Soybean Green Stem Caused by Bean Pod Mottle Virus

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ABSTRACT

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Soybean stems that remained green after pods matured, and to which leaf petioles were still attached, were infected with bean pod mottle virus. The virus was consistently detected serologically in extracts from the pulvini and commonly from the pith. Virus could be detected in extracts from seeds from infected plants but not from seedlings that developed from the seeds. Soybean seedlings could be successfully inoculated with this virus before the cotyledons opened. Soybean plants grown in the greenhouse and inoculated with this virus were stunted; some had terminal and/or axillary necrosis, and some remained green 200 days. Cultivars and experimental lines tested varied in response to bean pod mottle virus, tobacco ringspot virus, and tobacco streak virus.

The "Compendium of Soybean Diseases" (9) describes known diseases of soybean (*Glycine max*), including bean pod mottle, but does not include two symptoms that have been observed in Kansas—delayed maturity of soybean stems with concomitant retention of leaf petioles (but usually not blades) and necrosis of the growing point. The green-stem symptom were pronounced in 1974, especially in an irrigated experimental area, and in 1979 throughout much of the state's soybean-growing area. Terminal necrosis has been observed in the greenhouse and occasionally in the field. Although dead, the growing point is neither crooked nor stiff as with bud blight caused by tobacco ringspot virus (TRSV) (1) or tobacco streak virus (TSV) (2) or as with bean pod mottle virus (BPMV) combined with soybean mosaic virus (SMV) (7).

METHODS AND RESULTS

The green-stem syndrome was first observed in the fall of 1974 in an irrigated field of soybeans at the Ashland Experimental Farm south of Manhattan, KS. Predominant symptoms on maturing plants were thin, brown, dried pods with small seeds (9), few pods per node (9), light green-to-yellow stems that should have been tan to brown, light green petioles, and moderately green pulvini that should have abscised. Most leaf blades were gone or badly tattered.

Twelve plants with symptoms were sampled in early August and tested against antisera (AS) of TSV, TRSV, BPMV, and SMV with agar-double diffusion serology. Terminal portions of the plants were used for assay. All samples reacted positively against AS-BPMV and negatively against the others.

More soybean plants were tested as the season progressed, but because it became increasingly difficult to grind the plant tissue for virus extraction, various parts of the plants were tested for ease in grinding and strong reaction to AS-BPMV. Many plants had no pods, so extractions were made from pith at the nodes, pith between the nodes, petioles, pulvini, and stem growing point(s). The virus was consistently detected in pulvinal extracts. Pulvini were also the easiest to work with because they remained softer than other tissue as plants aged. In several plots all plants had green stems, and all samples reacted positively to AS-BPMV.

To determine if plants might have the virus but not pronounced green-stem symptoms, we harvested all plants in one row of a plot that chronologically should have been mature, then divided them into those with green stems (21 plants) and mature stems (eight plants). All 21 green-stemmed plants and seven of the eight without apparent green-stem symptoms reacted positively to AS-BPMV.

Twelve seeds from each plant were sown in soil in a greenhouse. Ten days later, when unifoliolate leaves were expanded, each plant was tested serologically for the virus; all reacted negatively.

Twelve more plants from the field, mature except for stem, were assayed, and 11 gave positive serologic reactions. Twelve seeds from each plant were sown and the seedlings tested for virus; all were negative. Germination of seed in these tests was 79.5%.

In a larger study, 1,089 seedlings were grown from 1,239 seeds harvested from a plot in which 50% of the plants tested reacted positively to AS-BPMV. Each seedling was observed for symptoms and assayed serologically when about 10 days old; all tests were negative.

To determine if the seeds contained detectable virus, we collected 268 seeds from 148 pods from plants with green stems in late October 1974. Although the pods were brown, the seeds were still moist, probably due in part to rainy weather. Seeds were grouped by size into five categories, then further subdivided into lots of five to six seeds. Seeds of each lot were combined, ground in buffer, and tested serologically against AS-BPMV. The five seed lots in the smallest-size category were tested as complete seeds; none of these gave a positive reaction. Seeds in the four categories of larger seeds had enough starch to obscure a precipitin line, so their cotyledons were removed and discarded. Eight of these lots, one to three from each of the four size categories, reacted positively.

Seedlings of the soybean cultivar Columbus, at ages up to 2 wk, were inoculated with BPMV to determine if the virus might move into very young seedlings. The inoculum source was infected Columbus plants, which we ground in 0.1 M phosphate buffer, pH 7.1, strained through cheesecloth, mixed

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with Carborundum, and sprayed on exposed parts of the plants using a DeVilbiss atomizer at about 90 psi air pressure. The youngest plants to develop symptoms were about 2.5 cm high when inoculated, with cotyledons still folded but green. To see if the virus could move into germinating seeds, we soaked 14 Columbus seeds in 10 ml of inoculum (BPMV in 0.1 M phosphate buffer, with 0.1% sodium sulfite, pH 7.1) at room temperature. Three to four seeds each were removed and planted after soaking for 2, 4, 24, or 48 hr in the inoculum. No virus symptoms were observed in any seedlings 2 wk later.

Soybean cultivars and breeding lines in the field differed in symptom expression, so plants were tested in a greenhouse to determine if the differences could be reproduced. In a preliminary test, six plants from each of 36 cultivars or lines were inoculated when 10 days old. Mosaic symptoms were apparent on most plants about 3 days later. One week after inoculation the emerging trifoliolate leaves were detached and tested serologically against AS-BPMV. All plants of all 36 cultivars or lines reacted positively.

At about 3 mo, symptoms varied widely among cultivars and lines. For example, all plants of K2-70-16 ((((Lincoln × Ogden) × Adams)[8] × Mukden) × Amsoy) were dead, although in K2-70-14 and K2-70-133 (same parentage as K2-70-16), some of the plants had died. The Kent cultivar plants had terminal necrosis, whereas Marshall plants were nearly symptomless. Some plants, especially those severely stunted, were extremely dark green (eg, Hark), whereas those of cultivars Cutler, Corsoy, Oksoy, and Pickett had several vestigial pods, as reported for TSV (3). Other cultivars and lines that displayed intermediate symptoms were K-1007 (Bonus × Cutler), Woodworth, Tracy, Amsoy, Amsoy 71, Arksoy, Williams, Forrest, Lee 68, Dare, Wayne, and D54-2437 (Roanoke × [Ogden × CNS] × [S-100 × CNS]). Thirteen private lines gave responses similar to those listed. Plants of 18 cultivars or lines lived to produce 537 seeds. All were planted and the seedlings tested serologically against AS-BPMV; all reacted negatively.

Three cultivars and one experimental line representing diverse reactions in the above test were planted in 20-cm pots, thinned to four plants per pot at 3 wk, and inoculated at 4 wk with BPMV. Two weeks later all plants had symptoms. K2-70-16 had severe terminal necrosis; Tracy and Cutler 71 had strong mosaic, leaf crinkling, and some necrosis; and Marshall had mild mosaic symptoms with no necrosis. In subsequent weeks Tracy became abnormally dark green; Cutler 71 was just slightly less dark. All except Marshall developed curled and cupped terminal leaves. As axillary shoots developed in K2-70-16, they also became necrotic. Marshall appeared to be almost free of symptoms until 4 wk after inoculation, when we observed one plant with mosaic symptoms on the upper leaves. Within another 4 wk, 50% of the Marshall plants had developed terminal necrosis; the others lacked obvious symptoms. As with the other plants, the onset of terminal necrosis seemed to be several days after periods of warm, sunny weather. The Marshall plants without pronounced symptoms averaged 190 cm tall at maturity (about 144 days from planting); Cutler 71 and Tracy plants each were about 91 cm at the same time, and K2-70-16 plants averaged 51 cm tall. Seed harvested from the plants ranged from slightly mottled (Marshall) to extremely mottled (Cutler 71). Tracy and K2-70-16 were still very green 200 days after planting. Noninoculated control plants were not maintained to maturity for height comparisons, but under Kansas field conditions Tracy is much taller than the others, Marshall is shorter, and Cutler 71 and K2-70-16 are intermediate and approximately equal in height.

The 29 entries in the 1974 Kansas Soybean Performance Test (6) were evaluated for responses to BPMV, TRSV, and TSV. Approximately 10 plants per treatment, five per 10-cm pot, were inoculated with the virus when 5 wk old. Overall symptom severity, terminal and axillary necrosis, and plant height were noted 6 wk after inoculation and compared with control plants. Results are summarized in Table 1, except that the 11 private cultivars or lines are not listed. We also determined the average plant height for each virus/host combination, as a percentage of the control, then averaged the percentages for all plants inoculated with that virus. The averages and the corresponding standard deviations were: BPMV (79.9%, 11.6), TRSV (61.1%, 15.6), and TSV (90.6%, 12.6).

DISCUSSION

Bean pod mottle is a common disease in Kansas soybeans (4), although the green-stem symptoms are sporadic. Because of the effect on breeding nursery material in 1974, several experimental lines were discarded. In 1979 bean pod mottle was widespread in Kansas and caused problems in field certification, as stem color and maturity are used in determining genetic purity (Lowell Burchett, Kansas Crop Improvement

Table 1. Reactions (ranked by height of check plants [CK]) of soybean cultivars and experimental lines[a] to bean pod mottle virus (BPMV), tobacco ringspot virus (TRSV), and tobacco streak virus (TSV), under greenhouse conditions

	Average height (cm)							Necrosis[e]							Overall symptom severity[e]		
								Axillary				Terminal					
Cultivar/line	CK	BPMV	TRSV	TSV	F^b	$\%P^c$	LSD[d]	CK	BPMV	TRSV	TSV	BPMV	TRSV	TSV	BPMV	TRSV	TSV
Corsoy	58.4	71.8	57.3	63.1	7.0	99.9	2.8	1	0	4	3	2	5	5	···	1	3
Hark	84.5	61.0	48.4	58.1	35.4	100.0	2.9	3	3	3	1	0	5	4	···	3	0
Amsoy 71	102.4	75.4	58.4	88.8	33.1	100.0	3.7	1	3	4	1	3	5	1	···	3	2
Woodworth	114.4	88.3	66.3	112.5	21.6	100.0	14.0	1	1	3	0	0	5	0	3	3	0
Williams	126.8	121.5	98.4	97.0	8.5	100.0	6.0	0	0	1	4	0	1	4	1	2	3
Tracy	140.6	102.4	82.1	125.9	39.4	100.0	11.2	1	2	3	1	0	5	0	3	2	1
Bonus	153.5	112.4	74.0	130.3	44.9	100.0	5.7	1	2	1	1	0	5	0	4	2	2
Cutler 71	153.7	123.3	82.0	93.4	16.2	100.0	9.0	1	1	1	2	1	5	3	1	2	3
Calland	155.7	128.3	105.8	132.3	6.1	99.8	9.4	0	1	4	1	0	5	0	5	3	2
Dare	156.0	140.5	71.0	136.9	37.8	100.0	6.9	0	3	1	0	0	5	3	2	2	2
Essex	156.5	127.0	72.9	142.9	56.4	100.0	14.0	0	1	2	0	0	5	0	1	3	1
Pomona	162.2	122.6	79.2	158.8	38.0	100.0	18.0	0	1	4	0	0	5	0	4	4	2
Columbus	164.8	121.9	70.7	122.8	61.8	100.0	5.5	1	1	3	1	0	5	0	2	3	2
Clark 63	165.6	136.3	83.1	110.9	29.7	100.0	7.3	1	4	1	2	0	5	3	2	2	2
Mack	174.1	121.3	102.0	183.1	43.4	100.0	17.3	0	3	3	0	2	5	0	4	3	2
Forrest	207.3	164.5	102.0	198.1	43.8	100.0	20.3	0	1	4	0	0	5	0	2	4	0

[a] Five-week-old plants were inoculated and observations made 6 wk later.
[b] F = variance ratio.
[c] Probability, expressed as a percentage, that differences are significant.
[d] LSD = least significant differences at the 0.05 probability level.
[e] Relative severity: 0 = no symptoms to 5 = severe symptoms. Overall symptoms include necrosis, chlorosis, plant height, and general appearance.

Association, *personal communication*).

The late-season symptoms contrast with earlier observations (9) that BPMV symptoms are not observed after pod set. That this was a problem in irrigated soybeans in 1974 and that moisture levels (including mid- to late-season rain) were adequate in 1979 suggest a possible relationship between moisture availability and this disease. Effects of early season moisture stress have been noted (9).

Walters (11) tested 70 cultivars or lines, and Scott et al (8) tested 169 commercial cultivars and 123 plant introductions for responses to BPMV; all were susceptible, though symptoms varied among plants (5). Our results confirm the apparent universal susceptibility. Some cultivars, such as Marshall, displayed high resistance, but none was immune. Although all were susceptible to some degree and virus could be serologically detected in seeds from infected plants, none of the seeds grown from infected plants produced infected seedlings, whether greenhouse or field grown, which confirms Skotland's report (10).

The terminal necrosis caused by BPMV alone does not cause the stem tip to curve, nor does it make the tip extremely brittle, as TRSV or TSV can do singly (1,3) or as BPMV does in combination with SMV (7). In a field of otherwise mature soybeans, the BPMV-infected plants lack leaf blades and are usually taller and less bushy than plants infected with TRSV or TSV. When BPMV is in a field, a high percentage of the plants usually are infected, compared with the scattered infection typical of TRSV and TSV. The green stems do not readily move through a combine, making harvest difficult.

We have occasionally isolated BPMV, TSV, and TRSV from a single plant. The plants thus affected have not been observed for an extended period to compare symptoms with singly infected plants.

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