 0.0001).
[c]Showing symptoms of *P. sojae* infection.

DISCUSSION

The Illinois severe isolate of SMV predisposed inoculated soybean plants to *P. sojae* seed infection. Predisposition was shown by inoculating the pathogens either alone or in combination onto soybean plants grown under controlled conditions. Our findings confirm the observation of Ross (9), who associated SMV with reduced seed quality and increased *P. sojae* seed infection. In our studies, inoculation with SMV did not predispose plants to infection by any

TABLE 2. Individual mean assay values for seeds of 25 soybean lines either noninoculated or inoculated with the Illinois severe isolate of soybean mosaic virus (SMV) in the field, 1977[a]

	Germination		Frequency of isolation of *Phomopsis sojae*		Frequency of moldy seeds[b]	
	SMV-inoc. (%)	Non-inoc. (%)	SMV-inoc. (%)	Non-inoc. (%)	SMV-inoc. (%)	Non-inoc. (%)
Midwest	93.5	88.0	4.8	9.3	0.3	0.5
PI 360.835	95.8	98.5	1.5	0.0	1.3	0.0
PI 181.549	97.5	95.5	8.3	5.0	1.0	1.0
Williams	76.0	95.0	59.0	11.3	2.3	1.5
PI 86.146	91.5	88.3	24.8	31.8	1.3	2.5
Mansoy	59.0	98.0	80.8	12.0	10.0	0.0
Hawkeye 63	52.3	96.0	73.0	23.0	7.3	3.5
Virginia	77.8	92.5	47.8	13.0	10.8	1.8
Manchu 2204	36.8	80.0	72.8	32.8	10.5	5.0
FC 04.007B	41.5	70.3	79.8	61.3	11.3	6.0
Seneca	56.3	71.3	82.5	72.8	8.8	8.5
PI 60.279	46.8	89.9	64.0	15.8	13.0	4.8
PI 70.019	28.3	81.0	80.3	37.3	15.3	4.0
Granger	58.5	72.7	68.5	46.3	15.0	5.0
Mukden	42.5	69.5	73.8	61.0	14.8	8.5
Funman	52.3	61.8	63.8	54.0	14.0	8.5
Manchu 606 Wisc.	28.5	53.5	88.0	54.8	13.5	10.0
Merit	21.8	52.3	79.8	60.8	18.8	7.8
PI 91.115	31.5	31.0	78.3	79.0	8.5	8.0
PI 68.680	24.5	39.8	83.0	56.8	18.0	9.0
Cloud	43.8	60.0	62.8	31.0	16.3	14.8
A. K. Harrow	20.8	47.5	95.3	81.8	19.3	12.5
PI 54.859	25.5	53.3	67.5	25.0	29.5	12.3
Magna	20.8	41.5	80.0	64.8	28.8	17.8
FC 31.678	6.3	13.0	90.8	62.7	45.5	28.3
$LSD_{P = 0.05}$	8.4		5.8		2.9	
$LSD_{P = 0.01}$	11.0		7.6		3.8	

[a]Means from four 100-seed replications.
[b]Showing symptoms of *P. sojae* infection.

TABLE 3. Results from seed assay in vitro and yield values for Williams soybeans noninoculated or inoculated at one of eight growth stages with the Illinois severe isolate of soybean mosaic virus

Growth stage at inoculation[a]	Means[b]				
	Yield (g/plot)	Germination (%)	100-seed weight (g)	*Phomopsis sojae* (% recovery)	Other seedborne fungi (% recovery)
Control (noninoculated)	458.2 a	95.2 a	24.1 a	13.0 a	4.8 a
R_5 (pod filling)	273.4 b	90.8 a	23.9 a	16.6 a	5.0 a
R_4 (2-cm pod)	294.5 b	88.8 a	24.1 a	15.0 a	7.8 ab
R_2 (0.5-cm pod)	274.9 b	75.4 b	19.9 b	35.2 b	8.6 ab
R_1 (flowering)	183.1 c	51.4 cd	17.2 b	65.6 c	8.2 ab
V_{20} (20th node)	108.7 d	49.5 cd	15.5 d	64.8 c	13.5 ab
V_{13} (13th node)	60.1 de	51.0 cd	16.2 cd	58.2 c	15.2 b
V_8 (8th node)	41.1 e	64.0 cd	20.1 b	39.3 b	10.0 ab
V_3 (3rd node)	22.7 e	45.2 d	16.6 cd	57.0 c	25.2 c

[a]Growth stages based on Fehr et al (4).
[b]Each mean based on five replications. Means with a common letter are not significantly different (a = 0.05) based on Duncan's multiple range test.

TABLE 4. Main effects of combined means for seeds with symptoms of infection by *Phomopsis sojae* (moldy seeds) from A. K. Harrow and Mansoy soybean plants noninoculated or inoculated with *P. sojae* or noninoculated or inoculated with the Illinois severe isolate of soybean mosaic virus (SMV)[a]

Treatment	Moldy seeds (%)
Inoculated with SMV	52
Noninoculated with SMV	31
Inoculated with *Phomopsis sojae*	64
Noninoculated with *P. sojae*	19

[a]Each mean is based on 16 observations. For mean comparisons, $LSD_{P=0.05}$ = 6.3 and $LSD_{P=0.01}$ = 8.7.

fungi other than *P. sojae*. The incidence of *C. kikuchii* and *C. dematium* var. *truncata* was reduced in SMV-inoculated plants.

Not all soybean lines were predisposed to *P. sojae* seed infection after inoculation with SMV. Midwest, PI 360.835, PI 86.146, and PI 181.549 were resistant to *P. sojae*, whether inoculated with SMV or not; Williams, however, was resistant to *P. sojae* if not inoculated with SMV but susceptible if inoculated with SMV. Cultivars appearing equally resistant to *P. sojae* when not infected with SMV showed differences in susceptibility when infected with SMV. This may indicate differences in degree or mechanism of *P. sojae* resistance among soybean lines. In breeding for higher seed quality in soybean, lines not predisposed to infection by *P. sojae* by SMV should be the preferred sources of *P. sojae* resistance.

Timing of SMV inoculation was important in determining seed quality losses induced by *P. sojae*. Germination was lowest if inoculation preceded flowering. In areas where predisposition to infection by *P. sojae* by SMV infection is likely, measures that reduce or prevent spread of the virus before pod development may promote production of high-quality seed.

Our findings may explain disagreement in the literature over the effect of SMV infection on soybean germination. Dunleavy et al (2), who associated low germination, seed coat cracking, and increased leachates with symptoms of soybean mosaic, may have examined effects of *P. sojae* accentuated by SMV infection. Gardner and Kendrick (5), who found no effect of SMV on soybean germination, probably examined soybean seed produced in seasons not favorable for *P. sojae* seed infection.

Knowledge of the interactions among pathogens is important to soybean breeding programs and to accurate assessment of yield losses.

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