Factors Associated with the Epidemiology of Soybean Mosaic Virus in Iowa

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ABSTRACT

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Soybean (*Glycine max*) grown under fully screened cages, half-screened cages, or without cages were rated for infection with soybean mosaic virus (SMV) by local-lesion indexing and by presence of seed-coat mottling on seeds harvested from the mother plants. Infected plants were detected in the half-screened cages and uncaged treatments but were rare in the fully screened cages. Seed-coat mottling was unreliable as an indicator of virus infection of mother plants and presence of infectious virus in seed. The distribution of SMV in the field suggested plant-to-plant spread from primary inoculum foci. It seems most probable that this primary inoculum consists of infected seedlings derived from SMV-infected seed.

Additional key word: potyvirus.

Soybean mosaic virus (SMV) causes a disease of soybeans (*Glycine max* [L.] Merr.) that is present in all the major soybean-growing areas of the United States. Significant yield losses (11,22–24,26), reduction in seed quality (11,12,23,24), decreased oil content (5), and decreased nodulation (27) have been reported to result from the disease caused by this virus.

Field observations on the pattern of occurrence of SMV-infected plants suggest that insect vectors are involved in the epidemiology of SMV in Iowa. Field experiments reported here confirm this, as do previous reports suggesting that the virus is transmitted by at least 20 aphid species (1,3,6,14). Recent work in our laboratory has defined some of the virus-vector relations in this system (16).

SMV also is transmitted in soybean seed (9,11,13,14). Seed-coat mottling is associated with seed transmission of the virus, but the relationship between mottling and virus transmission is inconsistent (11,19) and can be dependent upon environment, cultivar, and genotype (4,19,24,25).

SMV has a relatively narrow host range (2,8,20,21,29), limited almost entirely to the Leguminosae. Of the potential nonlegume hosts for this virus, only *Amaranthus* sp., *Chenopodium album, Setaria* sp., *Physalis virginiana, P. longifolia,* and *Solanum carolinense* are important to the Iowa soybean-producing area (D. Staniforth and D. Isely, *personal communication*). The most prevalent of these (*Amaranthus* sp., *C. album,* and *Setaria* sp.), however, are annual plants and barring transmission through seed of these hosts, are unlikely to be overwintering hosts for the virus. This observation suggested that infected soybean seed is the most likely source of the primary virus inoculum. We studied the association of SMV infection in plants with the production of mottled seed and the presence of infectious virus in seed. In addition, we monitored disease spread to better understand its epiphytology.

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MATERIALS AND METHODS

Caged plot experiments. The soybean cultivar Harcor was sown 11 May and 17 May in 1976 and 1977, respectively, near Ames, Iowa, at a rate of one seed per 3.8 cm in rows 1.2 m long and 76 cm apart. Every fourth row, a "spreader" row of cultivar Midwest soybean seed infected with SMV, was planted in the same manner as the Harcor seed to provide inoculum for natural spread of the virus.

Soybeans were grown either in 38.1-cm × 76.2-cm and 1.14-m-high wooden-framed cages fully screened with Saran® screen (32 meshes per linear 2.5 cm); in similar cages screened only halfway down from the top of the cage to create an environment similar to that of a full cage but to allow passage of insects into the cage; or not caged. Treatments consisted of the middle 18–20 plants in the row. Cages were placed in position before seedling emergence. Arrangement of the three treatments and five replications was in a completely randomized block. Virus infection was dependent upon natural spread from the spreader rows of Midwest soybeans.

Cages were removed on 25 August in 1976 and 22 August in 1977. All plants in the three treatments were tested for the presence of SMV by the local-lesion assay technique of Milbrath and Soong (17). Samples composed of a mixture of newly-emerged and older leaves were excised from plants by using a different single-edged razor blade for each plant. These leaves were bagged individually and placed on ice in an insulated cooler box until they were transported to the laboratory for indexing. In some treatments, fewer plants were indexed because of premature senescence caused by high temperatures in August. The percentage of seed with mottled coats was determined for all seed harvested from single plants in each treatment. Correlation coefficients between the local-lesion and mottled-seed criteria, as indicators of SMV infection of plants, were calculated for each treatment.

Seed harvested from plants in the three treatments were planted in a greenhouse that was routinely fumigated with insecticide at weekly intervals; the percentage seed transmission of SMV was

determined by examining seedlings for symptoms of SMV at weekly intervals from 1–3 wk and again from 5–6 wk after emergence of primary leaves. In 1976, seeds from plants grown in fully screened cages were not planted because these plants all were negative in local-lesion assays for the presence of SMV. In 1977, all seedlings that exhibited symptoms of SMV and many symptomless controls were indexed by Ouchterlony double-diffusion tests with 0.9% (w/v) Noble agar (Difco) dissolved in 0.05 M sodium borate, pH 7.2, containing 0.85% NaCl and 0.1% NaN_3. Antiserum prepared against pyrrolidine-degraded SMV isolate Ia 75-16-1 (homologous titer 1:16) (10) was placed in the center well. Extracts of plants were prepared by triturating leaf tissue in 0.05 M sodium borate, pH 7.2, with a mortar and pestle and squeezing the contents through two layers of cheesecloth. The filtrate was treated with an equal volume of 5% pyrrolidine in 0.05 M sodium borate, pH 7.2, and placed in peripheral wells. A total of 1,244 plants (11% of the seedlings grown in the 1977 greenhouse tests) were serologically indexed.

Determination of SMV spread in the field. The spread of SMV was monitored in experimental plots established near Ames, Iowa, in 1976 and 1977. In 1976, two experimental plots were planted at a rate of one seed per 3 cm of row with mottled seed hand-picked from a seed lot of cultivar Ontario soybeans known to be infected with the Ia SMV-0 isolate of SMV (16). Each plot consisted of two sets of four rows 76 cm apart and 6.1 m long, separated by a 61-cm alley. Plots were surrounded by a 4.6-m border sown with Ontario soybean seed free of seed-coat mottling. One plot, planted 10 May, was designated as the "corn environment" plot because several rows of cultivar Golden Bantam sweetcorn were planted north and south of the cultivar Ontario border. Large acreages of sweetcorn and soybeans were planted west and east of the plot, respectively. The other plot, planted 18 May and designated as the "soybean environment" plot, had soybeans immediately north and east and a dirt lane to the west and south of the bordering Ontario plants.

Six experimental plots in 1977 had the same format as those planted in 1976. Three "corn environment" plots, planted on 10 May, were within a 45- × 79-m field of Golden Bantam sweetcorn. The three "soybean environment" plots, planted 18 May, were surrounded by miscellaneous soybean cultivars. The Ontario seed used to plant the plots was free of mottling. On 3 June, plants from one plot in each environment were inoculated with the Ia SMV-0 isolate, which is not transmissible by the corn leaf aphid, and plants from another plot in each environment were inoculated with the Ia 75-16-1 isolate, which is transmissible by the corn leaf aphid (16). About 5% of the plants, chosen at random, in each of these two plots in each environment were inoculated by rubbing the primary leaves of plants with chilled infective plant sap containing 22-μm (600-mesh) Carborundum. All inoculated plants were marked. Plants from the third plot in each environment were not inoculated.

Plants were observed for SMV symptoms and marked with color-coded stakes on 2 June, 27 June–6 July, and 29 July in 1976 and on 3 June and 19–20 July in 1977. On 23–24 August 1976 and 19–20 August 1977 plants were examined and the location of virus-infected and uninfected plants was recorded. Fewer observations were made in 1977 than in 1976 because early appearance of symptoms seemingly was masked by heat and drought. Data were analyzed by Pielou's method to test for unsegregated versus segregated distribution of diseased and nondiseased plants (18) in 1976 and by the Vanderplank formula (28) in 1976 and 1977. A random distribution of diseased plants suggests an outside disease source, whereas a nonrandom plant-to-plant spread suggests spread mediated by a vector from a primary inoculum source within the field. Aphid species and their relative numbers in plots were monitored with yellow-pan traps placed at the height of the plant canopy.

RESULTS

Presence of SMV in treatments of caged-plot experiments. The percentage of SMV-infected Harcor soybeans in fully screened cages, half-screened cages, and in uncaged treatments as determined by local-lesion indexing and the presence of seed-coat mottling is shown in Table 1. As indicated by local-lesion indexing, the number of SMV-diseased plants in uncaged plots differs significantly from that in plots covered by fully screened or half-screened cages. The low percentage of SMV-infected plants in the fully screened cages in 1977, as indicated by local-lesion indexing, reflected a low background level of SMV infection in the Harcor seed used to plant the 1977 plots. Disease prevalence as indicated by mottled seed was closely correlated ($r = 0.76$) with that indicated by local-lesion indexing in 1976, but no correlation ($r = 0.08$) was detected in 1977.

Single soybean plants, either healthy or infected by SMV as indicated by local-lesion indexing, bore seeds with mottled seed-

TABLE 1. Infection of cultivar Harcor soybean plants with soybean mosaic virus in 1976 and 1977 as determined by local-lesion indexing and the presence of seed-coat mottling on seeds harvested from plants grown under three cage treatments

	Percentage of soybean mosaic virus-infected plants[a]			
	1976		1977	
Treatment	Local-lesion indexing	Mottled seed	Local-lesion indexing	Mottled seed
Uncaged	26.2 y[b]	39.0 y	33.0 y	75.6 z
Half-screened cage	1.0 z	13.8 z	13.4 z	92.2 y
Fully screened cage	0.0 z	1.2 z	4.4 z	79.2 z

[a] Figures are the mean of five replications for each year. Analysis of variance was conducted on transformed data (arc sine transformation).

[b] Within columns, means followed by different letters are significantly different ($P = 0.05$) by Duncan's multiple range test.

TABLE 2. Frequency distribution of percentage of mottled seed harvested from single cultivar Harcor soybean plants that indexed positive or negative for infection with soybean mosaic virus. Plants were grown under three different treatments in two different years

	Seed-producing plant indexed positive for SMV infection						Seed-producing plant indexed negative for SMV infection					
	Not caged		Half-screened cage		Fully screened cage		Not caged		Half-screened cage		Fully screened cage	
Frequency of mottled seed (%)	1976	1977	1976	1977	1976	1977	1976	1977	1976	1977	1976	1977
0.1–10	9	9	0	5	0	3	10	26	6	32	0	37
11–20	0	4	0	1	0	0	4	3	0	12	0	14
21–30	0	2	0	1	0	0	1	0	0	12	0	4
31–40	0	2	0	2	0	0	1	0	1	1	0	2
41–50	0	2	0	1	0	0	1	2	1	6	0	0
51–60	0	1	0	0	0	0	0	1	0	1	0	0
61–70	1	0	0	1	0	0	0	3	0	1	0	0
71–80	3	0	0	0	0	0	0	0	0	1	0	0
81–90	0	1	0	0	0	0	0	2	0	0	0	0
91–100	1	3	0	0	0	0	3	2	0	1	0	0

coats (Table 2). In either group of plants and irrespective of caging treatments, most frequently, only 10% or less of the seeds of a plant were mottled. Conversely, in 1976 and 1977, eight and three plants, respectively, in uncaged treatments and one plant in each year in half-screened treatments indexed positive for SMV infection but produced no mottled seeds.

Transmission of SMV in seed. Since seed transmission of SMV appears to be important in the epiphytology of the virus in Iowa, the seeds produced on plants grown in the three treatments were classified into groups based on the result of the local-lesion assay of the plant producing the seed and the presence or absence of a mottled seed coat. Detection of seed transmission of SMV in these groups was based on SMV infection of emergent seedlings after planting (Table 3). Statistical analyses were not made because the number of seeds planted in each category ranged from 0 to 3,034. The level of approximately 2% seed transmission from plants in the fully screened treatment in 1977 probably reflects the low level of infection in seed used to plant the plot in 1977, as noted previously.

Although levels of transmission were low, it is clear that seeds with and without mottling can transmit SMV. Further, SMV-infected plants may bear unmottled seeds which will transmit the virus.

In 1977, several plants that indexed negative for SMV bore seeds that produced seedlings in which SMV was detected by serological testing. Since the antiserum used was totally devoid of detectable reaction to healthy host antigens, these data may reflect enhanced sensitivity of the serological test as compared with the local-lesion assay. It also is probable that virus titer was higher in the young greenhouse-grown seedlings tested by serology than in the older field-grown mother plants tested by local-lesion indexing. Unfortunately, at the time the field-grown plants were indexed by local-lesion assay, quantities of available antiserum were too low to permit immunological testing of these plants.

Spread of SMV in the field. Results for 1976 and 1977 indicate that disease incidence in plants was much lower in 1977 than in 1976 (Table 4). The planting of infected seed in 1976 was expected to yield a random initial distribution of infected plants. In the "soybean environment," however, the initial pattern was nonrandom. During the 1976 growing season, the pattern remained nonrandom, and the observed number of doublets (pairs of adjacent diseased plants) was significantly greater than the expected number according to the Vanderplank formula (28) for all observations (Table 4). This suggests plant-to-plant spread. Identical conclusions were drawn when data were analyzed by Pielou's method (18) (data not shown). Similar results were obtained in 1977 with isolates Ia SMV-0 and Ia 75-16-1 (Table 4). Plants infected with Ia 75-16-1 were expected to assume a nonrandom distribution pattern inasmuch as a random number table was used to determine the inoculation pattern. As with Ia SMV-0, the final pattern was nonrandom, with the observed number of diseased doublets significantly greater than the expected number. In 1976, the number of diseased doublets steadily increased, whereas the rate of increase in percentage of plants infected decreased from 29 July to 23 August (Table 4). This relationship implies that most of the spread that occurred during this time can be attributed to an increase in the number of diseased doublets, reflecting plant-to-plant spread. In 1976 and 1977, most frequently, two adjacent plants constituting a disease doublet exhibited SMV symptoms at different observation dates. This suggests that infection of the two plants constituting a doublet occurred at different times rather than simultaneously.

Increase in disease incidence was greatest from early June to mid July (Table 4). Concomitant monitoring of aphid populations in the same fields showed that aphid populations during the time of maximum spread generally consisted of a mixed assortment of species, with no species predominating. When virus spread decreased, there were significant increases in corn leaf aphid populations (R. Hammond, *unpublished*).

The pattern of diseased plants in experimental plots planted in the "corn environment," as demonstrated by analysis according to Vanderplank (28) for 1976 and 1977 and Pielou (18) for 1976 (data not shown), remained random in 1976 and 1977 (Table 4). Disease incidence also was lower than in the "soybean environment" plots (Table 4), with virtually no disease spread occurring in 1977. Although analysis of spread patterns in the "corn environment" in 1976 indicated random disease spread, the rate of increase between 6 July and 29 July was very similar to that between 2 June and 6 July (Table 4). During the period 6 July to 29 July, however, the rate of diseased doublets increased in comparison with the rate between 2 June and 6 July (Table 4). This implies that the spread that occurred between 6 July and 29 July can be attributed to an increase in diseased doublets, suggesting plant-to-plant spread did occur in the "corn environment" during that time.

No SMV-infected plants were observed in the uninoculated plots in the "corn" or "soybean environments" in 1977.

DISCUSSION

No aphid species is known to or has been observed to colonize soybeans in Iowa; therefore, it is probable that migratory winged aphids are the most important aphid form involved in the spread of SMV. The migratory aphids must acquire the virus from some primary inoculum source, either SMV-infected weed hosts or SMV-infected soybeans introduced through infected seed. We believe that infected seed or artificially inoculated foci of infection provided the source of primary inoculum in this study.

TABLE 3. Transmission of soybean mosaic virus in cultivar Harcor soybean seeds produced on mother plants grown under three treatments

Seed category		Transmission by seed from plants in indicated treatments (%)							
		Fully screened cage		Half-screened cage		Not caged		Total[a]	
Local-lesion assay of mother plant	Quality of seed planted	1976[b]	1977	1976	1977	1976	1977	1976	1977
Positive	Mottled	...	0.0	NS[c]	1.8	2.9	7.1	2.9	5.2
Positive	Not mottled	...	NS	0.0	0.0	0.0	0.8	0.0	0.7
Positive	Not mottled, but some of the seeds on mother plant were mottled	...	1.3	NS	2.8	0.4	3.0	0.4	2.7
Negative	Mottled	...	2.4	0.0	7.3	0.0	6.9	0.0	7.2
Negative	Not mottled	...	2.3	NS	3.3	0.0	2.1	0.0	2.5
Negative	Not mottled, but some of the seeds on mother plant were mottled	...	2.0	0.0	2.8	0.0	1.8	0.0	2.0

[a] Data are the total percentage of seed transmission for each seed category planted. Data for 1976 exclude seed produced on plants in fully screened cages.
[b] Seeds harvested from mother plants grown in fully screened cages in 1976 were not planted because local-lesion assay data indicated that the mother plants were not infected.
[c] NS indicates that plants produced no seeds in this category.

TABLE 4. Occurrence and distribution pattern of soybean mosaic virus in cultivar Ontario soybeans in 1976 and 1977[a]

Environment	Observation date 1976	Observation date 1977	Plants infected: Number 1976	Plants infected: Number 1977	Plants infected: Percentage 1976	Plants infected: Percentage 1977	Doublets[b]: Observed 1976	Doublets[b]: Observed 1977	Doublets[b]: Expected[c] 1976	Doublets[b]: Expected[c] 1977	Two standard errors 1976	Two standard errors 1977	Distribution pattern[d] 1976	Distribution pattern[d] 1977
Soybean mosaic virus isolate Ia SMV-0														
Corn	2 June	3 June	73[e]	61	9.5	4.8	12	4	6.9	2.9	± 5.2	±3.4	R	R
	6 July	19–20 July	161	77	21.0	6.1	38	8	33.6	4.6	±11.6	±4.2	R	R
	29 July	19–20 August	224	79	29.2	6.2	71	9	65.2	4.9	±16.2	±4.4	R	R
	24 August		240		31.3		84		74.9		±17.4		R	
Soybean	2 June	3 June	71[f]	69	10.3	5.0	20	1	7.2	3.4	± 5.4	±3.6	N	R
	27 June	19–20 July	155	121	22.6	8.7	57	21	34.7	10.5	±11.8	±6.4	N	N
	29 July	19–20 August	224	163	32.6	11.8	101	31	72.7	19.1	±17.0	±8.8	N	N
	23 August		261		38.0		135		98.8		±19.8		N	
Soybean mosaic virus isolate Ia 75-16-1														
Corn		3 June		66[g]		4.4		0		3.2		±3.6		R
		19–20 July		73		5.4		0		3.9		±4.0		R
		19–20 August		76		5.6		0		4.2		±4.0		R
Soybean		3 June		73[h]		5.2		8		3.7		±3.8		N
		19–20 July		118		8.4		25		9.8		±6.2		N
		19–20 August		138		9.8		27		13.5		±7.4		N

[a] In 1976, plants were grown from seed infected with isolate Ia SMV-0; in 1977, about 5% of the plants in a plot were inoculated with isolate Ia SMV-0 or Ia 75-16-1.
[b] Doublets = pairs of adjacent diseased plants.
[c] Calculated according to the Vanderplank formula, $d = [\mu(\mu-1)]/n$, in which d = the expected number of doublets, μ = the total number of diseased plants, and n = the total number of plants examined in sequence.
[d] Designates random (R) or nonrandom (N) distribution of plants infected with soybean mosaic virus in the experimental plot suggesting an outside disease source (R) or spread mediated by a vector from a primary inoculum source occurring within the field (N).
[e] Number of infected plants in a total of 776 and 1267 plants in the corn environment in 1976 and 1977, respectively.
[f] Number of infected plants in a total of 687 and 1383 plants in the soybean environment in 1976 and 1977, respectively.
[g] Number of infected plants in a total of 1349 plants in the corn environment in 1977.
[h] Number of infected plants in a total of 1405 plants in the soybean environment in 1977.

Application of Pielou's method or Vanderplank's test to field-map data provided strong evidence for plant-to-plant spread of SMV from primary inoculum foci in the "soybean environment" plots. Plants immediately adjacent to primary inoculum foci generally were the first to become infected, both in 1976, when infected seedlings from SMV-infected seed and in 1977, when artificially inoculated seedlings constituted the primary inoculum. These findings provide strong evidence that SMV-infected seed are the source of primary inoculum.

Results in the "corn environment" plot provided little evidence for plant-to-plant spread. This lack of evidence, however, does not exclude the possibility that the inoculum source is within the crop. Indeed, the 1976 data suggested that plant-to-plant spread did occur in the "corn environment" plot during the period 6 July to 29 July. Recent investigations have shown that barrier rows of corn substantially reduce the spread of the aphid-transmitted cucumber mosaic virus (7). The pattern of occurrence of virus-infected plants depends on the pattern of vector movement, which is influenced by many variables, such as inherent behavior of the aphid species, duration of previous flight, host status of the plant, and wind patterns. It is entirely possible that the difference in spread reflects differences in aphid movement caused by alterations in the microenvironment between the two environments.

In 1976, the greatest spread of SMV occurred before the end of July. This observation is significant inasmuch as yield reduction and potential for seed transmission appear greatest when plants are inoculated early (24). In Iowa, soybean plants generally flower near the 2nd and 3rd wk in July. When plants are inoculated earlier, the potential for seed transmission and yield reduction should be enhanced.

Although resistance to SMV in soybean has been documented previously (11,14,24,26), attempts to incorporate resistance to a range of pathogenic variants of the virus into commercial soybean cultivars have met with little success. Therefore, on the basis of the data implicating seed as the primary inoculum source, it seems that currently the most prudent control measure is the use of SMV-free seed.

The association of seed-coat mottling with seed transmission suggests an attractive means for certification of seed for absence of virus. Although the correlation might not be absolute (19), it would be useful if the amount of mottled seed could be used as an indicator of the amount of SMV infection in a seed-production field. In 1976, a reasonable correlation was obtained between the percentage of SMV-infected plants and mottled seed produced on these plants; in 1977, no correlation was evident. Therefore, on a year-to-year basis, the presence of mottled seed is an unreliable indicator of SMV infection of the mother plant. It follows that certification of seed-production fields for absence of virus cannot be based on the presence of mottled coats on harvested seed. Instead, some easily automated technique must be developed to detect virus in seed. The newly developed ELISA procedure (15) or other serological methods under development may achieve this.

Our data confirm previous reports (11,19) indicating that both infected and apparently uninfected plants produce mottled seed. Under the conditions of these experiments, only about 10% of the seeds on Harcor soybean plants were mottled.

Analysis of seed harvested from mother plants grown under fully screened cages, half-screened cages, or uncaged confirmed previous reports (11,19) that mottled and nonmottled seeds may contain infectious SMV. Unexpectedly, some plants that indexed negative for SMV infection by the local-lesion assay produced seed that contained infectious virus. This finding suggests that the indexing procedure was not sufficiently sensitive to detect all infected plants grown in the field. In most treatments of this experiment, increased SMV seed transmission was associated with an increase in percentage of seed-coat mottling.

The number of infected plants in the half-screened cages was considerably lower than that in the uncaged treatments and greater, but not statistically different ($P = 0.05$), than that in the fully screened cages. This suggests that virus vectors alight at the top of the plant canopy. When the plant canopy grew into the upper, screened portion of the cage, plants may have been protected from SMV infection by prevention of insect landing.

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