

## Identification of Field Tolerance to Bean Pod Mottle and Soybean Mosaic Viruses in Soybean

J. H. Hill,\* N. C. Koval, J. M. Gaska, and C. R. Grau

### ABSTRACT

Development of a method to identify field tolerance to bean pod mottle and soybean mosaic viruses (BPMV and SMV) in soybean [*Glycine max* (L.) Merr.] allowed evaluation of 33 soybean accessions for field response to BPMV and SMV. Based on measurement of parameters that included relative level of virus antigen in seed and mottling of soybean seed coats, three accessions showed tolerance to SMV and BPMV (PI 561353, M90-18411, PI 507353), eight were tolerant to SMV (MN 1301, M92-160047, M91-113037, PI 423826A, A99-216031, NE 3001, U96-2408, Colfax), and four were tolerant to BPMV (PI 184042, M93-326056, M95-255017, Spansoy 201). Treatment of seed with the insecticide imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) did not effect disease control. The relative level of virus antigen in seed harvested from experimental plots was shown to correlate significantly with virus incidence in plots. The data suggest that quantitative assay of virus seed antigen may provide a useful estimate of relative virus incidence in test plots and aid identification of field tolerance to seed-borne viruses.

BEGINNING IN 1998, BPMV has become an increasing problem for soybean producers in the north central United States. Recently, Giesler et al. have summarized properties of the virus as well as its pathology to soybeans (Giesler et al., 2002). Since the late 1990s, losses due to the disease have included reduced yield and seed quality conferred by mottling of soybean seed coats or hilum bleeding. The principal insect vector of the virus is the bean leaf beetle (*Cerotoma trifurcata*) (Hopkins and Mueller, 1984). A low level of transmission through soybean seed may also occur (Krell et al., 2003).

One solution for disease control has been developed and depends on well-timed insecticides to manage bean leaf beetle populations. (Krell et al., 2004). However, the most effective long-term method for control of disease caused by virus infection is host plant resistance. Resistance to BPMV has not been reported for commercially available cultivars of *Glycine max*. Ross (1986) previously reported resistance of four soybean germplasm lines that exhibit mild symptoms after mechanical inoculation with BPMV. A recent report, using mechanical inoculation of 115 accessions of *G. max* soybeans with BPMV in the greenhouse, has identified 11 acces-

sions that expressed mild symptoms of virus infection. All accessions, however, became systemically infected, and field performance of these lines was not reported (Zheng et al., 2005). In the same report, 12 accessions of *G. tomentilla* did not become systemically infected with BPMV. However, incorporation of this resistance into *G. max* is hampered by the difficulty of making interspecific crosses. Recent evaluation of 52 U.S. ancestral accessions found no resistance to BPMV (Zheng et al., 2005). Personal observations as well as those reported by growers suggest that soybean cultivars vary significantly in response to disease caused by BPMV, and that severity of leaf symptoms does not positively correlate with the presence of BPMV. Therefore, since leaf symptoms could not be reliably used, an alternative approach explored the potential for resistance or tolerance. Studies were established to search for field tolerance under conditions of high disease incidence. Under these conditions, tolerant accessions may have reduced virus incidence resulting in higher yields and improved seed quality. No controlled inoculation of plants was employed, and all spread of endemic virus occurred through activity of insect vectors naturally present at experimental locations.

Since primary grower concerns are centered on reductions in yield and seed quality, the measurement of field tolerance included data on yield and evaluation of seeds harvested from soybeans grown where virus disease was present. Since seed quality can be negatively impacted by discoloration or mottling (hilum bleeding) of seed coats, assessment of seed quality for purposes of this study included the percentage of seed with mottled seed coats, as well as the relative amount of virus antigen in a seed sample. Percentage of seed with mottled seed coats and the amount of virus antigen were the basis for development of a virus seed index (Krell et al., 2005).

Compounding the difficulty of identification of tolerance to BPMV was presence of SMV, whose symptoms are difficult to differentiate from those of BPMV in the field (Hill, 1999). Associated with infection by SMV is mottling of soybean seed coats, which is indistinguishable from that caused by BPMV. Similar to BPMV, the virus is also transmitted through seed but at potentially higher levels (Hill, 1999). Further, infection of soybean plants by both viruses can cause synergism (Calvert and Ghabrial, 1983). For SMV, resistance to a variety of SMV strains is differentially conferred by three resistance genes, *Rsv1*, *Rsv3*, and *Rsv4* (Hill, 1999).

Since an efficient vector of SMV (Hill et al., 2001) was increasingly prevalent after 2001 (Lee Burrows et al.,

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**Abbreviations:** BPMV, bean pod mottle virus; ELISA, enzyme-linked immunosorbent assay; OD, optical density; SMV, soybean mosaic virus.

2005, Ragsdale et al., 2004, Wedberg et al., 2001), the possibility that SMV could be an important part of the virus disease complex in experimental plots to identify virus disease resistance was evident. Therefore, soybean accessions were also screened for field tolerance to SMV in the last two of the three years the study was conducted, using methods similar to those used for BPMV. The objective of this study was to determine if a method to identify field tolerance to BPMV and/or SMV could be developed. In the process, several candidate soybean accessions were identified.

## MATERIALS AND METHODS

### Experimental Field Design

For the initial studies, a set of cultivars was selected from plots to evaluate lines for resistance to brown stem rot [*Phialophora gregata* (Allington and Chamberlain) W. Gams]. In 2001 and 2002, the cultivars were planted at the Arlington Agricultural Experiment Station, Columbia County, WI, and near Janesville, Rock County, WI. For the 2002 trials only, seed of an additional set of the same cultivars was treated with the insecticide imidacloprid (Gaucho 4F, 29.6 mL/22.7 kg; Gustafson, Marsing, ID, and Bayer CropScience, Research Triangle Park, NC). Additional accessions, cultivars, breeding lines, and plant introductions were evaluated for reaction to BPMV and SMV. The expanded set of accessions, based on field and greenhouse results (Grau, unpublished), were added to the trial at Rock County in 2002, and the experiment was repeated in 2003 at the West Madison Agricultural Experiment Station, Dane County, WI.

Individual plots were planted at 3.0 m × 7.6 m, and four rows were spaced 76 cm apart. Plots were managed for weeds using standard agronomic practices. The previous year's crop was corn at all Rock County locations and soybean at the Columbia County and Dane County locations. A randomized complete block design with three to four replications was used at each location.

To determine virus incidence, the most fully expanded terminal trifoliolate leaf was collected from each of 20 plants chosen randomly in each plot, and each leaf was assayed separately by enzyme-linked immunosorbent assay (ELISA). Leaves were immediately placed on ice for transport and stored at -20°C until assayed for BPMV and SMV. In 2001, samples were collected at soybean growth stages (Fehr and Caviness,

1977) V5/V6, R2, and R5 at both locations. An additional sampling time was added in 2002, with samples being collected at soybean growth stages V1, V5/R2, R3/R4, and R6 at both locations. Sampling at the Dane County site in 2003 was intensified to seven times during the growing season, including soybean growth stages V1, V5, R2, R3, R4, R5, and R6/7. Protocols previously described (Krell et al., 2003) or diagnostic kits from Agdia (Elkhart, IN) were used for the ELISA. Leaf samples were extracted using 0.05 M phosphate buffer, pH 7.2 (Sigma, St. Louis, MO). Absorbance at 405 nm (generally greater than 0.80) was measured using an EL 800 Universal Microplate Reader (Bio-Tek, Winooski, VT).

Plots were also visually assessed for virus symptoms three times in 2001 at both locations, starting when symptoms began to appear. Symptoms attributed to viruses were rated visually in each plot by estimating the percentage of symptomatic tissue visible at the top of the plant canopy. In 2002, the Columbia County site was assessed three times and Rock county was assessed five times beginning at growth stages R2/R3. Virus assessment in 2003 at Dane County began at growth stages R3/4 and was done weekly for 4 wk. Only data from the last sampling date are shown.

### Seed Analyses

Samples of 100 seeds were evaluated from each replicate to determine the percentage of seed coat discoloration for a soybean seed sample. Any seed showing brown or black seed coat discoloration was counted as mottled. The relative amount of BPMV or SMV antigen in a sample of 100 seeds was determined by ELISA in a manner similar to that previously described (Krell et al., 2005). In summary, batches of 100 seeds were ground at setting no. 6 for 1 min in 100 mL of 0.05 M sodium borate, pH 7.2, with a Brinkmann Polytron homogenizer and model PT 20 ST probe generator (Brinkman Instruments, Westbury, NJ). Extracts were squeezed through two layers of cheesecloth and 0.1-mL samples were used per well. Positive and negative controls were included in each ELISA plate. Optical density (OD) at 405 nm was recorded at six timed intervals during a 20- to 150-min period after addition of the enzyme substrate. The mean OD<sub>405</sub> of four replicate wells was used to generate a regression equation that relates hydrolysis time to OD. A standard hydrolysis time of 60 min after substrate addition was used to calculate the OD that corresponds to the amount of virus antigen in each homogenized seed sample (sample OD). A similar calculation was made for the negative healthy seed control (negative control

**Table 1. Response of soybean cultivars to natural infection by bean pod mottle virus (BPMV) at two Wisconsin locations in 2001.**

Cultivar	Seed mottling		BPMV AG†		BPMV virus SI‡		Yield		Virus symptoms	
	Columbia County	Rock County	Columbia County	Rock County	Columbia County	Rock County	Columbia County	Rock County	Columbia County	Rock County
	%						kg ha <sup>-1</sup>		%	
Spansoy 201	—	5 a	—	4.01 bcd	—	18.7 a	—	4287 cd	—	10 ab
Mycogen 5261	1 a§	8 ab	0.94 a	4.64 d	1.2 a	45.7 ab	4361 e	4656 e	4 a¶	4 a
H2494	1 a	16 b	0.99 ab	2.81 a	1.0 a	41.5 a	4193 de	4508 de	3 a	20 a-c
Asgrow 2301	5 a	20 b	0.96 a	4.10 cd	4.4 a	83.1 b	3870 cd	4159 c	4 a	26 a-c
Pioneer 92B62	4 a	44 c	1.40 c	3.60 bc	4.7 a	151.8 c	3393 b	4065 bc	3 a	37 cd
Mark 9824RR	3 a	58 c	1.08 b	3.30 ab	3.6 a	195.9 d	3541 bc	3769 b	63 c	79 e
Asgrow 2101	53 b	47 c	0.93 a	3.97 b-d	45.5 b	184.5 cd	2506 a	2997 a	26 b	56 de
Spansoy 250	11 a	45 c	0.93 a	3.85 bc	10.4 a	177.1 cd	4011 de	3957 bc	5 a	61 de
LSD <sub>0.05</sub>	15	11	0.10	0.78	17.3	38.5	467	368	9	27

† BPMV antigen (AG) is used as a measurement of relative amount of BPMV in seed samples harvested from experimental plots. A value of 1.0 or less designates no detectable virus antigen in the sample.

‡ Virus seed index (SI) is calculated as the product of the percentage of seed coat mottling and the BPMV antigen.

§ Means of four replications followed by the same letter are not statistically different at  $P \leq 0.05$ .

¶ Estimated percentage of symptomatic tissue visible at the top of the plant canopy in experimental plots on 20–21 Aug. 2001 (R6).

OD). For each ELISA plate, the relative virus antigen content of each sample was calculated as relative amount of virus antigen in seed sample = (sample OD)/(negative control OD plus two standard deviations). Therefore, calculated values of virus antigen in seed samples are relative to 1.0, which designates no detectable antigen. Similar to a previous report, sample well-to-well variation was less than 5%, and regression equations yielded coefficient of determination ( $R^2$ ) values greater than 0.99 (Krell et al., 2005).

The number calculated as the product of percentage seed coat mottling and relative antigen was arbitrarily defined as the “virus seed index” for each seed sample. Using this criterion, a seed sample with a low virus index was regarded as superior in quality to a sample with a high virus index. To obtain an overall assessment of tolerance to both BPMV and SMV, a virus seed index was also calculated as the sum of the seed index for each virus.

### Data Analyses

Differences between virus index, relative antigen content, seed coat mottling, seed yield, and percentage symptomatic

plants were analyzed using the general linear model procedure (SAS Institute, Inc., 1999). Differences generating significant F-value differences were further compared by examining the least significant difference.

## RESULTS AND DISCUSSION

The initial experiments were established in 2001 at two sites using a set of soybean cultivars (Table 1). The mean value of relative BPMV antigen for all cultivars was significantly higher ( $t$  test,  $P < 0.01$ ) at the Rock County site (mean = 3.8) than at the Columbia County site (mean = 1.0). This was supported by observation of few bean leaf beetles at the Columbia County site as compared to the Rock County site and virus incidence data (not shown), based on ELISA of leaf samples, collected at growth stage R5, that showed mean incidence (mean = 100%) of BPMV significantly greater ( $t$  test,  $P < 0.01$ ) at Rock County than at Columbia County (mean = 0.0%). Apparent viruslike symptoms observed

**Table 2. Response of soybean cultivars to natural infection by bean pod mottle and soybean mosaic viruses (BPMV and SMV) at two Wisconsin locations in 2002.**

Cultivar	Seed mottling		BPMV AG <sup>†</sup>		BPMV virus SI <sup>‡</sup>	
	Columbia County	Rock County	Columbia County	Rock County	Columbia County	Rock County
	%					
Spansoy 201	1 a§	8 a	2.05 a	4.98 ab	1.6 a	36.7 a
Mycogen 5261	1 a	21 b	1.80 a	5.27 ab	2.7 a	100.7 ab
Mark 9824RR	2 a	36 c	2.10 a	4.55 ab	4.4 a	170.2 bc
H2494	0.5 a	47 de	2.07 a	4.10 a	2.1 a	189.2 cd
Asgrow 2301	2 a	33 c	3.56 b	6.97 cd	6.6 a	230.0 c-e
Spansoy 250	4 a	46 de	2.15 a	5.33 ab	5.6 a	249.2 de
Colfax	1 a	38 cd	3.95 b	7.37 d	5.6 a	275.7 e
Asgrow 2101	20 b	48 e	3.11 ab	5.80 bc	70.3 b	275.0 e
LSD <sub>0.05</sub>	10	10	1.38	1.30	41.2	77.8
Cultivar	SMV AG <sup>¶</sup>		SMV virus SI		BPMV + SMV AG	
	Columbia County	Rock County	Columbia County	Rock County	Columbia County	Rock County
Spansoy 201	1.43 a-d	0.97 ab	1.4 a	7.8 a	5.46 b	6.04 a-c
Mycogen 5261	1.23 a	1.01 ab	1.7 a	21.1 b	2.90 a	5.62 ab
Mark 9824RR	1.30 ab	0.98 ab	2.9 a	34.9 c	3.40 a	5.53 ab
H2494	1.22 a	0.87 a	1.2 a	41.5 c	3.29 a	4.97 a
Asgrow 2301	1.70 b-d	1.04 bc	2.3 a	38.5 c	5.05 b	8.07 d
Spansoy 250	1.34 a-c	0.97 ab	6.8 a	44.4 cd	3.47 a	6.30 bc
Colfax	1.51 a-d	1.03 bc	1.5 a	39.3 c	5.46 b	8.51 d
Asgrow 2101	1.81 d	1.19 c	47.9 b	56.6 c	4.92 b	6.95 d
LSD <sub>0.05</sub>	0.45	0.17	23.7	12.8	1.42	1.29
Cultivar	BPMV + SMV virus SI		Yield		Virus symptoms <sup>#</sup>	
	Columbia County	Rock County	Columbia County	Rock County	Columbia County	Rock County
	-kg ha <sup>-1</sup>					
Spansoy 201	3.5 a	60.5 a	3823 bc	3863 c	3 ab	7 a
Mycogen 5261	4.3 a	103.6 a	4361 e	3722 c	2 ab	22 c
Mark 9824RR	7.4 a	205.0 b	3910 c	3272 a	15 cd	37 e
H2494	3.3 a	232.0 bc	4468 e	3695 bc	0.5 a	13 b
Asgrow 2301	9.1 a	259.7 cd	3910 cd	3312 ab	11 bc	9 ab
Spansoy 250	21.2 a	293.4 de	4052 d	3803 c	1 ab	33 d
Colfax	5.5 a	320.6 e	3420 a	3333 ab	29 e	73 f
Asgrow 2101	118.2 b	314.7 e	3628 ab	3077 a	27 de	21 c
LSD <sub>0.05</sub>	64.2	44.9	228	385	10	5

<sup>†</sup> BPMV antigen (AG) is used as a measurement of relative amount of BPMV in seed samples harvested from experimental plots. A value of 1.0 or less designates no detectable virus antigen in the sample.

<sup>‡</sup> Virus seed index (SI) is calculated as the product of the percentage of seed coat mottling and the virus antigen.

<sup>§</sup> Means of eight replications followed by the same letter are not statistically different at  $P \leq 0.05$ . Data from four replications per soybean variety of seeds treated or not treated with imidacloprid were combined for analysis because pairwise comparison revealed no significant differences ( $t$  test,  $P = 0.05$ ) for all characters measured.

<sup>¶</sup> SMV seed antigen (AG) is used as a measurement of relative amount of SMV in seed samples harvested from experimental plots. A value of 1.0 or less designated no detectable virus antigen in the sample.

<sup>#</sup> Estimated percentage of symptomatic tissue visible at the top of the plant canopy in experimental plots on 19–20 Aug. 2000 (R6).

at the Columbia County site (Table 1) were presumably due to presence of SMV, where mean incidence was 18% as compared to 4% at Rock County.

In 2002 and 2003, seed tests were conducted for both BPMV and SMV because of increased populations of the soybean aphid and the inability to discern BPMV from SMV based on symptoms on foliage or seed (Tables 2 and 3). In 2002, based on reports that well-timed application of foliar insecticide may reduce final disease incidence and improve yield and seed quality (Krell et al., 2004), an additional set of seeds of the soybean cultivars selected for the 2001 test was treated with the insecticide imidacloprid to reveal any effect on the characters measured. Although application of imidacloprid as a seed treatment may reduce beetle populations after emergence (Cullen et al., 2003), pairwise comparisons of these cultivars in 2002 showed no significant differences (*t* test,  $P = 0.05$ ) in all characters measured for each soybean cultivar (data not shown). Therefore, imidacloprid did not confer disease control in these tests and the data were combined for analysis.

As in 2001, mean incidence of BPMV (mean = 99.9%), based on ELISA of leaf samples collected at growth stage R6, was significantly (*t* test,  $P < 0.01$ ) higher at Rock County in 2002 than at Columbia County (mean = 2.9%) (Table 2). For SMV, mean incidence of SMV was greater at Columbia County (mean = 27.4%) than at Rock County (mean = 0.4%) in plants sampled at R6. Consistent with this, mean values of relative BPMV antigen for all accessions was significantly higher ( $P < 0.01$ ) at Rock County (mean = 5.5) than at the Columbia County site (mean = 2.6). For SMV, significantly different ( $P < 0.01$ ) values were reported at Rock County (mean = 1.0) and Columbia County (mean = 1.4) (Table 2). Both the incidence data and relative virus antigen were a reflection of the insect vector prevalent at each location (data not shown).

Tests were conducted at the Dane County location in 2003 where both bean leaf beetles and soybean aphids were present (data not shown). Consistent with this observation, ELISA of leaf samples collected from

soybean plants at R6/7 showed both SMV and BPMV were detected but that mean incidence (mean = 43.1%) of SMV was significantly different ( $P < 0.01$ ) than that of BPMV (mean = 4.8%). In agreement with these data, the mean relative antigen for all cultivars in the set of commercial cultivars used in the 2001 and 2002 tests was 1.7 and 2.8 for BPMV and SMV, respectively (Table 3).

The expanded set of soybean accessions was evaluated where BPMV was prevalent at Rock County in 2002, and where SMV was prevalent at the Dane County location in 2003 (Table 4). Mean relative BPMV antigen for all accessions at Rock County in 2002 was 6.8 and was 1.1 for SMV. In contrast, mean relative SMV antigen (mean = 2.6) was higher than mean relative BPMV antigen (mean = 1.7) at Dane County in 2003. Again, both the virus incidence data (2002: mean BPMV = 98.7%, SMV = 0.0%; 2003: mean BPMV = 6.6%, SMV = 32.5%) and relative virus antigen were a reflection of the insect vector prevalent at each location (data not shown).

The assumption that soybean accessions with higher incidence of infection will result in higher levels of seed-borne virus (relative antigen) was tested by regression analysis. The highly significant ( $P < 0.01$ ) coefficient of determination ( $R^2 = 0.77$ ) between virus incidence, as determined by ELISA of leaf samples, and relative virus antigen suggested the validity of this hypothesis. The significance of this relationship suggests that comparative analysis of seed-borne virus antigen can provide a relative assessment of virus incidence in experimental plots and precludes the necessity of collecting and assaying leaf samples to determine differences in virus incidence among different plots. Thus a homogeneous mixture of harvested seed may represent a better source of plant material to assay than leaves collected from the crop canopy.

Based on both relative antigen level in seed and virus incidence, as determined by ELISA of leaf samples, Rock County in 2001 and 2002 were sites most appropriate to characterize soybean accessions for tolerance to BPMV. Columbia County in 2002 and Dane County in 2003 were locations to characterize soybean accessions for tolerance to SMV. The biological variability

**Table 3. Response of soybean cultivars to natural infection by bean pod mottle and soybean mosaic viruses (BPMV and SMV) in Dane County, Wisconsin in 2003.**

Cultivar	Seed mottling	BPMV AG†	BPMV virus SI‡	SMV AG§	SMV virus SI	BPMV + SMV AG	BPMV + SMV virus SI	Yield	Symptoms¶
	%							kg ha <sup>-1</sup>	%
Spansoy 201	76 cd#	1.42 a	109.5 b	3.00 ab	229.7 c	4.43 ab	339.2 cd	2365 a	28 a-d
Mycogen 5261	28 b	1.70 ab	47.5 a	3.39 bc	96.4 ab	5.09 bc	143.9 ab	3366 b	26 a-c
H2494	11 ab	1.67 a	19.8 a	2.10 ab	24.9 ab	3.77 ab	44.7 a	4502 c	7 a
Asgrow 2301	79 cd	1.73 ab	137.0 b	4.72 c	377.6 d	6.45 c	514.6 e	3454 b	32 b-d
Spansoy 250	79cd	1.72 ab	135.8 b	3.05 ab	251.5 c	4.77 ab	387.7 c-e	3359 b	44 cd
Colfax	1 a	1.61 a	1.6 a	1.88 a	1.9 a	3.49 a	3.5 a	4327 c	14 ab
Asgrow 2101	90 d	2.24 b	205.1 c	2.65 ab	240.7 c	4.90 a-c	445.8 de	2862 ab	50 d
Pioneer 92B62	73 c	1.89 ab	136.4 b	1.94 a	141.9 bc	3.83 ab	278.3 bc	2956 b	22 a-c
Mark 9924	78 cd	1.40 a	106.5 b	2.93 ab	223.0 c	4.34 ab	329.5 cd	3131 b	80 e
LSD <sub>0.05</sub>	17	0.57	57.8	1.40	115.7	1.60	153.6	621	24

† BPMV antigen (AG) is used as a measurement of relative amount of BPMV in seed samples harvested from experimental plots. A value of 1.0 or less designates no detectable virus antigen in the sample.

‡ Virus seed index (SI) is calculated as the product of the percentage of seed coat mottling and the virus antigen.

§ SMV antigen (AG) is used as a measurement of relative amount of SMV in seed samples harvested from experimental plots. A value of 1.0 or less designates no detectable virus antigen in the sample.

¶ Estimated percentage of symptomatic tissue visible at the top of the plant canopy in experimental plots on 29 Aug. 2003 (R6/R7).

# Means of four replications followed by the same letter are not statistically different at  $P \leq 0.05$ .

**Table 4. Response of soybean accessions and lines to natural infection by bean pod mottle and soybean mosaic viruses (BPMV and SMV) in Rock County, WI, in 2002 and in Dane County, WI, in 2003.**

Accessions	Seed mottling	BPMV AG‡	BPMV virus SI§	SMV AG‡	SMV virus SI§	BPMV + SMV AG	BPMV + SMV virus SI§	Yield	Symptoms¶
	%							kg ha <sup>-1</sup>	%
Year†									
2002									
PI 184042	10 ab#	4.81 ab	44.3 a	1.17 b-d	11.8 a-c	5.99 ab	56.1 a	3393 c-e	37 gh
M 93-326056	11 ab	6.75 b-h	71.7 ab	1.17 b-d	13.0 a-d	7.92 b-f	84.6 ab	3205 b-d	5 a-c
PI 561353	12 a-c	6.91 c-i	83.1 a-c	1.04 a-d	12.7 a-d	7.95 b-f	95.8 a-c	2943 bc	1 a-c
M 90-184111	16 a-d	5.28 a-c	86.4 a-d	1.02 a-d	16.7 a-g	6.30 a-c	103.0 a-d	3581 d-f	2 a-c
PI 507353	16 a-d	6.02 a-f	94.4 a-e	0.97 a-c	15.3 a-f	6.99 a-d	109.7 a-e	3091 b-d	15 b-e
M 95-255017	23 b-f	5.13 a-c	116.2 a-f	1.03 a-d	23.5 a-h	6.16 ab	139.9 a-f	3783 e-g	13 a-d
MN 1301	47 hi	5.02 a-c	239.4 e-i	0.97 a-c	53.2 l	5.99 ab	285.4 g-j	3192 b-d	15 c-f
M 92-160047	30 ef	5.35 a-c	164.9 a-h	0.98 a-c	29.7 d-i	6.34 a-c	194.6 a-h	2970 bc	4 a-c
SD 96-755	45 g-i	4.19 a	191.0 a-h	1.01 a-d	45.6 i-l	5.21 a	236.6 d-i	3071 b-d	72 i
Bell	47 hi	4.18 a	191.5 a-h	1.02 a-d	47.9 j-l	5.20 a	239.4 d-i	3077 b-d	13 a-d
IA 1008	27 d-f	7.74 e-j	200.8 b-h	1.17 b-d	32.7 g-k	8.91 d-g	233.5 c-i	3239 b-d	0.3 ab
M 91-113037	35 f-h	5.89 a-e	207.2 b-h	1.03 a-d	36.2 h-l	6.94 a-d	244.1 e-i	3400 c-e	6 a-c
M 94-209136	50 i	5.58 a-d	283.3 g-j	0.96 a-c	48.5 kl	6.51 a-c	330.2 h-k	3447 c-f	10 a-d
PI 511356	50 i	6.12 a-g	305.3 h-j	1.06 a-d	53.8 l	7.18 a-e	359.1 i-k	1391 a	0 a
U 96-2408	7 a	9.28 j	63.9 ab	0.97 a-c	6.5 a	10.25 g	70.3 ab	4421 i-k	73 i
PI 398311	11 ab	9.31 j	100.5 a-e	0.91 a	9.9 ab	10.19 g	110.1 a-e	2748 b	11 a-d
PI 423826A	25 c-f	6.40 b-g	157.0 a-g	1.08 a-d	27.6 b-h	7.48 a-e	184.4 a-g	2936 bc	8 a-c
A 99-216031	20 b-d	7.95 e-j	159.2 a-h	0.93 ab	19.0 a-h	8.88 d-g	179.7 a-g	4179 g-j	2 a-c
NE 3001	24 c-f	6.49 b-g	161.5 a-h	1.18 cd	30.4 d-j	7.67 b-f	191.9 a-h	4878 k	40 h
SD 97-456	22 b-e	7.97 f-j	172.5 a-h	0.93 a-c	20.0 a-h	8.91 d-g	192.4 a-h	4461 jk	30 f-h
NE 2701	26 d-f	8.96 ij	231.0 c-i	1.07 a-d	27.4 b-h	10.03 g	258.4 f-i	4461 jk	7 a-c
Savoy	34 e-g	7.46 d-j	252.2 f-j	0.94 a-c	31.9 f-k	8.40 c-g	284.1 g-j	4361 h-j	35 gh
LN 98-4446	21 b-e	8.17 g-j	278.9 g-j	1.05 a-d	21.9 a-h	9.22 e-g	198.6 b-h	4340 h-j	0 a
SD 97-230	45 g-i	7.81 f-j	359.8 ij	1.13 a-d	53.1 l	8.94 d-g	412.9 jk	4206 g-j	23 d-g
IA 2021	45 g-i	8.59 h-j	391.4 j	1.11 a-d	49.3 kl	9.71 fg	440.7 k	3924 f-i	28 f-h
Collfax	25 c-f	9.20 j	233.2 d-i	1.24 d	29.6 c-i	10.44 g	262.8 f-i	3863 e-h	73 i
LSD <sub>0.05</sub>	13	2.06	148.2	0.25	18.0	2.13	140.1	510	15
2003									
PI 184042	40 b-g	1.30 a	50.6 b-f	2.79 b-g	111.7 c-g	3.84 a-c	162.3 c-g	1626 ab	0 a
M 93-326056	39 b-g	1.70 a-c	70.1 c-g	3.79 g	156.4 f-i	5.49 e	226.5 f-i	2936 d-g	7 ab
PI 561353	18 a-d	1.54 a-c	29.5 a-e	2.49 a-f	43.5 a-d	4.04 a-e	73.0 a-d	2365 b-e	10 a-c
M 90-184111	20 a-d	1.45 ab	25.1 a-d	2.38 a-f	43.5 a-d	3.83 a-c	68.6 a-d	3915 i	19 a-e
PI 507353	12 a-c	1.73 a-c	23.9 a-d	2.54 b-f	33.6 a-c	4.28 a-e	57.5 a-c	2775 d-g	9 ab
M 95-255017	55 e-j	1.78 a-d	98.3 g-i	3.45 fg	193.9 g-j	5.23 c-e	290.6 h-k	2547 c-f	43 f-h
MN 1301	12 a-c	1.43 ab	18.4 a-d	1.92 a-c	20.6 ab	3.61 ab	39.0 ab	2909 d-g	7 ab
M 92-160047	21 a-c	1.24 a	28.4 a-d	2.26 a-f	52.4 a-d	3.50 ab	80.8 a-d	2533 c-f	21 b-e
SD 96-755	60 f-j	1.81 a-d	108.6 h-j	2.70 b-g	160.6 f-i	4.51 a-e	266.8 g-j	2735 c-f	30 c-f
Bell	86 jk	1.81 a-d	160.9 kl	2.70 b-g	229.1 ij	4.51 a-e	390.0 kl	2936 d-g	8 ab
IA 1008	85 i-k	1.66 a-c	141.9 jk	2.56 b-f	217.3 h-j	4.22 a-e	359.2 j-l	2211 b-d	66 i
M91-113037	11 a-c	1.42 ab	17.0 a-c	2.50 a-f	28.1 a-c	3.92 a-d	45.1 a-c	1975 a-c	13 a-d
M94-209136	33 a-f	1.82 a-d	58.7 c-g	3.20 e-g	99.7 b-f	5.03 b-e	158.4 c-g	2956 d-g	20 a-e
PI 511356	15 a-c	1.42 ab	21.2 a-d	2.93 c-g	43.2 a-d	4.26 a-e	64.4 a-d	1639 ab	8 ab
U96-2408	1 a	1.90 b-d	3.0 a	1.80 a-c	2.1 a	3.70 a-c	5.2 a	3843 hi	3 ab
PI 398311	31 a-f	1.81 a-d	42.1 a-e	1.68 ab	47.7 a-d	3.49 a	89.8 a-e	1398 a	7 ab
PI 423826A	15 a-c	2.35 d	38.5 a-e	1.35 a	21.6 ab	3.70 a-c	60.2 a-d	1720 ab	6 ab
A99-216031	21 a-e	1.67 a-c	34.7 a-e	2.55 b-f	50.4 a-d	4.22 a-e	85.6 a-d	3931 i	55 g-i
NE 3001	6 ab	1.73 a-c	9.6 ab	1.76 a-c	9.3 a	3.50 ab	18.8 a	3763 hi	12 a-c
SD 97-456	76 h-k	2.08 cd	159.4 kl	3.34 fg	256.7 j	5.42 de	416.1 l	3514 g-i	33 d-f
NE 2701	59 f-j	1.68 a-c	98.8 g-j	2.47 a-f	147.8 e-i	4.15 a-e	246.6 f-j	2547 c-f	19 a-e
Savoy	34 a-f	2.11 cd	70.0 e-g	1.95 a-d	67.6 a-e	4.07 a-e	137.6 b-f	2499 c-f	61 hi
LN 98-4446	45 c-h	1.69 a-c	61.2 d-g	3.22 e-g	144.4 e-h	4.63 a-e	178.2 d-h	3494 g-i	7 ab
SD 97-230	53 d-i	1.71 a-c	85.7 f-h	2.38 a-f	117.3 d-g	4.09 a-e	202.9 e-i	3729 hi	44 f-h
IA 2021	80 h-k	1.71 a-c	134.9 i-k	3.13 d-g	252.9 j	4.84 a-e	387.8 kl	3118 e-h	38 e-g
LSD <sub>0.05</sub>	36	0.59	43.4	1.21	84.0	1.53	118.3	764	20

† Data from 2002 and 2003 were analyzed independently.

‡ BPMV or SMV antigen is used as a relative amount of BPMV or SMV in seed samples harvested from experimental plots. A value of 1.0 or less designates no detectable antigen in the sample.

§ Virus seed index (SI) is calculated as the product of the percentage of seed coat mottling and the BPMV or SMV antigen.

¶ Estimated percentage of symptomatic tissue visible at the top of the plant canopy in experimental plots on 20 Aug. 2002 (R6) or 29 Aug. 2003 (R6/R7).

# Means of four replications followed by the same letter are not statistically different at  $P \leq 0.05$ .

that occurs within years and locations, due in part to different insect vector populations that influence which virus is most prevalent at a location, dictates comparative yearly evaluation of accessions at each location for the traits measured.

The results from this study reveal that it is not practical to establish rigid quantitative limits to identify accessions that are field tolerant or sensitive to virus infection. In this study, specific attention was directed

toward both seed mottling and relative virus antigen. This is illustrated by data for Mycogen 5261 whose percentage of mottled seed ranged from 9.7% to 27.5% in the years 2001–2003 (Tables 1–3). Relative to the accessions in the test, the Mycogen 5261 ranks among the lowest two in 2001 to 2002 and lowest three in 2003 for percentage of seed coat mottling. However, in 2001 seed from the Mycogen 5261 had the highest relative antigen content of those tested at the Rock County

location for BPMV, and, in 2002 and 2003, it ranked fifth highest for BPMV. In contrast, data for H2494 show that it is low for relative BPMV antigen at Rock County in 2001 and 2002, years in which incidence of BPMV was high at this location. However, in 2002 the percentage of mottled seed was high at this site. This was unlikely due to SMV, since relative SMV antigen was low in 2002 at this location. Factors other than virus infection are known to cause mottling of soybean seed coats (Srinivasan and Arihara, 1994; Takahashi and Abe, 1994; Takahashi et al., 1996; Cober et al., 1998).

In more extensive tests of a larger set of soybean accessions (Table 4), the high BPMV relative antigen content in 2002 of accessions PI 398311, SD97-456, U96-2408, and A99-216031 did not suggest they were tolerant to BPMV (Table 5), even though the percentage of mottled seed in these lines was in the lower half of those lines tested (Table 4). In contrast, seed from Bell and SD96-755 were highly mottled, making them unacceptable for consideration as tolerant (Table 5), but contained a relatively low BPMV antigen content in 2002 (Table 4). The data affirm previous reports (Krell et al., 2005) that mottling is an unreliable indicator of virus presence in seed. If mottling is used as a sole selection criterion for tolerance, selection of an accession that may have high levels of virus antigen but low percentage of seed-coat mottling could increase the potential risk through seed transmission for introduction of virus into a field the following growing season.

Analyses of the smaller set of soybean cultivars showed that the cultivar Spansoy 201 in 2001 and 2002 was tolerant to BPMV with a relatively low virus seed index (Tables 1 and 2), despite the especially high incidence of BPMV at the Rock County location. The SMV antigen content of this cultivar at the Columbia County location in 2002 suggested its sensitivity to SMV. This was confirmed in 2003 when SMV antigen content placed it in the bottom half of the cultivars tested, and resulted in a higher BPMV + SMV virus seed index, relative to other lines in the test, than occurred in the previous 2 yr (Table 3). Therefore, this cultivar exhibited field tolerance to BPMV but was sensitive to SMV.

All accessions in this study (Tables 1–4) were placed into groups based on their response in the field trials (Table 5). Initially, accessions that had a relatively low virus seed index were selected as having potential field tolerance to one or both viruses. Then, examination of the components of the seed index allowed accessions

with specific tolerance to one or both viruses to be identified. Accessions that had both a low mottling percentage and antigen content were regarded as superior. Our assessment suggested at least three accessions were tolerant to both SMV and BPMV, while eight accessions were tolerant to SMV and four were tolerant to BPMV. Others in the test were unacceptable due to either consistent or occasional occurrence of some combination of high antigen value and/or percentage of seed coat mottling. Placement into a group is obviously dependent on the selection criteria used by the investigator.

Due to the wide range of seed yield potential among the accessions in the test, meaningful comparisons of seed yield with relative virus antigen level is impractical. For example, seed yield of LN 98-4446 was higher than that of PI 507353; conversely, LN 98-4446 has a higher relative antigen content than PI 507353 (Table 4).

The data confirm anecdotal evidence that leaf symptoms are an unreliable selection criterion for resistance. For example, LN 98-4446 showed virtually no leaf symptoms in the field. However, it had high BPMV and SMV seed antigen levels in 2002 and 2003, respectively (Table 4), demonstrating no positive association of virus incidence with evident symptoms.

Both BPMV and SMV were easily detected in seeds from the same plot in 2003 (Tables 3 and 4). This raises concern for the synergistic interaction of BPMV with SMV and is expected to be a future problem when inoculum and vectors of both viruses are present. Increased titer of BPMV in SMV-infected plants may result in increased levels of BPMV antigen in some accessions (Anjos et al., 1992). The significant increase in loss of seed yield potential when soybeans are infected by both viruses (Ross, 1969) argues for incorporation of virus resistance into soybeans used for commercial production.

The results demonstrate that interpretation of data to identify field tolerance to virus disease requires correct identification of viruses prevalent at the field site where the test is conducted. This becomes particularly critical when two viruses, with different insect vectors, cause similar phenotypic symptoms. Therefore, in this study it was important to monitor incidence of both BPMV and SMV at the experimental sites. This was especially important in 2002 and 2003 when both soybean aphids and bean leaf beetles were prevalent, but sometimes in different geographic areas.

The seed-borne nature of both viruses suggested that one criterion to identify field tolerance might be the relative amount of virus antigen in seed lots harvested from plants in experimental plots. The approach described is designed to screen large numbers of lines for tolerance to BPMV and SMV. The concept may also be useful for other viruses that are seed-borne. Specificity of the antisera allows discrimination of two viruses with closely similar symptoms. Initial screening, under conditions of high disease incidence, allows selection of lines with acceptable seed yield potential and seed with relatively low mottling percentage (e.g., <25%). Subsequent evaluation of the selected lines for relative antigen content may be easily mechanized. Vector prevalence parallels incidence of SMV or BPMV, and the

**Table 5. Field reactions of soybean accessions to bean pod mottle and soybean mosaic viruses (BPMV and SMV).**

Reaction phenotype	Accession
Tolerant to SMV/ BPMV	PI 561353, M90-18411, PI 507353
Tolerant to SMV/ not BPMV	MN 1301, M92-160047, M91-113037, PI 423826A, A99-216031, NE 3001, U96-2408, Colfax
Tolerant to BPMV/ not SMV	PI 184042, M93-326056, M95-255017, Spansoy 201
Sensitive to BPMV and SMV	SD96-755, Bell, IA 1008, M94-209135, SD97-456, NE2701, LN 98-4446, SD97-230, IA 2021, PI 511356, Savoy, PI 398311, Pioneer 92B62, Spansoy 250, Mycogen 5261, Asgrow 2101, Asgrow 2301, Mark 9824RR (9924), H2494

relative amount of virus antigen in seed lots correlates significantly with virus incidence in plots from which the seed was harvested.

This is believed to be the first report of field tolerance to BPMV in *G. max*, for which no resistance genes have been identified (Wang et al., 2005; Zheng et al., 2005). Subsequent incorporation of naturally occurring resistance genes to SMV into BPMV field tolerant cultivars may eliminate potential for synergism between the two viruses and yield more effective management tactics for disease caused by these two viruses.

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