Serologic Detection of Feathery Mottle Virus Strains in Sweet Potatoes and *Ipomoea incarnata*

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ABSTRACT

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The enzyme-lined immunosorbent assay technique was tested for detection of sweet potato feathery mottle virus in greenhouse and field-grown hosts. Virus was detected in partially purified preparations at a concentration of 32 ng/ml and in infected sap from sweet potatoes at dilutions up to 1:6,250 (w/v) but only if the leaves had symptoms. The reactions, using all homologous and heterologous combinations, were identical for the four isolates in *Ipomoea incarnata* and in sweet potatoes. Feathery mottle virus was also readily detected by serologically specific electron microscopy in partially purified virus preparations, infected *I. incarnata* leaves, and sweet potato leaves with virus symptoms but not in symptomless leaves.

Additional key words: chlorotic leaf spot virus, internal cork virus, russet crack virus

Feathery mottle virus (FMV) is an aphid-transmitted virus of the potyvirus group (3). Four isolates of FMV have been purified, characterized, and shown to be serologically closely related: ordinary (FMV-O), russet crack (FMV-RC), internal cork (FMV-IC), and chlorotic leaf spot (FMV-CLS) (3). Some isolates of FMV cause economical losses (4,5,12). The virus is difficult to detect because it does not always cause obvious symptoms in the foliage of infected plants.

Because serologic techniques have not been introduced for the detection of viruses in sweet potatoes, researchers working in breeding or in certification programs use indexing methods (14) that require considerable time and greenhouse space. The objectives of this work were to test the feasibility of more rapid and convenient serologic methods such as ELISA (6,7) and serologically specific electron microscopy (2,8).

MATERIALS AND METHODS

Four isolates used in this study were maintained, transmitted, and purified as described (3).

The ELISA test was done by Clark and Adams' method (6,7), with some modifications. For FMV-O the optimal concentration of coating γ-globulin was 1 μg/ml and the plates were incubated for 4 hr at 30 C (9). Enzyme-conjugated γ-globulin was incubated in the plates for 5–6 hr at room temperature. Results were scored visually or by diluting the reacted substrates 1:4 and determining the absorbance at 405 nm. In the latter method, samples were not scored as positive unless the absorbance was at least twice that of the healthy controls.

Crude leaf extracts or partially purified virus preparations were incubated in coated plates for 16–18 hr at 4 C. The extraction buffer was phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20 plus 2% polyvinyl pyrrolidone (40,000 mol wt, Sigma Chemical Co., St. Louis, MO 63170) and 0.2% ovalbumin.

The leaf sap was extracted with a hand sap extractor by adding half the extraction buffer to the leaf sample wrapped in nylon mesh, squeezing, and repeating this with the remainder of the buffer. Leaf samples of about 0.5 g were extracted at a 1:5 dilution (w/v), or in one trial at 1:10 dilution, and subsequently adjusted to higher dilutions with the same extraction buffer. Other samples of about 0.1 g were extracted at a 1:50 dilution (w/v) with a Polytron homogenizer (Brinkman Instruments, Westbury, NY 11590). The two extraction methods were compared using a composite sample of 1 cm^2 leaf pieces of equal weight from infected or healthy plants of the specified hosts.

Virus concentration in partially purified preparations was determined spectrophotometrically (3). The preparations were first diluted to twice the final concentration with 0.025 M borate buffer, pH 9, and then mixed with an equal volume of phosphate-buffered saline containing Tween plus 2% polyvinyl pyrrolidone and 0.2% ovalbumin before being placed in the wells.

Serologically specific electron microscopy, described by Brlansky and Derrick (2, 8), was done as outlined (3), and the grids were examined at ×3000. Partially purified FMV-O diluted in 0.05 M Tris buffer, pH 7.5, was used at about 100 μg/ml and leaf samples were extracted at 1:5 (w/v) dilution with the same buffer.

RESULTS

Detection of FMV with ELISA. FMV-O was detected at concentrations as low as 32 ng/ml with enzyme-conjugated, homologous γ-globulin at dilutions of 1:100 and 1:200 (Fig. 1). For economy, the 1:200 dilution was used as standard for subsequent experiments. Three other antisera gave similar results at this concentration. Each of the four isolates was detected at concentrations of 32 ng/ml with their homologous antisera.

In two experiments, coating and enzyme-conjugated γ-globulins of each isolate were used to detect each of the four isolates in sap of *I. incarnata*. The sap was extracted at 1:10 dilution (w/v) with a hand sap extractor and tested at this dilution and at 1:50. Strong homologous and heterologous reactions were obtained by visual scoring for each of the 16 combinations of four antisera and four isolates at 1:10 dilution. The reactions were weaker but distinct when the samples were diluted at 1:50. No reaction was seen with healthy sap or extraction buffer controls. These results confirm the

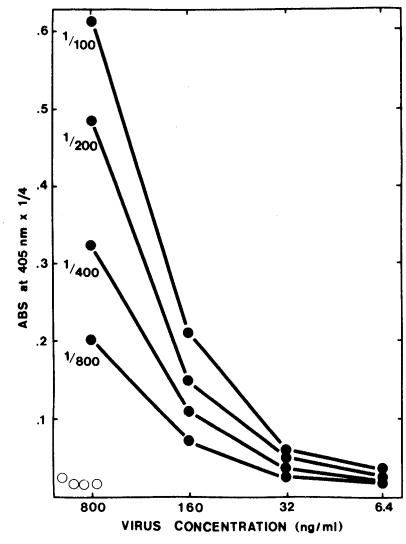

Fig. 1. Relationship between concentration of partially purified feathery mottle virus (FMV-O), dilution of enzyme-labelled γ-globulin, and absorption values of reactants in enzyme-linked immunosorbent assay. • = partially purified virus; o = sap from healthy plants at $A_{260} = 4.0$.

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cross-reactivity of these antisera and virus isolates observed with partially purified virus (3).

Sweet potato cultivars Jersey, Porto Rico, and Garnet growing in the greenhouse and inoculated with each of the four virus isolates were extracted with a hand sap extractor and tested with FMV-O antiserum. Positive readings were obtained from leaves with symptoms of any of the four virus isolates, and the results were combined for simplicity (Table 1). Symptomless leaves from six of the infected plants gave negative results.

Samples extracted with the Polytron homogenizer had significantly higher absorption values at 1:50 dilution (w/v) than those extracted with the hand sap extractor (Table 2).

When samples were tested after fivefold dilutions in extraction buffer, FMV was detected at dilutions as high as 1:6,250 in the Polytron-homogenized extract and only at 1:1,250 with the hand sap extractor samples. In further trials with the Polytron homogenizer, the best tissue/buffer ratio was 1:100 for leaves of infected *I. incarnata* (Fig. 2). Absorbance values were higher if the samples were clarified by low-speed centrifugation at 8,000 rpm for 10 min in a SS-34 rotor before placing them in the plates. This experiment was repeated with similar results.

The ELISA method was used to detect FMV in 12 mature, field-grown Jewel plants with chlorotic spots or rings in the leaves. Leaf samples weighing 50 mg were cut out with a cork borer, ground in a mortar, diluted 1:100 with extraction buffer, and homogenized with the Polytron. The absorbance was 0.101 ± 0.027 for samples from leaves with symptoms and 0.019 ± 0.004 for samples from adjacent symptomless leaves on the same shoots of the same plant. The absorbance value was 0.018 ± 0.003 for six virusfree plants grown in an isolated foundation block. Five infected plants each of cv. Garnet and L4-131 had positive reactions from leaves with symptoms but not from adjacent symptomless leaves on the same shoots.

Serologically specific electron microscopy. The average number of particles per electron micrograph was significantly higher when the grids coated with FMV antiserum were compared with those with normal serum. With partially purified preparations, they were increased from 9 to 1,120 and with sap from *I. incarnata* from 3 to 136. In another experiment, sap from infected *I. incarnata* was extracted and fivefold dilutions were made with 0.05 M Tris buffer, pH 7.5. Virus was detected at dilutions up to and including 1:625. In another experiment, leaves of an infected

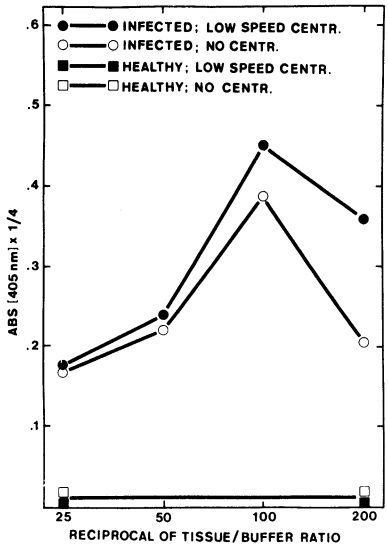

Fig. 2. Effect of tissue/buffer ratio and low-speed centrifugation on the absorption value of reactants in enzyme-linked immunosorbent assay, with samples extracted from *Ipomoea incarnata* with a Polytron homogenizer.

Table 1. Detection of feathery mottle virus with ELISA in sweet potato plants growing in the greenhouse

Cultivar	Plants[a] (no.)	Absorbance at 405 nm × 1:4[b] Average	Range
Infected			
Porto Rico	8	0.172 ± 0.061	0.101–0.275
Garnet	10	0.111 ± 0.033	0.085–0.186
Jersey	6	0.124 ± 0.036	0.089–0.179
Healthy			
All cultivars	8	0.026 ± 0.004	0.022–0.031

[a] Samples were extracted with hand sap extractor and phosphate-buffered saline containing Tween + 2% polyvinyl pyrrolidone + 0.2% ovalbumin at 1:5 dilution (w/v).
[b] Absorbance values for fourfold dilutions of reacted substrates.

Table 2. Comparison of two sample extraction methods for detection of feathery mottle virus (FMV) in sweet potato cultivar Porto Rico and *Ipomoea incarnata* by enzyme-linked immunosorbent assay

Host	Virus[a] isolate	Symptoms	Sample extracted with[b]: Polytron homogenizer	Hand sap extractor
Porto Rico	FMV-O	Faint	0.063 ± 0.004	0.032 ± 0.001
	FMV-CLS	Faint	0.064 ± 0.001	0.035 ± 0.001
	FMV-IC	Distinct	0.401 ± 0.022	0.120 ± 0.006
	None	None	0.012 ± 0.001	0.014 ± 0.001
I. incarnata	FMV	Distinct	0.397 ± 0.011	0.130 ± 0.006
	None	None	0.017 ± 0.001	0.016 ± 0.001

[a] O = ordinary, CLS = chlorotic leaf spot, IC = internal cork.
[b] Absorbance values at 405 nm for fourfold dilution of reacted substrates. Each value is the average of two replicates from one leaf sample from one plant. Samples were extracted with the Polytron homogenizer at 1:50 dilution (w/v) in buffer. In the case of hand sap extractor, samples were extracted at 1:5 dilution and further diluted to 1:50.

Table 3. Comparison of enzyme-linked immunosorbent assay and serologically specific electron microscopy for detection of feathery mottle virus in an infected sweet potato plant growing in the greenhouse

Sample leaf[a]	Sap dilution 1:100	1:500	1:2,500	1:12,500
1, with symptoms	0.160[b] (84)[c]	0.069 (14)	0.041 (0)	0.019 (0)
2, with symptoms	0.100 (24)	0.042 (5)	0.025 (0)	0.021 (0)
3, faint symptoms	0.046 (4)	0.021 (0)	0.017 ...	0.018 ...
4, symptomless	0.018 (0)	0.018 ...	0.017 ...	0.019 ...
5, symptomless	0.019 (0)	0.018 ...	0.018 ...	0.019 ...
Virusfree check	0.016 (0)	0.016 ...	0.018 ...	0.018 ...

[a] Leaves numbered acropetally on one runner.
[b] Average of absorbance values at 405 nm for fourfold dilutions of reacted substrates of duplicate samples extracted with a Polytron homogenizer.
[c] Number of virus particles seen in 5 min at approximately ×3000. Samples for serologically specific electron microscopy were extracted in a Polytron homogenizer.

sweet potato plant were tested for the presence of virus using both serologically specific electron microscopy and ELISA. Virus was detected with both techniques but only in leaves with symptoms (Table 3).

DISCUSSION

FMV was detected with ELISA at concentrations of 32 ng/ml in partially purified preparations and in dilutions up to 1:6,250 (w/v) of sap of *I. incarnata* or sweet potato growing in the greenhouse. ELISA can confirm the presence of FMV strains in the foliage of sweet potatoes or other *Ipomoea* spp. growing in the greenhouse or field if there are foliar symptoms. Negative results were obtained with symptomless leaves on the same runners of these plants. The virus is unevenly distributed in the plant and therefore the value of the ELISA test is limited. It only can be used to confirm the presence of FMV and its relative concentration in infected plants with symptoms. Although serologically specific electron microscopy was not extensively studied, our results indicate that FMV can be detected with this method in *Ipomoea* spp. with symptoms and grown in the greenhouse.

Use of the Polytron homogenizer for sample extraction improved the sensitivity of ELISA when samples were extracted at high dilution (1:100). Inhibition of specific reactions by plant sap has been observed with the ELISA technique in black currents infected with Arabis mosaic virus at 1:10 but not 1:100 (7). The situation may be similar in *Ipomoea* spp.

The cross-reactivity of these antisera permits detection of different strains of FMV with one antiserum in the ELISA technique. Similar results were obtained with other elongated viruses (1,10,13), but strain specificity seems to be a problem with some isometric viruses. Koenig (10) found that conjugates prepared to one strain of Andean potato latent virus failed to detect other serologically closely related strains. A similar phenomenon was observed with barley yellow dwarf isolates (11).

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