

## Complementation Between Middle and Bottom Components of Broad Bean Stain Virus and Echantes Ackerbohnmosaik-Virus

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### SUMMARY

Jack bean (*Canavalia ensiformis*) and French bean (*Phaseolus vulgaris*) cv. The Prince were useful diagnostic hosts for broad bean stain virus and Echantes Ackerbohnmosaik-Virus. Bottom and middle component of each virus complemented one another to give greatly increased infectivity but there was no complementation between components from the different viruses. Bottom and middle components of each virus were indistinguishable serologically.

### INTRODUCTION

Broad bean stain virus (BBSV) and Echantes Ackerbohnmosaik-Virus (EAMV) are both members of the cowpea mosaic virus group but are not serologically related to one another (Gibbs & Smith, 1970; Gibbs & Paul, 1970).

Although all of the same size, particles of BBSV sediment as three components with sedimentation coefficients of 60, 100 and 127S (Gibbs, Guissani-Belli & Smith, 1968); particles of EAMV sediment as two components with sedimentation coefficients of 98 and 119 S (Paul, 1961, 1962). Although EAMV produces only two components, throughout this paper its 98 S component will be called middle component, by analogy with the 100 S component of BBSV. In cowpea mosaic virus, the type-member of the group, middle (95 S) and bottom (115 S) components are both necessary for infectivity and the separated nucleoprotein components are not infective (Van Kammen, 1968). Top (58 S) component particles contain no RNA and do not contribute to infectivity (Van Kammen, 1971).

This paper gives diagnostic hosts for BBSV and EAMV, and presents evidence that, with each virus, middle and bottom components are both needed to produce infection.

### METHODS

*Virus isolates.* BBSV and EAMV were isolated from field bean (*Vicia faba*) growing at Rothamsted. Their identities were confirmed using antisera prepared earlier at Rothamsted and with other antisera supplied by Dr M. Hollings. The isolates were maintained in the glasshouse by manual inoculation of field bean plants (*V. faba* cv. Maris Bead).

*Diagnostic hosts.* Primary leaves of a number of leguminous species were dusted with carborundum (600 mesh) and rubbed with sap from infected field bean. Local and systemic symptoms were recorded and the presence of virus in uninoculated tip leaves checked by inoculating French bean (*Phaseolus vulgaris* cv. The Prince) and by examining sap in the electron-microscope.

*Virus purification.* BBSV and EAMV were purified from pea plants (*Pisum sativum* cv.

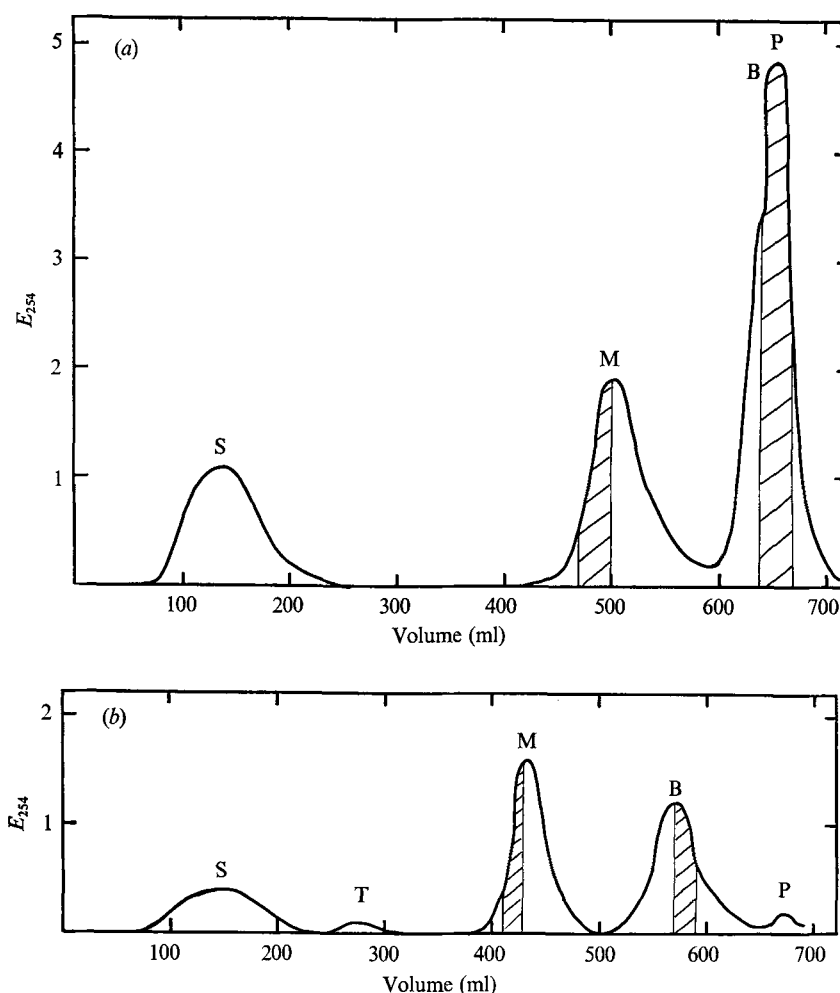


Fig. 1.  $E_{254}$  of fractions displaced from the zonal rotor after centrifuging (a) EAMV, (b) BBSV. B, M, T, bottom, middle, top component; P, pellet; S, small mol. wt. material diffused from origin. Fractions in shaded areas were pooled for infectivity tests.

Onward) manually inoculated about 3 weeks earlier. Leaves (100 g) were homogenized in an M.S.E. Atomix with 200 ml 0.1 M-ascorbic acid neutralized with borax. Then 100 ml of an equal volume mixture of butanol and chloroform was added and the homogenizer run at full speed for 1 min. The resulting emulsion was strained through muslin and centrifuged for 30 min at 12000 g. The separated aqueous phase was given two cycles of high and low speed centrifuging (3 h at 80000 g and 10 min at 12000 g), resuspending the first pellets in 0.1 M-ammonium acetate, pH 7, containing 0.02 M-EDTA and the final pellets in 0.1 M-ammonium acetate, pH 7. Yields were about 20 mg of BBSV and about 50 mg of EAMV.

*Separation of nucleoprotein components.* The components were separated in an M.S.E. BXIV zonal rotor. An isokinetic gradient of 15 to 26 % sucrose in 0.1 M-ammonium acetate, pH 7, was pumped into the rotor, while it was rotating at 2500 rev/min, using an M.S.E. automatic variable gradient former. About 20 mg purified BBSV, or about 50 mg purified EAMV, in 5 ml 0.1 M-ammonium acetate, pH 7, with 5 % (w/v) sucrose was layered on top

of the gradient using a mechanical syringe driven at 2.5 ml/min, followed by an overlay of 130 ml 0.1 M-ammonium acetate, pH 7, pumped with the gradient former. The rotor was then accelerated to 35000 rev/min (100000 g) and run for 135 min (BBSV) or 195 min (EAMV) at 18 to 20 °C. At the end of the run the rotor was decelerated to 2500 rev/min and the contents displaced by pumping 40 % sucrose to the periphery of the rotor. The  $E_{254}$  of the effluent was monitored with an Isco model UA 2 u.v. analyser using a 2 mm path length flow cell. Fractions of 10 ml were collected and the analyser trace was used as a guide when pooling fractions from the leading edge of the bottom component zone and from the trailing edge of the middle component zone (Fig. 1). The bottom zone of EAMV had begun to pellet on the periphery of the rotor and the components were therefore not as well separated as those of BBSV. The combined fractions were dialysed overnight against 0.05 M-ammonium acetate, pH 7, and concentrated by centrifuging for 3 h at 80000 g and resuspending the pellets in 0.01 M-phosphate buffer, pH 7.5. Each component was then diluted in 0.01 M-phosphate buffer, pH 7.5, to a known concentration (using extinction coefficients of 10 for bottom component and 8 for middle component) and a number of 1 ml samples frozen at -20 °C ready for assay.

*Infectivity tests.* BBSV was assayed on French bean (*Phaseolus vulgaris*) cv. The Prince or jack bean (*Canavalia ensiformis*) and EAMV on French bean cv. The Prince. Dilutions ranging from 0.5 µg/ml to 5 µg/ml of each component were made in 0.07 M-phosphate buffer, pH 7.5. Infectivities of mixtures were compared on opposite primary leaves with infectivities of single components having the same concentration as they had in the mixture.

*Preparation of antisera.* Rabbits were given a single intra-muscular injection of 15 mg unfractionated virus emulsified with an equal volume of Freund's complete adjuvant and bleedings were taken at 2 week intervals. Titres reached a maximum after 4 to 6 weeks of 1/2048 for BBSV and 1/1024 for EAMV in Ouchterlony double diffusion tests in 1 % agar.

## RESULTS

### *Diagnostic hosts*

BBSV and EAMV both infected *Dolichos lablab* and mung bean (*Phaseolus aureus*) but the symptoms were not sufficiently distinctive to be of diagnostic value.

The two viruses could be distinguished on French bean cv. The Prince because the chlorotic lesions on inoculated primary leaves caused by BBSV were much larger and more diffuse than those caused by EAMV (Fig. 2*a, b*). BBSV often became systemic in French bean cv. The Prince but EAMV never did and this host was used successfully to select BBSV from a mixture of the two viruses. The two viruses could not be distinguished on French bean cv. Tendergreen in which both gave large diffuse chlorotic lesions and BBSV usually became systemic while EAMV sometimes did. French bean cv. Processor gave small brown local necrotic spots and flecks when inoculated with BBSV but seemed to be immune to EAMV.

BBSV gave very characteristic lesions on inoculated primary leaves of jack bean (*Canavalia ensiformis*). The lesion was a chocolate brown spot 1 to 2 mm in diam. surrounded by a chlorotic halo (Fig. 3*a*) and BBSV never became systemic. By contrast, EAMV caused only faint chlorotic lesions (Fig. 3*b*) and frequently became systemic. Jack bean was used to separate EAMV from a mixture of the two viruses.

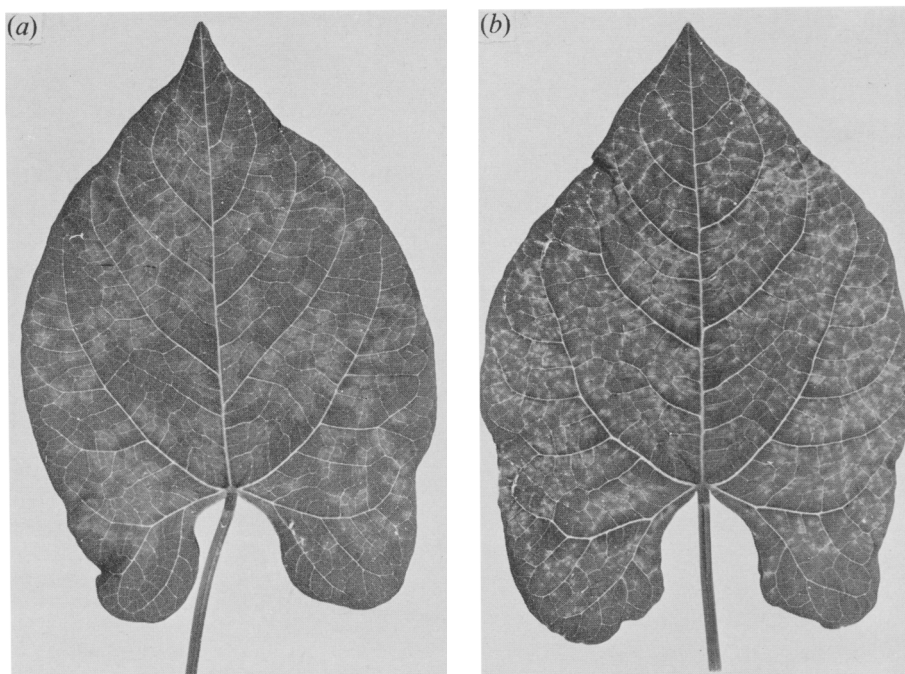


Fig. 2. Lesions of (a) BBSV, (b) EAMV on inoculated primary leaf of French bean cv. The Prince.

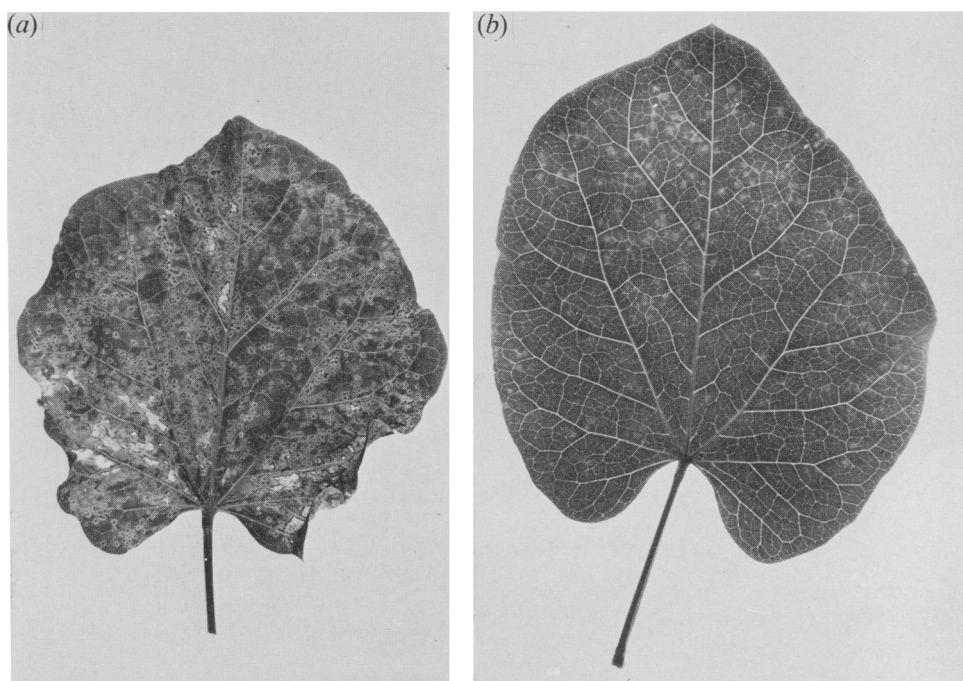


Fig. 3. Lesions of (a) BBSV, (b) EAMV on inoculated primary leaf of jack bean.

Table 1. *Infectivities of BBSV and EAMV bottom and middle components and their homologous and heterologous mixtures on French bean cv. The Prince*

	BBSV*			EAMV*		
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Expt. 6
Bottom component	2.4†	0.5	0.3	3.1	2.6	1.8
Middle component	0.2	0.1	0	0.1	0	0.2
Bottom + homologous middle	230	165	83.3	61.1	75.8	36.7
Bottom + heterologous middle	—	—	0.5	—	—	1.0

\* Concentration of each component was 1 µg/ml (Expt. 1), 0.5 µg/ml (Expt. 2, 3) and 2.5 µg/ml (Expt. 4 to 6).

† Number of lesions/leaf, average of 6 to 18 leaves.

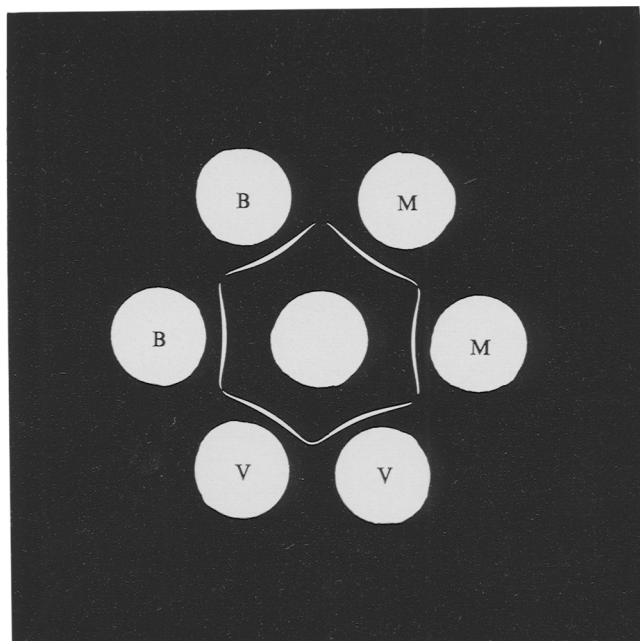


Fig. 4. Reaction of BBSV antiserum (centre well) in Ouchterlony double diffusion test with bottom (B) and middle (M) components of BBSV and their mixture (V).

#### *Infectivity of components*

The infectivities of the separate components of BBSV and their mixture are shown in Table 1. Bottom component was more infective on its own than middle component but even its infectivity was usually only 0.3 to 1 % of that of that of the mixture. Only in experiments (not shown) where the mixture gave very large numbers of lesions was this proportion exceeded, presumably because the dilutions used were outside the straight part of the dilution curve. Similar results were obtained when assays were done on jack bean. Table 1 also compares the infectivities of bottom and middle components of EAMV and their mixture. Bottom component was again more infective on its own than middle component but did not usually exceed 5 % of the infectivity of the mixture.

With both BBSV and EAMV infectivity is greatly increased by mixing bottom component with middle component and it seems likely that, if perfectly separated, each component alone would be non-infective. Much of the infectivity of EAMV bottom component on its own may have been caused by contamination with aggregates of middle component in the pelleted part of the bottom component zone (Fig. 1*a*).

One assay experiment included inocula prepared by mixing the bottom component of BBSV or EAMV with the middle component of the other virus. Table 1 shows that such mixtures were no more infective than the bottom component alone.

### *Serology of components*

Antisera to the unfractionated virus preparations were used to test whether the bottom and middle components of BBSV or EAMV differ from each other antigenically. Fig. 4 shows that in double diffusion tests BBSV antiserum gave only one precipitation line when tested against unfractionated virus and that the precipitation line given by bottom component was continuous with that given by middle component. No spur was formed, suggesting that bottom and middle components are antigenically identical. Similar results were obtained with the components of EAMV. The bottom and middle components of cowpea mosaic virus too are serologically indistinguishable (Bruening & Agrawal, 1967).

### DISCUSSION

BBSV and EAMV have been difficult to distinguish from each other without using serological tests (Gibbs & Smith, 1970). However the results presented here show that inoculation of jack bean provides a ready means of distinguishing between the two viruses and that French bean cv. The Prince and jack bean will separate BBSV and EAMV, respectively, from a mixture.

Like most other members of the cowpea mosaic virus group that have been investigated (Wood & Bancroft, 1965; Van Kammen, 1968; Kassanis, White & Woods, 1973), BBSV and EAMV show strong complementation between the two nucleoprotein particles. It is unlikely that the apparently 'empty' 60 S particles of BBSV contribute to infectivity, although they were not tested.

Components from different virus strains sometimes complement each other but only when they are closely serologically related (Kassanis *et al.* 1973). Although BBSV and EAMV are assigned to the same virus group, they are not serologically related and it is not surprising, therefore, that the middle component of one does not complement the bottom component of the other to give increased infectivity.

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