Increased Incidence of *Phomopsis* sp. in Seeds from Soybeans Infected with Bean Pod Mottle Virus

R. E. STUCKEY, Associate Extension Professor, and S. A. GHABRIAL, Associate Professor, Department of Plant Pathology, University of Kentucky, Lexington 40546, and D. A. REICOSKY, Assistant Professor, Department of Crops and Soil Science, Michigan State University, East Lansing 48824

ABSTRACT

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The incidence of *Phomopsis* sp. in soybean (*Glycine max*) seeds increased as much as fivefold, depending on the cultivar, in plants inoculated with bean pod mottle virus and compared with uninoculated control plants. Soybean mosaic virus and bean yellow mosaic virus had little or no effect on seed infection by *Phomopsis* sp. Infections with bean pod mottle virus and soybean mosaic virus, regardless of inoculation sequence, increased seed infection by *Phomopsis* to levels equal to or significantly greater than infection with bean pod mottle virus alone. Seed infection by *Cercospora kikuchii* was much lower than that by *Phomopsis* sp. and did not appear to be influenced by virus infection. Yields from plants infected with both bean pod mottle virus and soybean mosaic virus ranged from 35 to 43% of controls in soybean cultivars susceptible to these two viruses. Soybean fields with a high incidence of bean pod mottle virus are more likely to have high levels of *Phomopsis* sp. seed infection and should not be saved for planting.

Additional key words: *Diaporthe*, predisposition, seed germination

Diaporthe phaseolorum (Cke. & Ell.) var. *sojae* Wehm. (anamorph *Phomopsis sojae* Leh.), the pod and stem blight fungus, is recognized as a major cause of poor quality and reduced germination of soybean (*Glycine max* (L.) Merr.) seed (9,11,14; J. A. Balles, *unpublished*). Recent studies with variants of *D. phaseolorum* recognize three distinct types of fungal isolates in association with soybean seed infection (7–9). They are *Phomopsis* sp., along with the teleomorphs *D. phaseolorum* var. *sojae* and *D. phaseolorum* var. *caulivora* Athow and Caldwell. Distinction among these variants is based on the production and morphology of perithecia and on the degree of virulence on seed (7,8). Furthermore, *Phomopsis* sp. and *D. phaseolorum* var. *sojae* are also associated with pod and stem blight, whereas *D. phaseolorum* var. *caulivora* is associated with stem canker (9). Because the majority of the fungal isolates in this study were classified as *Phomopsis* sp., this will be used for all isolates of *D. phaseolorum* from soybean seed.

An increase in the susceptibility of soybean to seed infection by *Phomopsis* sp. was reported as a result of infection with soybean mosaic virus (SMV) (6). Because recent surveys of virus diseases of soybean in Kentucky (5) have indicated that SMV, bean yellow mosaic virus (BYMV), and bean pod mottle virus (BPMV) are widespread, it was of interest to determine whether infections with these viruses also affect the susceptibility of soybean seed to seedborne fungi. The present paper reports the results of a 3-yr study on the effect of single and double virus infections in several cultivars of soybean on the incidence of seed infection by *Phomopsis* sp. and the purple seed stain fungus, *Cercospora kikuchii* (T. Matsu. & Tomoyasu) Chupp.

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MATERIALS AND METHODS

All field experiments were conducted at the University of Kentucky farm in Lexington in row or hill plots. Row plots, used in 1977 and 1978, were arranged in a randomized split-plot design with three replicates. Each plot consisted of three rows 6.1 m long and 76 cm apart. The virus treatments were main effects, and the cultivars were subeffects. Blocks of the main effects were separated by three rows of the soybean cultivar York, which is resistant to BYMV and SMV. The entire experimental plot was surrounded by three to six rows of York soybean and further surrounded by three to six rows of sweet corn (*Zea mays* L.). These measures were taken to reduce virus spread by vectors into and between plots.

Hill plots (13) were used in 1978 and 1979. In each hill, 15 seeds were planted within a linear distance of 46 cm. The hills were spaced 91 cm in one direction and 76 cm in the other. Each cultivar-virus treatment combination was replicated nine times. All treatments were completely randomized within each of three blocks (each block contained three replicates). Corn and soybean barriers were used between blocks and around the entire field, as described for row plots. Hills were thinned to eight plants per hill when the plants had reached the third node (V3) stage of growth (4) or earlier.

The SMV, BPMV, and BYMV isolates used in this study have been previously described (5). The viruses were increased in either *G. max* cv. Dare (SMV, BPMV) or *Phaseolus vulgaris* L. cv. Bountiful (BYMV). Infected tissues, harvested 14 days after inoculation, were homogenized in a Waring Blendor with 0.05 M potassium phosphate buffer, pH 7.0 (1:5 w/v), and the homogenates were filtered through two layers of cheesecloth. Carborundum (600 mesh) was added to the filtrates (2 g/L), and the inoculum was applied with cheesecloth pads to the primary leaves and first trifoliates of test plants. The percentage of plants showing symptoms typical of the virus used as inoculum was recorded 2–3 wk after inoculation.

The following soybean cultivars were used: Kent (IV), Essex (V), James (V), Williams (III), Cutler 71 (IV), and York (V). These soybean cultivars are commonly grown in Kentucky (16) and represent three different maturity groups (III, IV, and V). The soybean cultivars Kent, Essex, and James are susceptible to the three viruses used; the cultivars Williams and Cutler 71 are susceptible to SMV and BPMV but resistant to BYMV. The soybean cultivar York is resistant to both SMV and BYMV but susceptible to BPMV.

Seeds were collected from all plots at maturity or as soon after as weather permitted. Thus, several harvests were necessary per season because of differing cultivar maturities. In row plots, the center 4.9 m of the middle row was harvested. In hill plots, all plants were harvested. Seeds were dried to 7% moisture content and weighed. The weights were adjusted to 13% moisture content for yield comparisons.

For determination of the presence of seed-infecting fungi, 50–100 seeds per replicate were taken at random from harvested seed of row plots. Seeds from plants that showed symptoms not typical of the virus inoculated were discarded for seed assay purposes. For the hill plots, 50 seeds per hill were assayed from each of the nine replicates. In cases where insufficient seeds were harvested to make up a 50-seed sample, seeds from the three replicate hills within a block were pooled to make a total of 100 seeds and used for assay. Seeds used for the assay were surface-disinfected by immersion in 10% sodium hypochloride for 3 min, aseptically transferred to sterile petri dishes (9 cm) containing acidified Difco potato-dextrose agar (9), and incubated at room temperature. Five seeds were placed in each dish, and the incidence of *C. kikuchii*, *Phomopsis* sp., and other fungi was recorded after 7 days. Seed germination tests were determined with the wet paper towel method according to the standards set forth by the Association of the Official Seed Analysts (1).

RESULTS

In 1977, the percentage of virus-inoculated plants that showed symptoms varied from 40 to 90%, and no significant differences in yield were obtained between treatments of virus-inoculated and uninoculated control plants. The low level of virus-infected plants in the row plots (40–50% in some plots) probably reflects the inefficiency of the manual method of inoculation. Seed testing in 1977 was, therefore, limited to one cultivar (Essex). Plants showing symptoms typical of those of the virus used as inoculum were tagged, and seeds were harvested by hand at maturity. A significantly higher incidence of *C. kikuchii* and *Phomopsis* sp. was found in seeds from BPMV-infected than from uninoculated plants of the soybean cultivar Essex (Table 1). Significantly fewer healthy seeds and lower germination rates were also observed (Table 1). All other virus treatments were not significantly different from the control.

Failure to obtain 100% virus-infected plants in row plots in 1977 led to the approach of including hill plots in 1978 experiments. With the hill plots, monitoring the success of mechanical inoculation and roguing of uninfected plants could be more conveniently and efficiently performed. With few exceptions, all virus-inoculated plants showed symptoms. Significantly lower yields were obtained from plants in hill plots of cultivars susceptible to and inoculated with BPMV, SMV, or a combination of the two viruses in mixed inocula 1:1 (v/v) compared with control plants (Table 2). In all cultivars (not York) susceptible to BPMV + SMV, the double inoculation treatment resulted in more severe host symptoms than with either virus alone. The cultivar York is resistant to BYMV and SMV. The yields of the doubly infected cultivars were significantly reduced, averaging 40% of the control yield (Table 2). The BYMV treatment did not affect the yield of any of the cultivars tested. Unlike the hill plots, significant differences in yield among treatments were not demonstrated in the 1978 row plot experiments.

In 1978, the incidence of *Phomopsis* sp. in uninoculated control plants varied from 66% in Williams to 12% in Essex (Table 3). The earlier maturing the cultivar, the more susceptible it was to *Phomopsis* sp. seed infection. A statistically significant increase in the levels of *Phomopsis* sp. seed infection was obtained with all cultivars as a result of single inoculation with BPMV but not with SMV or BYMV (Table 3). Plants doubly infected with BPMV + SMV had the highest incidence of *Phomopsis* sp. seed infection in all cultivars tested; with the cultivars Essex and James, this treatment was significantly higher than the BPMV treatment (Table 3). A lower incidence of *C. kikuchii* was found in seeds from plants inoculated with BPMV, SMV, or both viruses together than in uninoculated control plants in cv. Williams, but not in other cultivars (Table 3).

In 1979, all virus treatments significantly reduced yield of all cultivars when compared with respective uninoculated control treatments with the exception of the SMV treatment on York, a cultivar resistant to SMV but susceptible to BPMV (Table 4). In the SMV-susceptible Essex, double infection with BPMV and SMV, regardless of the sequence of inoculation, increased incidence of seed infection by *Phomopsis* sp. more than single virus inoculation treatments (Table 4). Unlike the previous 2 yr, a significantly higher incidence of *Phomopsis* sp. was recorded in seeds of Essex, susceptible to BPMV and SMV, as a result of inoculation of SMV alone. York, resistant to SMV and susceptible to BPMV, had significantly higher seed infections by *Phomopsis* sp. with the BPMV or the BPMV and SMV inoculation sequence than with the SMV treatment (Table 4). No reduction in seed infection by *C. kikuchii* comparable with that recorded in 1978 was observed in the cv. Williams as a result of infection with BPMV or SMV.

DISCUSSION

Infection of several soybean cultivars with BPMV, but not with SMV or BYMV, significantly increased the incidence of *Phomopsis* sp. in seeds during 3 consecutive years. Because soybean fields with high incidences of BPMV are more likely to have high levels of *Phomopsis* sp. seed infection, seeds from such fields should not be saved for planting. Although BPMV is not seedborne, it is efficiently transmitted by certain chrysomelid beetles (12). The epidemiology of BPMV in Kentucky is not known, and soybean varieties resistant to BPMV are not commercially available. The use of insecticidal sprays to reduce the vector population (15) and/or fungicidal sprays for *Phomopsis* control (3,14) may prove economical in areas where BPMV is present.

The soybean cultivars commonly grown in Kentucky are all susceptible to *Phomopsis* sp., with the early maturing cultivars showing the highest levels of seed infection (Table 3). The cultivar

Table 1. Effect of infection with bean pod mottle virus (BPMV), bean yellow mosaic virus (BYMV), or soybean mosaic virus (SMV) on seed germination and infection by *Cercospora kikuchii* and *Phomopsis* sp. in soybean cultivar Essex (maturity group V) grown in row plots in 1977

Virus	Seed infection (%)[y]		Seed healthy (%)	Seed germination (%)[z]
	Cercospora	*Phomopsis*		
BPMV	10.5 a	27.5 a	57.0 a	61.0 a
BYMV	4.5 b	0.5 b	91.5 b	79.0 b
SMV	4.0 b	6.5 b	82.5 b	85.5 b
Control	3.5 b	5.0 b	84.5 b	82.0 b

[y] Values are means of four replicates (50 seeds per replicate). Means in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Values are means of four replicates (200 seeds per replicate). Means in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Mean yield of five soybean cultivars grown in hill plots and inoculated with bean pod mottle virus (BPMV), soybean mosaic virus (SMV), bean yellow mosaic virus (BYMV), or both BPMV and SMV in 1978

Virus	Yield (grams per hill) of cultivar[y]				
	Williams (III)	Kent (IV)	Essex (V)	James (V)	York (V)
BPMV + SMV[z]	62 a	107 a	86 a	62 a	220 ab
BPMV	107 b	157 b	133 b	103 b	201 a
SMV	136 b	204 c	148 b	111 b	240 bc
BYMV	176 c	244 d	203 c	137 bc	271 c
Control	174 c	247 d	211 c	160 c	263 c

[y] Maturity groups are given in parentheses. Values are means of nine replicates. Means in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Inocula for BPMV and SMV were mixed (1:1, v/v) prior to application to test plants.

Williams was reported to be resistant to *Phomopsis* sp. in Illinois in the absence of SMV infection (6). This cultivar was the most susceptible to *Phomopsis* sp. in this study regardless of virus infection. Similarly, the cultivar James, reported to be resistant to *Phomopsis* sp. seed infection in Delaware (2), was shown to be highly susceptible to *Phomopsis* sp. in Kentucky (Table 3). Differences in environmental conditions between two regions during seed maturation in a given soybean cultivar may account for these discrepancies. Wet warm conditions during the seed-filling period (beginning of seed development to physiologic maturity) were correlated with high incidences of *Phomopsis* sp. in seeds of several soybean cultivars (Balles, *unpublished*).

Table 3. Incidence of seed infection with *Cercospora kikuchii* and *Phomopsis* sp. in five soybean cultivars grown in hill plots and previously inoculated with bean pod mottle virus (BPMV), soybean mosaic virus (SMV), bean yellow mosaic virus (BYMV), or both BPMV and SMV in 1978

		Seed infection (%)[y]		
Cultivar[x]	**Virus**	***Cercospora***	***Phomopsis***	**Seed healthy (%)**[y]
Williams (III)	BPMV + SMV[z]	1.1 a	94.7 a	3.1 a
	BPMV	2.9 ab	85.3 a	8.7 a
	SMV	4.4 b	72.6 b	18.2 b
	BYMV	5.1 bc	71.1 b	20.4 b
	Control	7.6 c	66.4 b	24.7 b
Kent (IV)	BPMV + SMV	3.1 a	76.4 a	13.8 a
	BPMV	5.3 a	70.7 a	16.4 ab
	SMV	5.1 a	55.3 b	30.0 bc
	BYMV	6.2 a	53.1 b	30.9 bc
	Control	5.1 a	52.9 b	33.8 c
Essex (V)	BPMV + SMV	2.4 a	59.1 a	24.5 a
	BPMV	2.4 a	43.6 b	35.3 ab
	SMV	4.5 a	18.0 c	53.7 cd
	BYMV	4.3 a	14.9 c	49.3 bc
	Control	4.7 a	12.2 c	67.6 d
James (V)	BPMV + SMV	2.4 a	81.3 a	9.8 a
	BPMV	2.2 a	58.9 b	29.8 b
	SMV	3.1 a	40.7 c	46.9 c
	BYMV	2.7 a	40.4 c	48.7 c
	Control	5.3 a	39.6 c	39.1 bc
York (V)	BPMV + SMV	8.0 a	36.4 a	36.7 a
	BPMV	9.6 a	30.0 ab	44.9 a
	SMV	8.2 a	21.8 bc	53.8 a
	BYMV	11.1 a	13.1 c	54.7 a
	Control	8.2 a	12.9 c	52.4 a

[x] Maturity groups are given in parentheses.
[y] Values are means of nine replicates (50 seeds per replicate). For each cultivar, treatments that are followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Inocula for BPMV and SMV were mixed (1:1, v/v) prior to application to test plants.

Table 4. Effect of single or double infection with bean pod mottle virus (BPMV) and soybean mosaic virus (SMV) on yield and percentage of seed infection by *Cercospora kikuchii* and *Phomopsis* sp. in three soybean cultivars grown in hill plots in 1979

			Seed infection (%)[y]		
Cultivar[w]	**Virus**	**Yield**[x]	***Cercospora***	***Phomopsis***	**Seed healthy (%)**[y]
Williams (III)	BPMV	176.9 b	4.0 a	89.7 ab	3.7 a
	SMV	135.2 a	3.3 a	87.3 ab	8.7 a
	BPMV→SMV[z]	133.8 a	2.7 a	94.0 b	3.7 a
	SMV→BPMV[z]	120.6 a	2.3 a	92.7 ab	4.0 a
	Control	229.2 c	2.7 a	86.3 a	9.3 a
Essex (V)	BPMV	155.9 b	1.9 ab	40.3 b	55.0 b
	SMV	125.8 ab	2.0 b	49.7 b	46.0 b
	BPMV→SMV[z]	144.7 ab	1.3 ab	69.7 c	26.3 a
	SMV→BPMV[z]	121.0 a	1.9 ab	70.3 c	26.7 a
	Control	280.6 c	0.3 a	20.3 a	74.3 c
York (V)	BPMV	237.8 a	1.7 a	33.0 b	60.3 a
	SMV	300.6 b	1.3 a	18.0 a	75.0 b
	BPMV→SMV[z]	204.6 a	1.0 a	31.7 b	63.3 a
	SMV→BPMV[z]	235.6 a	0.7 a	26.7 ab	65.0 ab
	Control	337.8 b	1.3 a	22.3 a	69.9 ab

[w] Maturity groups are given in parentheses.
[x] Values are mean weights (grams per hill) of nine replicates. Each replicate represents the weight of seeds harvested from all plants in the hill. Means in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[y] Values are means of nine replicates (50 seeds per replicate). Means in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Plants were inoculated with the first virus (BPMV or SMV) and 1 wk later with the second virus.

Susceptible cultivars doubly infected with BPMV and SMV produced the lowest yields, and the seeds showed the highest incidence of *Phomopsis* sp. seed infection. Significant differences in *Phomopsis* sp. infections were obtained between plants doubly infected with BPMV + SMV and those singly infected with BPMV in the Essex and James cultivars (Table 3). Inoculation with SMV alone, however, did not consistently alter the level of *Phomopsis* sp. seed infection. Doubly infected plants were severely stunted and exhibited varying degrees of necrosis. The apparent synergistic response of plants to double virus infections may have rendered them more susceptible to *Phomopsis* sp. infection. Hepperly et al (6), using a highly virulent strain of SMV, reported a marked increase in *Phomopsis* sp. seed infection in SMV-inoculated plants in several soybean cultivars. The Kentucky isolate of SMV used in this study is a mildly virulent strain and may not have been as efficient as that of Hepperly et al (6) in predisposing infected plants to *Phomopsis* sp.

The significantly lower incidence of *C. kikuchii* found in seeds of the cv. Williams inoculated with SMV, BPMV, or a combination of the two viruses (Table 3) supports a similar observation by Hepperly et al (6) in the case of SMV-inoculated plants. This finding, however, may be unrelated to virus infection because there may be an alternative interpretation. Williams, the earliest maturing cultivar, had the highest incidence of *Phomopsis* sp. Assuming that *Phomopsis* sp. infection greatly reduces the chances for *C. kikuchii* infection (double seed infections were recorded in less than 2% of the seeds), there would be fewer seeds available for *C. kikuchii* infections. When the number of seeds infected by *C. kikuchii* over the total number of seeds not infected by *Phomopsis* sp. is expressed as a percentage of the *C. kikuchii* infection of available seeds, insignificant treatment effects of *C. kikuchii* are observed. Levels of *C. kikuchii* infection in 1978 then become 21, 20, 16, 18, and 23% for the treatments BPMV + SMV, BPMV, SMV, BYMV, and uninoculated control, respectively. Infections with BPMV or SMV evidently do not affect the level of seed infection with *C. kikuchii*, in contrast to their effect on *Phomopsis* sp.

infection.

Although examples of predisposition of virus-infected plants to fungal pathogens have been reported in several crops (10), the mechanisms underlying these interactions as well as those in the *Phomopsis* sp.-BPMV system are unknown. Nevertheless, awareness of such interactions is important because of their economic implications.

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