Green Stem Disorder of Soybean

H. A. Hobbs and **C. B. Hill,** Department of Crop Sciences, University of Illinois, Urbana 61801; **C. R. Grau** and **N. C. Koval,** Department of Plant Pathology, University of Wisconsin, Madison 53706; **Y. Wang** and **W. L. Pedersen,** Department of Crop Sciences, University of Illinois, Urbana 61801; and **L. L. Domier** and **G. L. Hartman,** USDA-Agricultural Research Service and Department of Crop Sciences, National Soybean Research Center, 1101 W. Peabody Dr., University of Illinois, Urbana 61801

ABSTRACT

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Green stem disorder of soybean (*Glycine max*) is characterized by delayed senescence of stems with normal pod ripening and seed maturation. Three different field research approaches were designed to determine the relationship of green stem disorder to *Bean pod mottle virus* (BPMV) and other potential factors that may be involved in causing this disorder. The first research approach surveyed green stem disorder and BPMV in individual plants monitored in several commercial soybean fields during three growing seasons. Leaf samples from maturing plants (growth stage R6) were tested by enzyme-linked immunosorbent assay (ELISA) for BPMV. The percentage of monitored plants infected with BPMV at growth stage R6 in some fields was higher than the incidence of green stem disorder at harvest maturity. Many plants infected with BPMV did not develop green stem disorder, and conversely, many plants that had green stem disorder were not infected with BPMV. According to a chi-square test of independence, the data indicated that green stem disorder was independent of BPMV infection at growth stage R6 (P = 0.98). A second research approach compared green stem disorder incidence in an identical set of soybean entries planted in two locations with different levels of natural virus infection. Despite differences in virus infection, including BPMV incidence, 20 of 24 entries had similar green stem disorder incidence at the two locations. A third research approach completed over two growing seasons in field cages showed that green stem disorder developed without BPMV infection. BPMV infection did not increase green stem disorder incidence in comparison to controls. Bean leaf beetle, leaf hopper, or stinkbug feeding did not have an effect on the incidence of green stem disorder. The cause of the green stem disorder remains unknown.

Delayed or incomplete maturation of soybean plants or plant parts can interfere with soybean (*Glycine max* (L.) Merr.) seed harvesting, especially when large sections of fields mature at different rates. Green stem disorder, as defined in this research, is a disorder of soybean with the principal characteristics being a nonsenescent stem with normal, mature pods and seeds. Because the green, tough, and pliable stems of plants with green stem disorder are more difficult for the knives of the combine to cut, ground speed must be slowed while keeping engine speed high, reducing the fuel efficiency of the combining operation. Combine cylinder speed must also be increased to reduce the risk of clogging the opening between the concave and cylinder with moist plant material that does not collapse as readily as dry material during the threshing process. Furthermore, moisture from the stems can be transferred to seed during threshing, reducing the grade and storability of seed. These problems encourage growers to delay harvesting areas in fields where green stem disorder is prevalent until a hard frost kills the green stem tissue. The potential for green stem disorder is very high in sensitive cultivars. We have observed green stem disorder incidence of over 90% in plots of sensitive cultivars in yield trials (6).

Schwenk and Nickell (14) reported in 1980 that *Bean pod mottle virus* (BPMV) was a cause of green stem of soybean. They noted, however, that many plants infected with BPMV did not develop green stem symptoms. Symptoms of green stem that they described included green stems after pod maturity, attached leaf petioles, thin pods with small seeds, few pods per node, and often terminal necrosis of the stem. Other soybean disorders with similarities to green stem disorder have been reported. Higher percentages of plants with green stems at harvest were observed in fungicide-treated plots in soybean experiments in Louisiana (11). Senescence of soybean genotypes was delayed by infestation by a stinkbug species in Brazil (9). Boethel et al. (1) demonstrated that delays in soybean maturity called "green bean syndrome" resulted from southern green stinkbug (*Nezara viridula*) infestation of soybean. Maturity of infested plants was delayed by several days or weeks, with leaves not senescing and pods not ripening (1).

Plants with green stem disorder as defined in this research have green, yellow-green, or yellow, moist, nonsenescent stems with normal, ripe, dry, brown pods containing mature, dry seeds (harvest levels of seed moisture generally below 20%). Sometimes petioles remain attached to the stem. Patterns of distribution of green stem disorder plants that we have observed range from scattered plants in some commercial fields to near 100% incidence in small plot cultivar tests. It is the most common type of delayed maturity found in Illinois (6) and Wisconsin (4) and is distinguished from other types of delayed maturity because pods and seeds ripen normally.

The goal of this research was to examine the relationship of green stem disorder in soybean to BPMV infection and other possible causes of this disorder.

MATERIALS AND METHODS

Evaluation of green stem disorder. For the purposes of this research, plants were classified as having green stem disorder when they had moist, nonsenescent green, yellow-green, or yellow stems with dry, ripe, brown pods (Fig. 1) containing dry, mature seeds (generally below 20% seed moisture). Plants with both green stems and green pods were not included in this definition of green stem disorder because that condition was considered to be delayed plant maturity, not green stem disorder, and could have numerous possible causes, such as severe virus infection, insect infestation, late plant emergence, or microclimatic conditions nonconducive to ripening.

Field monitoring surveys. In 2000, 2001, and 2002, sampling areas were

Corresponding author: G. L. Hartman
E-mail: ghartman@uiuc.edu

* The *e*-Xtra logo stands for "electronic extra" and indicates that Figure 1 appears in color in the online edition.

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marked in a grid pattern with flags in two or three soybean production fields per year at growth stage R6 in Champaign Co., IL. In each field, four to six rows separated by 30 m were marked at 30- to 40-m intervals. All fields except one were monitored in a 1- to 1.5-ha area, using 20 flag locations. The sole exception (field 2000A) was monitored in a 3- to 4-ha area using 48 flag locations. At each flag location, four plants were marked with orange spray paint, a total of 80 plants for each of the smaller field surveys and 192 plants for the single larger field survey; leaf samples (youngest developed leaves) were collected from each marked plant and tested fresh or after storage at –20°C for BPMV. BPMV presence or absence was determined by enzyme-linked immunosorbent assay (ELISA) (2) using commercial BPMV coating antibodies and alkaline phosphatase-conjugated BPMV antibodies (Agdia, Inc., Elkhart, IN) and Agdia protocols. Absorbance was read at 405 nm using a Biotek EL 340 plate reader, with a positive threshold value of twice the healthy soybean leaf negative control absorbance. A day or two before the plants were harvested with a combine, when the mean seed moisture content was generally below 20%, marked plants were collected for visual assessment of green stem disorder symptoms. Selected stem samples from plants with green stem disorder symptoms were tested for the presence of BPMV with ELISA. Results from ELISA on leaf samples were compared with green stem disorder symptom observations of the harvested marked plants.

Cultivars planted in each of the fields surveyed were: 2000A, Limagrain LG 9280 STS (MG II); 2000B, UAP Dyna-gro 3370 RR (MG III); 2001A, UAP Dyna-gro 3271 NRR (MG II); 2001B, Limagrain LGC 3033 RR (MG III); 2002A, UAP Dyna-gro 3321 NRR (MG III); 2002B, UAP Dyna-gro 3292 NRR (MG II); and 2002C, UAP Dyna-gro 3292 NRR (MG II). Growers chose the cultivars to plant; growers' fields were chosen for monitoring by researchers based on growth stage in field. The numbers 2000, 2001, and 2002 indicate year; letters A, B, and C represent individual fields.

Green stem disorder incidence of soybean entries at two locations with differing BPMV incidence. Twenty-one F_2 derived lines, from the cross between soybean cultivars Bell and Colfax, were evaluated for green stem disorder along with the parents and the cultivar Dwight at the West Madison Agricultural Research Station, Madison, WI, and Rock County Farm near Janesville, WI, in 2002. At both locations, the soil type was Plano silt loam, and the previous year's crop was corn. A randomized complete block design was used, and each soybean line was replicated three times. Plots were planted 15 May at West Madison and 6 May at the Rock County Farm. Seeding rates were 27 to 30 seeds per meter of row. Rows were spaced at 76 cm within individual plots of 3.0 × 7.6 m. Imazamox (Pursuit, 2.3 liters ha^{-1}), thifensulfuron methyl (Pinnacle, 18.3 ml ha^{-1}), and quizalofop (Assure II, 0.716 liters ha^{-1}) were applied for weed suppression at the Rock County Farm. Metribuzin (Sencor, 1.8 kg ha^{-1}) and pendimethalin (Prowl, 0.6 liters ha^{-1}) were applied for weed control at West Madison. The plot at West Madison was irrigated twice during the growing season, 1.65 cm ha^{-1} on 29 June and 1.13 cm ha^{-1} 6 July. Lambda-cyhalothrin (Warrior 1EC, 233.8 ml ha^{-1}) was applied on 31 May to suppress bean leaf beetle (*Cerotoma trifurcata* Forster) populations at West Madison. Plots were harvested mechanically on 11 October at Janesville and 26 September at West Madison with an Almaco SP20 Plot Combine (Allen Machine Co., Nevada, IA).

Single leaflets of the youngest trifoliolate were collected randomly from 20 soybean plants in each plot. Plants were sampled 16 August at Rock County Farm and 21 August at West Madison. Plants were at growth stages R5 to R6 at each location. Leaflets were chilled on ice in the field and later stored at 4°C. The 20 leaves from each plot were bulked and placed into a 7.6 × 7.6 cm grinding bag (Agdia Inc.), ground in 1 ml of phosphate-buffered saline (PBS, pH = 7.2), and 100 µl was loaded onto a 96-well ELISA plate (Agdia Inc.). Sap was assayed using double antibody sandwich ELISA for BPMV, *Soybean mosaic virus* (SMV), and *Tobacco streak virus* (TSV). Samples were diluted 1:5 in a 96-well polystyrene microtiter plate (ProBind, Becton-Dickenson, Franklin Lakes, NJ) in PBS for BPMV, SMV, and TSV (Sigma-Aldrich, St. Louis, MO). Antibodies for BPMV and SMV were obtained from John Hill, Iowa State University. Antibodies for TSV were obtained from Agdia, Inc.

Indirect ELISA was used for *Alfalfa mosaic virus* (AMV). Leaf samples were ground in carbonate buffer (Sigma) at pH 9.6, and 100 µl was added to each well. Plates were washed with PBS Tween 3 times after each step. After 2 h of incubation, 200 µl of PBS-BSA was added to each well and incubated for 1 h. Anti-AMV antibody, obtained from Ana Mondjana, University of Wisconsin-Madison, was added at a dilution of 1:10 K to chilled PBS-Tween and incubated for 2 h at room temperature. Then 100 µl of anti-rabbit enzyme conjugate diluted 1:20 K in chilled PBS-Tween was added and incubated for 2 h.

For all viruses, positive, negative, and buffer controls were included on each plate. Substrate (1 mg/ml *p*-nitrophenol phosphate in 10% diethanolamine, pH 9.6) was added and plates were incubated in darkness until being read. Sample absorbance values were read using an EL800 Universal MicroPlate Reader (Biotek Instruments Inc., Winooski, VT). Samples with absorbance values (405 nm) that exceeded the mean plus two standard deviations of the negative control were regarded as positive. Background, as measured in buffer and negative control wells, was minimal for all ELISA assays. All reactions were performed at room temperature.

Symptoms attributed to viruses were visually rated in each plot. Symptom severity was assessed for each plot by estimating the percentage of the upper canopy symptomatic at growth stage R3 on 24 July at Janesville and R3/R4 on 7 August at West Madison. Symptom severity was assessed for each plot by estimating the percentage of the upper canopy symptomatic for symptoms of mosaic, mottle, and

Fig. 1. Soybean plant with normal maturation (left), and plant with green stem disorder (right), which has mature brown pods but a green stem.

rugosity. Symptomatic and asymptomatic plants were not tested for virus infection status.

The incidence of green stem disorder was assessed at both sites on 24 September by a visual estimate of the percentage of plants having delayed senescence of stems with normal pod ripening and seed maturation. The incidence of seed with mottled seed coats was assessed for 100 randomly selected seeds per plot.

Field cage experiments. In 2002 and 2003, experiments were conducted at the University of Illinois Crop Science Research and Education Center (CSREC) South Farm, Urbana, IL in cages designed to exclude insects as small as aphids. The soil types at the location were Drummer silty clay loam (fine-silty, mixed, mesic Typic Haplaquolls) and Flannagan silty loam (fine, montmorillonitic, mesic Aquic Argiudolls).

Cages consisted of a galvanized steel frame covered with a polypropylene 52 × 52 mesh screen (about 20 threads per linear cm; 50% shade factor) with vinyl, reinforced mud flaps that folded out 0.3 m from the mesh and were covered with soil to anchor the cages (Redwood Empire Awning and Furniture Co., Santa Rosa, CA). Cages dimensions were 2 m high, 1.5 m wide, and 3 m long in 2002, and were 2 m high, 3 m wide, and 3 m long in 2003.

In 2002 and 2003, single-row plots of four soybean cultivars were planted in blocks inside each cage. Two blocks in 2002 and three blocks in 2003 of four cultivars were planted inside each cage. Rows of cultivars within each block were 0.3 m in length and 0.6 m apart. Each treatment had 58 to 88 plants in 2002, and 86 to 110 plants in 2003. Numbers of plants of each cultivar ranged from 16 to 24 per treatment in 2002 and 17 to 35 per treatment in 2003. Total plant numbers of each cultivar ranged from 108 to 126 in 2002 and 154 to 199 in 2003.

Treatments were applied to separate cages with replications (blocks) nested within treatments. The treatments in the 2002 experiment were: (i) caged control, (ii) BPMV inoculation at growth stage V2 (early), (iii) BPMV inoculation at growth stage R3 (late), (iv) inoculation with green stem disorder homogenate at growth stage V2, (v) bean leaf beetle infestation, and (vi) an uncaged control. The isolate used in BPMV inoculations was BPMV AS. Bean leaf beetles are the primary vectors of BPMV in the north central states (3). Treatments in the 2003 experiment were: (i) caged control, (ii) leafhopper infestation, (iii) stinkbug infestation, (iv to vi) inoculations with three different isolates (98, AS, and WP2) of BPMV at the V2 growth stage, and (vii) inoculation with green stem disorder homogenate at growth stage V2.

Cultivars planted in 2002 were Merschman Cherokee XRR (MG II), Beck 323 RR (MG III), Gateway 2R260 (MG II), and Asgrow AG 3903 (MG III). The same cultivars were not available in 2003, and they were substituted with Hughes 441 RR (MG II), Mark MRK RR 0129CTB (MG II), Stine S2463-4 (MG II), and Kruger K-232+ RR (MG II). All of the cultivars possessed the Roundup Ready trait and were tolerant to the herbicide glyphosate.

BPMV isolates 98 and AS were originally collected from infected soybean on the CSREC South Farm, Urbana. BPMV isolate WP2 was originally collected from a soybean field in Carmi, IL. Nucleic acid hybridization analysis indicated that the isolates 98, AS, and WP2 belonged to BPMV subgroups I, II, and I/II (5), respectively (S. Ghabrial, University of Kentucky, *personal communication*). All BPMV isolates were shown to be free of other common soybean viruses by ELISA, and were maintained in dried, refrigerated plant material and by continuous greenhouse transfer using mechanical inoculation.

BPMV inoculum was prepared from infected soybean leaf tissue. Green stem disorder homogenate was prepared from frozen tissue of plants with green stem disorder that did not have BPMV as determined by ELISA. Tissue was ground in chilled 0.025 M potassium phosphate buffer, pH 7.1, in a mortar with a pestle. To transfer BPMV, or test for mechanical transmissibility of a hypothetical green stem disorder-inducing factor in the green stem disorder homogenate, the leaves of healthy soybean plants were sprinkled with Carborundum abrasive and were mechanically inoculated using pestles with the BPMV isolates or "green stem disorder homogenate", respectively. The presence or absence of BPMV was determined in leaf samples (youngest developed leaves) collected from each plant at growth stage R6 in each treatment (cage) by ELISA. In cages with BPMV inoculation treatments, only plants verified by ELISA as infected with BPMV were included in assessment of green stem disorder incidence.

Insects were collected using sweep nets. Bean leaf beetles and green stinkbugs were periodically collected from nearby soybean fields during the growing season and put in the appropriate cages. Leafhoppers, principally the potato leafhopper (*Empoasca fabae*), were collected from nearby alfalfa and soybean fields. Bean leaf beetles and leafhoppers were put in cages starting at V2 plant stage, and stinkbugs starting at R3, when they were first available from soybean fields.

After sowing, a systemic insecticide, imidacloprid (1% G Marathon, Olympic Horticultural Products, Mainland, PA) was top-dressed on the surface of each row in all cages except those where insects were introduced, at a rate of 4.2 ml of granules per meter of row.

Green stem disorder symptoms were assessed for each individual plant in both experiments a few days before growers were planning to harvest. Mean seed moisture content was generally less than 20%.

Statistical analyses. All statistical analyses were performed with the aid of JMP Version 5.1 (SAS Institute Inc., Cary, NC). A chi-square test of independence was performed on the counts of plants with or without green stem disorder symptoms and infected or not infected with BPMV at growth stage R6 in the field monitoring experiments to analyze the independence between BPMV infection and green stem disorder development (8). Green stem disorder incidence data (percentage of plants with green stem disorder) in the cage and field experiments were first transformed using the arcsine-square root method before performing analysis of variance, and least square means were detransformed for presentation in the tables. Mean separation was done by calculating the LSD at $P = 0.05$ when treatment means were significantly different ($P < 0.05$) in the ANOVA.

RESULTS

Relationship of BPMV infection and green stem disorder in plants monitored in production fields from 2000 to 2002. A higher percentage of plants monitored were infected with BPMV than had green stem disorder symptoms in both fields in 2000 and in one field in 2001 (Table 1). BPMV infection was not detected by ELISA in collected leaf samples from two of the three fields monitored in 2002 (2002B and C), while a number of the plants in the two fields had green stem disorder symptoms (Table 1). Moreover, BPMV was not detected by ELISA in 28 stem samples collected from plants with green stem disorder at harvest time from fields 2002B and 2002C (data not shown).

A chi-square test of independence was conducted that compared the numbers of plants from the field survey that were BPMV-infected at growth stage R6 with green stem disorder at harvest, BPMV-infected at growth stage R6 with normal stems at harvest, BPMV-free plants at growth stage R6 with green stem disorder at harvest, and BPMV-free plants at growth stage R6 with normal stems at harvest. For each of the four classes of plants, expected versus observed numbers of plants were 12 versus 13, 62 versus 61, 105 versus 104, and 493 versus 494, respectively. The probability of obtaining a larger χ^2 than calculated by chance alone was 0.98; therefore, the null hypothesis of independence was not rejected. The results indicated that green stem disorder was independent of BPMV infection at growth stage R6.

Comparison of green stem disorder incidence in F_2-derived entries at two locations with different levels of virus

infection. The level of BPMV differed at the two locations based on incidence of detection by ELISA, symptom severity, and incidence of mottled seed. Virus rating at the Janesville location ranged from 22 to 68% for the different entries, with a mean of 34%. Virus rating at West Madison was 0% for all entries. Seed mottling at Janesville ranged from 13 to 68% for the different entries, with a mean of 39%. Seed mottling at West Madison ranged from 0 to 16% with a mean of 4%. Overall percentage of bulked samples (20 leaves combined) for all plots that were positive by ELISA for BPMV was 90% at Janesville versus 68% at West Madison. Incidences of BPMV were significantly different at the two locations ($P = 0.0015$).

Table 1. Number and percentage of soybean plants in fields that were infected with *Bean pod mottle virus* (BPMV) at growth stage R6, developed green stem disorder symptoms at harvest maturity, and had both BPMV infection and green stem disorder symptoms based on data from seven fields in the 2000 to 2002 growing seasons in Illinois

		Number (percentage) of plants with		
Field[y]	Total no. of plants	BPMV	Green stem disorder	BPMV + green stem disorder
2000A	192	39 (20.3%)	17 (8.9%)	4 (2.1%)
2000B	80	13 (16.3%)	6 (7.5%)	0 (0%)
2001A	80	5 (6.3%)	5 (6.3%)	1 (1.3%)
2001B	80	11 (13.8%)	4 (5%)	2 (2.5%)
2002A	80	6 (7.5%)	49 (61.3%)	5 (6.25%)
2002B	80	0 (0%)	14 (17.5%)	N/A[z]
2002C	80	0 (0%)	22 (27.5%)	N/A
Combined	672	74 (11.0%)	117 (17.4%)	12 (1.8%)

[y] Cultivars used in each field were as follows: 2000A, Limagrain LG 9280 STS; 2000B, UAP Dyna-gro 3370 RR; 2001A, UAP Dyna-gro 3271 NRR; 2001B, Limagrain LGC 3033 RR; 2002A, UAP Dyna-gro 3321 NRR; 2002B, UAP Dyna-gro 3292 NRR; 2002C, UAP Dyna-gro 3292 NRR. Numbers 2000, 2001, and 2002 indicate year of field trial; A, B, and C were separate fields monitored in those years. Growers chose cultivars; fields were chosen for monitoring by researchers based on soybean growth stage.

[z] Not applicable.

Table 2. Percentage of green stem disorder among F_2 derived lines from the cross between soybean cultivars Bell and Colfax at two locations with different levels of *Bean pod mottle virus* (BPMV) incidence in Wisconsin in 2002

	Green stem disorder percentage at location[x]	
Entries	Janesville (higher BPMV incidence)[y]	West Madison (lower BPMV incidence)
Bell	0 l[z]	1 kl
Colfax	100 a	38 fgh
Dwight	84 bcde	82 bcde
LD99-16402	35 fghi	59 efg
LD99-16415	7 ijkl	0 l
LD99-16417	33 ghij	42 fg
LD99-16433	45 fg	84 bcde
LD99-16437	39 fgh	40 fg
LD99-16440	14 hijk	3 kl
LD99-16442	95 ab	86 bcd
LD99-16446	61 defg	13 hijk
LD99-16452	0 l	0 l
LD99-16454	7 kl	7 jkl
LD99-16458	0 l	6 kl
LD99-16460	1 kl	2 kl
LD99-16465	2 kl	3 kl
LD99-16466	90 bc	86 bcd
LD99-16468	94 ab	90 bc
LD99-16469	86 bcd	67 cdef
LD99-16473	5 kl	4 kl
LD99-16474	78 bcde	36 gh
LD99-16477	3 kl	1 kl
LD99-16479	0 l	1 kl
LD99-16489	3 kl	2 kl

[x] There was a highly significant cultivar × location interaction ($P < 0.001$).

[y] Virus rating at the Janesville location ranged from 22 to 68% for the different entries, with a mean of 34%. Virus rating at West Madison was 0% for all entries. Symptoms attributed to viruses were rated in each plot by visual methods. Symptom severity was assessed for each plot by visually estimating the percentage of the upper canopy symptomatic for mosaic, mottle, and rugosity. Symptomatic and asymptomatic plants were not tested for virus infection status. Seed mottling at Janesville ranged from 13 to 68% for the different entries, with a mean of 39%. Seed mottling at West Madison ranged from 0 to 16% with a mean of 4%. Overall percentages of bulked samples (20 leaves combined) for all plots that were positive by enzyme-linked immunosorbent assay (ELISA) for BPMV were 90% at Janesville versus 68% at West Madison. Incidences of BPMV were significantly different at the two locations ($P = 0.0015$).

[z] Levels of green stem disorder not followed by the same letter are significantly different by least square means differences Students' *t* test.

At both the Janesville and West Madison locations, overall incidence of green stem disorder was similar (Table 2), with a significant correlation of incidence among the entries between the two locations ($r = 0.95$, $P < 0.0001$) despite a higher BPMV incidence at the Janesville location. However, a highly significant cultivar × location interaction for incidence of green stem disorder was found ($P < 0.001$). LD-16446, LD-16474, and Colfax had significantly higher green stem disorder incidence at Janesville, while LD99-16433 had significantly higher green stem disorder incidence at West Madison (Table 2). There was no significant correlation ($r = 0.12$, $P = 0.16$) between BPMV incidence and green stem disorder incidence.

Effects of BPMV infection and insect feeding on green stem disorder incidence in cage experiments. There were no significant differences ($P > 0.05$) among the treatments for green stem disorder incidence in the cage experiments in 2002 and 2003 (data not shown). In addition, individual single degree of freedom comparisons of differences between the treatments and the caged control in 2002, and between the treatments and the caged control in 2003 were not significant ($P > 0.05$). Therefore, none of the treatments in the experiments had an effect on the incidence of green stem disorder.

Virus inoculations in the 2002 and 2003 seasons successfully infected from 76 to 98% of plants inoculated (data not shown); only those plants verified to be BPMV-infected by ELISA were included in assessments of relationship between virus infection and green stem disorder. Early or late infection, or infection with different BPMV subgroups, did not significantly affect green stem disorder incidence (data not shown).

In both years, all leaf samples (youngest developed leaves) from individual plants at growth stage R6 in caged controls (not inoculated with BPMV, and not exposed to any insect species) were found to be free of BPMV when tested by ELISA (data not shown).

Infestation with bean leaf beetles, leafhoppers, or stinkbugs did not have a statistically significant effect ($P > 0.05$) on green stem disorder incidence (data not shown). Approximately 800 bean leaf beetles were collected from fields in the vicinity of the experiment and placed in the bean leaf beetle cage during the 2002 season. The beetles caused extensive damage on the foliage, destroying 40 to 90% of leaf surface area. They were not tested for

the presence of BPMV to determine if they were viruliferous, but two of 87 plants in the cage were BPMV ELISA-positive at growth stage R6. About 750 leafhoppers were collected and placed in the leafhopper cage in 2003. They caused no discernable damage to the soybean plants. Ten stinkbugs were captured and placed in the stinkbug cage in 2003. These subsequently reproduced, and approximately 100 individuals were counted at growth stage R6. They caused minor damage to pods and developing seeds.

Significant differences in incidence of green stem disorder among cultivars were found in both years (Table 3). In 2002, green stem incidence was significantly lower in Merschmann Cherokee than in the other three lines tested (Table 3). In 2003, green stem incidence was significantly lower in Hughes 441 and Kruger K-232 than in Mark MRK 0129 (Table 3). Green stem incidence in Mark MRK 0129 was significantly lower than in Stine S2463-4 (Table 3).

DISCUSSION

Surveys showed that soybean green stem disorder was independent of BPMV infection in several fields planted with different cultivars over 3 years, experiments with entries planted at two field locations with differing BPMV incidence, and in field cage experiments.

Redinbaugh et al. (13) observed no green stem disorder symptoms after inoculating soybean plants with BPMV in the field at both early and late plant development stages. Despite their hypothesis that BPMV caused green stem, Schwenk and Nickell (14) reported that not all field BPMV-infected soybean plants in a row developed green stem disorder. Their description of green stem symptoms (14) is similar to the description defined in our research, in that plants with the disorder have green stems with leaf petioles frequently remaining attached to the stems at pod maturity; however, their description of the disorder also included smaller than normal pods and seed size, and fewer pods per node. Those characteristics were not commonly observed in our research to be associated with the green stem disorder but rather with virus infection, usually BPMV, that resulted in a delay in the maturation of the whole plant and not just the stem. These differing observations may have resulted in different conclusions regarding the role of BPMV and green stem.

Higher virus symptom ratings and percentages of seed with seed coat mottling (7) reflected the higher BPMV incidence at Janesville compared with West Madison. Three entries had higher green stem disorder incidence at the location (Janesville) with the higher BPMV incidence, one entry had lower green stem disorder incidence at that location, and 20 entries had approximately the same green stem disorder incidence at both locations. It is unlikely that the differential response of four of the entries for incidence of green stem disorder between the two Wisconsin locations was caused by the effects of BPMV infection. Incidences of BPMV infection were not significantly different among the entries. There may have been another undetected factor, such as an environmental, biotic, or physical factor, that affected green stem disorder development and produced a differential response among the entries at each location. Relative sensitivity to green stem disorder among cultivars was consistent across locations in another study (6).

Samples of plants from the field surveys in commercial fields in Illinois were tested for the presence of other viruses such as AMV, SMV, *Tobacco ringspot virus* (TRSV), and TSV, and none were detected. In Illinois, SMV, TRSV, TSV, and AMV are present, but are encountered infrequently in commercial fields compared with BPMV, which is widely distributed (10). In Wisconsin, SMV, TSV, and AMV were present in both field locations of West Madison and Janesville, but at much lower incidence levels than BPMV, with one exception: TSV at West Madison was present at only slightly lower levels than BPMV. It is unlikely that TSV had a significant effect on green stem disorder incidence at West Madison, as green stem disorder was reported to be at low incidence in the presence of high incidence of TSV in Wisconsin (12).

Although the same cultivars were not tested in the field cage experiments, because of lack of available seed, or in the field monitoring experiment, because of different season market availability of and grower's choice in soybean cultivars, it is improbable that the variability among cultivars for incidence of green stem disorder was a response to BPMV infection, because there is no known resistance to BPMV in soybean (16,17). No BPMV resistance was found in over 700 cultivars entered into state variety tests conducted in 2002 (15). Furthermore, no BPMV resistance was found in over 3,000 plant introduction accessions after screening them as part of a larger project to evaluate soybeans for disease resistance (C. B. Hill, *unpublished data*).

In summary, BPMV incidence at R6 stage was higher than green stem disorder incidence at harvest maturity in some of the fields in the field surveys, thereby demonstrating that some marked BPMV-infected plants did not develop green stem disorder. Green stem disorder symptoms developed without BPMV infection in the field surveys and the field cage experiments, and BPMV infection did not increase green stem disorder incidence in comparison to the caged controls in cage experiments. Field experiments with identical entries at two locations in Wisconsin with differing BPMV incidence showed similar levels of green stem disorder incidence in most entries. A preponderance of evidence indicated that green stem disorder symptom development was independent of BPMV infection. The cause of the green stem disorder remains unknown.

Table 3. Mean green stem disorder incidence of soybean cultivars in field cage[y] experiments in Illinois during the 2002 and 2003 growing seasons

Year	Cultivar	Mean green stem disorder incidence (%)
2002	Asgrow AG3903 (MGIII)	21.8 a[z]
	Gateway 2R260 (MGII)	17.0 a
	Beck 323 (MGIII)	10.8 a
	Merschman Cherokee (MGII)	2.0 b
	Overall mean	11.4
2003	Stine S 2463-4 (MGII)	22.0 a
	Mark MRK 0129 (MGII)	7.0 b
	Kruger K-232+ (MGII)	0.2 c
	Hughes 441 (MGII)	0.1 c
	Overall mean	3.8

[y] Experiments conducted in field cages covered with fine mesh effective in excluding aphid and beetle virus vectors.

[z] Means followed by the same letters were not significantly different by least square means differences Students' *t* test ($P = 0.05$). Each cultivar was represented by 108 to 126 plants in 2002 and 154 to 199 plants in 2003.

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