

## Distribution of Bean Pod Mottle Virus in Soybeans in Kentucky

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### ABSTRACT

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A survey of bean pod mottle virus (BPMV) incidence in soybeans in Kentucky was carried out during the 1985 through 1987 seasons. An ELISA method based on detecting BPMV in extracts from bulked samples of the bean leaf beetle, the vector of BPMV, was used to predict virus incidence in the soybean fields from which beetles were collected. For this survey, beetles were collected from 382 fields in 28 counties in western Kentucky. The results showed BPMV to be widespread in soybeans in those counties; it was detected in about 66% of the soybean fields surveyed. Virus distribution patterns varied with the county; some counties had consistently low virus incidence, others had consistently high incidence. In general, virus incidence was higher in 1987 than the preceding 2 yr.

Bean pod mottle virus (BPMV) is widespread in many of the soybean (*Glycine max* (L.) Merr.) growing areas in the southern and eastern states (7,9,10). Soybean yield losses of 10–40% have been reported as a result of BPMV infection (4–6,9). The economic importance of BPMV is not limited to yield reduction. Infection by BPMV has also been reported to predispose soybean plants to infection by the pod and stem blight fungus *Phomopsis longicolla* T. W. Hobbs, a major cause of poor seed quality in soybean (8,11). Furthermore, BPMV interacts synergistically with

soybean mosaic virus (SMV) to drastically reduce yields in doubly infected plants (1,6).

Currently, no soybean cultivars with resistance to BPMV are commercially available. Formulation of proper control strategies thus requires an understanding of the epidemiology of BPMV in soybeans in Kentucky. Because earlier limited virus surveys made during the 1970s (2) suggested that BPMV was spreading throughout the soybean-growing areas of the state, it was important to update this information and present a more complete description of BPMV distribution. Our paper reports the results of a 3-yr survey that included 382 soybean fields in 28 counties.

In an earlier study (3), Ghabrial and Schultz developed an ELISA procedure to detect BPMV in extracts of bean leaf beetles (*Ceratoma trifurcata* Forst) the vector of BPMV. We used this method in our study to predict BPMV incidence in the soybean fields from which the

beetles were collected. The level of virus incidence in the individual soybean fields was rated on the basis of the amount of BPMV antigen found in extracts from bulked beetle samples. ELISA readings of such extracts reflect virus incidence in the fields from which the beetles were collected because BPMV accumulates in beetles that feed on infected plants; its concentration declines in virus-containing beetles that feed on healthy plants (3).

### MATERIALS AND METHODS

**Beetle collection and virus identification.** Bean leaf beetles were collected using sweep nets from soybean fields in various counties during the period from July to September in 1985, 1986, and 1987. Soybean fields surveyed were selected at random and beetles were collected from random sites within the field. Beetle samples collected from a given soybean field were pooled; at least 25 beetles were used per field.

Extracts from bulked beetle samples were prepared by grinding the beetles in a mortar and pestle along with 0.02 M phosphate-buffered saline (PBS) containing 2% polyvinylpyrrolidone, 0.05% Tween 20, and 0.02% sodium azide (PBS-PVP-T), pH 7.4. The conditions for ELISA were standardized as described by Ghabrial and Schultz (3). Because beetle extracts can be stored for several months at 4 C with virtually no changes in their ELISA absorbance values (3), standards of extracts from virus-free and virus-containing beetles with known

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ELISA absorbance values were included in each test. As a result, comparisons among various locations could be made. The ELISA plates were read at 15-min intervals during a period of 1 hr.

**Rating of virus incidence.** A rating for BPMV incidence in the soybean fields surveyed was made based on the ELISA absorbance values ( $A_{405\text{ nm}}$ ) of extracts from bulked beetle samples. The ELISA tests were conducted under standardized conditions (described below). ELISA readings made 1 hr after adding the substrate were used to rate the soybean fields. The rating system was composed of six classes. A rating of 1 was given to soybean fields when the ELISA absorbance readings of beetle extracts were less than 0.1; such a field was designated as having no virus incidence. Soybean fields for which ELISA readings ranged from 0.1 to 2.0 were given ratings of 2 through 5 as follows: 2 = readings of 0.1–0.499 (low), 3 = readings of 0.5–0.999 (moderate), 4 = readings of 1.0–1.499 (moderate-high), and 5 = readings of 1.5–1.999 (high). A rating of 6 was given when the ELISA readings were higher than 2.0; a field rated 6 was designated as having very high virus incidence.

## RESULTS AND DISCUSSION

Soybeans are grown mainly in the western region of Kentucky. We selected fields in 28 western counties (Fig. 1) as the sites for our survey of BPMV incidence in soybean fields. The fields surveyed varied in size from 20 to 200 acres, with an estimated average size of 75–100 acres. The results of this 3-yr survey (1985 through 1987) indicated that BPMV is widespread in soybeans; the virus was detected in 254 of 382 soybean fields surveyed (Table 1). Furthermore, BPMV was detected in all 28 counties at least once during the 3 yr (Table 1). Virus incidence was consistently high during the 3-yr period in Caldwell, Henderson, Simpson, and Webster counties, where BPMV was detected in almost all soybean fields surveyed. On the other hand, BPMV incidence was consistently low in four other counties (Hickman, Galloway, Livingston, and Lyon), where BPMV was detected in only 50% of the fields surveyed.

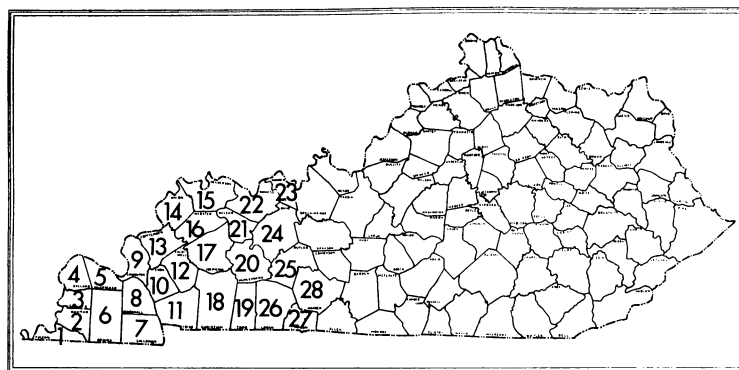
BPMV incidence was relatively high in nine of the 28 counties surveyed in at least 2 yr (Table 1). BPMV was detected in 112 of the 128 fields included in the survey from these counties. Close to one-third of all the fields surveyed had high virus incidence during the 1985–1987 period.

Neighboring counties tended to have similar levels of virus incidence; this was particularly evident in the first year of the survey (Fig. 1, Table 1). It is also noteworthy that, even though BPMV was not detected during the first 2 yr

in Marshall, Daviess, and Hancock counties (Table 1), a very high virus incidence was manifest in these counties during the third year. (Approximately 14 soybean fields were surveyed each year from these three counties.) When all counties are considered, virus incidence was highest in 1987 (Table 1). As a result, we believe that virus surveys should be carried out for a minimum of three consecutive years to get meaningful information on BPMV distribution.

The ELISA method with beetle extracts proved to be efficient and highly

reliable in predicting the incidence of BPMV in soybean fields. In eight instances during the survey, we took leaf samples from plants in the same row that we sampled for beetles and assayed them individually for BPMV by ELISA. In all cases, there was a complete agreement in the predicted incidence of BPMV from the ELISA test results with leaf and beetle samples. This result confirmed an earlier report (3) on the reliability of ELISA with beetle extracts in predicting BPMV incidence in the soybean fields from which the beetles were collected.



**Fig. 1.** Map of Kentucky showing the locations of the 28 western counties included in a survey of bean pod mottle virus in soybeans. Numbers were assigned to the various counties; these same numbers were used in Table 1 to cross-reference virus incidence.

**Table 1.** Incidence of bean pod mottle virus (BPMV) in soybean fields in 28 counties in western Kentucky

No. <sup>b</sup>	County Name	Fields surveyed		Virus incidence ratings <sup>a</sup>		
		Total number	BPMV detected	1985	1986	1987
1	Fulton	13	9	2.3	2.3	5.4
2	Hickman	18	9	2.1	1.8	2.5
3	Carlisle	14	10	2.4	1.8	5.0
4	Ballard	11	8	3.3	1.3	4.4
5	McCracken	10	8	ND <sup>c</sup>	1.6	5.2
6	Graves	17	12	1.4	3.7	4.8
7	Calloway	18	8	1.8	1.2	2.8
8	Marshall	12	4	1.0	1.0	5.2
9	Livingston	12	6	1.7	2.6	2.3
10	Lyon	11	6	3.0	1.2	2.7
11	Trigg	18	10	2.3	1.9	6.0
12	Caldwell	12	11	4.3	4.2	4.7
13	Crittenden	11	7	4.8	2.0	1.5
14	Union	25	20	3.6	2.0	6.0
15	Henderson	15	15	5.0	4.2	4.4
16	Webster	12	12	4.3	4.3	5.6
17	Hopkins	15	9	1.6	1.8	5.8
18	Christian	24	13	3.1	1.3	6.0
19	Todd	13	11	5.5	2.5	5.4
20	Muhlenberg	11	9	4.0	2.0	6.0
21	McLean	12	7	1.0	2.2	5.0
22	Daviess	17	5	1.0	1.0	5.8
23	Hancock	12	4	1.0	1.0	6.0
24	Ohio	5	4	ND	2.2	ND
25	Butler	14	13	5.3	2.5	5.6
26	Logan	12	10	6.0	2.5	3.4
27	Simpson	9	9	6.0	5.3	5.0
28	Warren	9	5	ND	2.8	2.6
Totals		382	254			

<sup>a</sup>Ratings are on a 1–6 scale in which 1 = ELISA absorbance reading less than 0.1 (no virus incidence), 2 = ELISA reading of 0.1–0.499 (low), 3 = ELISA reading of 0.5–0.999 (moderate), 4 = ELISA reading of 1.0–1.499 (moderate-high), 5 = ELISA reading of 1.5–1.999 (high), and 6 = ELISA reading higher than 2.0 (very high).

<sup>b</sup>Numbers are those used in Figure 1 to indicate the locations of the various counties.

<sup>c</sup>ND = Not determined.

Because collecting beetles is less time-consuming than collecting leaf samples and does not require close examination of plants for disease symptoms, we were able to include a large number of fields in our survey.

The ELISA test using beetle extracts allowed us to identify areas in the state where BPMV incidence has been consistently high. We have visited several of the soybean fields predicted to have very high (rating of 6) virus incidence (Table 1) and, in all cases, actual virus incidence was extremely high—approaching 100%—as judged by visual assessment and by ELISA testing of leaf samples collected at random. Such soybean fields have been selected as sites for conducting epidemiological studies aimed at identifying the primary sources of BPMV inoculum in soybean fields in

Kentucky. Furthermore, we are encouraging growers in such localities to apply insecticidal spray for beetle control early in the season, as this practice may prove economically feasible in fields with a potential for high virus incidence.

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