Purification and Properties of Sweet Potato Feathery Mottle Virus

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ABSTRACT

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An isolate of sweet potato feathery mottle virus was recovered from Georgia Red sweet potato plants exhibiting interveinal chlorotic spotting and vein mottling. The virus was readily sap-transmissible when diluted 10-fold in 0.05 M potassium phosphate buffer, pH 7.2. It was nonpersistently transmitted by *Aphis gossypii*, *A. craccivora*, *Lipaphis erysimi*, and *Myzus persicae*, but was not transmitted by *Rhopalosiphum maidis* or *R. padi*. Properties in *Ipomoea nil* sap were: thermal inactivation point 60-65 C, dilution end-point between 10^{-3} and 10^{-4}, and it remained infectious in sap less than 12 hr. Twenty-seven plant species from seven families were inoculated but only eight *Ipomoea* spp. became infected. A purification procedure is described which results in 8-10 mg virus/kg infected tissue. The purified virus particles measured approximately 850 nm in length. Purified virus had a 260/280 ratio of 1.18. Antisera produced to a purified virus had a titer of 1:1,024 in microprecipitin tests and 1:16 in agar double-diffusion tests.

There are many reports on viruslike diseases of sweet potato, *Ipomoea batatas* (L.) Lam.; e.g., mosaic (2, 6), feathery mottle (3, 5), internal cork (16), and russet crack (4). However, only limited information is available on the viruses that cause these diseases. This has resulted in the previously discussed (3) unwarranted appearance of new virus names (13). For example, sweet potato feathery mottle virus (SPFMV) first was used for the syndrome of leaf spotting and vein banding in sweet potato (5). A mechanically and nonpersistently aphid-transmitted agent later was associated with the disease (11, 17, 18). Also, SPFMV has been used in reference to a whitefly-transmitted agent (8) and to a disease complex of three agents (9). Most recently, SPFMV has been more strictly used to refer to the virus and not the disease (3, 15).

Criteria such as biological and biochemical properties and serological relationships are needed for classification of viruses causing foliar and root diseases of sweet potatoes. Reported here are biological properties, an extended host range, and a purification procedure for an isolate of SPFMV. Its relation to other sweet potato viruses is discussed.

MATERIALS AND METHODS

Georgia Red sweet potato plants exhibiting chlorotic spots served as the initial virus source. Isolations were made by approach-grafting leaf petioles to *Ipomoea nil* 'Scarlet O'Hara' seedlings in the one- to two-true-leaf stage which then were covered with plastic bags for 4 days. The virus was maintained in *I. nil* plants.

Transmission.—The most suitable buffer for mechanically transmitting the virus was determined using systemically infected *I. nil* source leaves and inoculating the cotyledons of *I. nil* seedlings. Distilled water at pH 7.2 and several buffers at a concentration of 0.05 M were tested on 25 seedlings in each of three trials.

Aphid transmissibility was determined using *Aphis gossypii* Glover reared on *Cucumis melo* L. Groups of 10 apterous adults were starved approximately 1 hr, given 5-min acquisition access periods on detached, systemically infected *I. nil* leaves, and inoculation access periods on cotyledons of healthy *I. nil* seedlings overnight. Inoculation access periods were terminated by spraying plants with 1.0% nicotine sulfate (v/v).

To reduce the possibility of a mixed virus culture, transmissions were made using aphids which were permitted only a single acquisition probe on the virus source. Fifty apterous adults were starved a minimum of 30 min. Then each aphid was allowed a single probe lasting 10-20 sec and transferred to separate *I. nil* plants. Three serial transmissions were made at approximately 14-day intervals, each time using as a virus source a plant infected in the previous transmission. The resulting virus culture was maintained in *I. nil* plants in a screened area of the greenhouse and used as the virus source for further identification studies. Periodically during the course of this study Georgia Red cuttings which indexed negative for the virus were graft-inoculated to assure that pathogenicity had not been lost.

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Virus-vector relations.—Studies were conducted to identify potential vector species and determine optimal acquisition and inoculation probing times. Aphid species commonly occurring in the sweet potato-growing region of North Carolina were tested for ability to transmit this virus. Aphid species tested and rearing plants were: *Aphis craccivora* Koch, [*Vigna unguiculata* (L.) Walp]; *Aphis gossypii* Glover, (*Cucumis melo* L.); *Lipaphis erysimi* Davis, (*Raphanus sativus* L.); *Myzus persicae* (Sulzer), [*Brassica juncea* (L.) *Coss*]; *Rhopalosiphum maidis* (Fitch) and *Rhopalosiphum padi* (L.), (*Hordeum vulgare* L.). Species were tested by allowing 20 groups of 10 apterous adults 5-min acquisition access periods on excised healthy or systemically infected *I. nil* leaves. Each group was placed on one *I. nil* seedling overnight and then killed with 1.0% nicotine sulfate. Species which did not transmit were tested further to assure that they actually probed the virus source plant: single apterous adults that were observed to make 10- to 20-sec acquisition probes on an infected leaf then were removed and caged overnight in groups of 10 on cotyledons of *I. nil* seedlings.

The virus acquisition and inoculation probing times were determined using *Aphis gossypii* and *Myzus persicae* that had been starved for 30 to 180 min. Acquisition times were determined using individual aphids that had been given uninterrupted probes on an infected *I. nil* leaf, transferred to individual *I. nil* seedlings for 2 hr, and then killed with 1.0% nicotine sulfate. Inoculation times were determined by giving starved apterous adults a 10- to 20-sec acquisition probe, then timing initial probes on healthy *I. nil* seedlings.

In vitro properties.—The following tests were conducted using 0.05 M potassium phosphate buffer, pH 7.2, and mortars and pestles chilled to 4 C. Longevity in vitro was determined using *I. nil* sap, sap plus buffer (equal volumes), and sap plus buffer containing 0.05 M sodium diethyldithiocarbamate (DIECA). Samples of each treatment were incubated at room temperature and at 4 C. Each sample was assayed on five *I. nil* plants 0 to 12 days after preparing sap and the test was conducted twice.

Dilution end point of virus in systemically infected *I. nil* leaves 14 days following inoculation was determined by triturating systemically infected leaves in a mortar and pestle, collecting the sap by squeezing through cheesecloth, and inoculating 10 plants in each of three trials with dilutions (10^{-1} to 10^{-5}) made with 0.05 M phosphate buffer, pH 7.2.

Thermal inactivation point was measured by triturating in ten volumes of buffer systemically infected *I. nil* leaves 14 days following inoculation. Two milliliter aliquots were incubated 10 min at temperatures from 40-100 C and used to inoculate 10 *I. nil* seedlings in each of three trials.

Host range.—Plants representing 27 species from seven families were mechanically inoculated using systemically infected *I. nil* leaves triturated in 10 volumes of 0.05 M potassium phosphate buffer pH 7.2. Plants were observed daily for symptom development. Twenty-one days after inoculation, immature, intermediate and fully expanded leaves were collected separately from each species and indexed on *I. nil* seedlings.

Purification.—A previously reported (3) attempt to purify sweet potato viruses from *Ipomoea* spp. met with only limited success. Comparisons of buffer systems, organic solvent clarifying agents, and stabilizing agents were based on relative infectivity and absorbance. Purification trials were conducted by homogenizing systemically infected *I. nil* leaves in three volumes 0.5 M buffer (1:3, w/v) containing a stabilizing agent. The homogenate was clarified with an organic solvent and centrifuged to remove large debris followed by two cycles of precipitation with 4% polyethylene glycol 6000 (PEG) (4 g/100 ml homogenate) plus 1.75% NaCl (1.75 g/100 ml homogenate). The precipitate was resuspended in 0.05 M buffer and insoluble material removed by low-speed centrifugation. This was followed by one cycle of differential centrifugation, resuspending the pellet in 0.05 M buffer containing 0.005 M disodium ethylenediaminetetracetate (EDTA) overnight, and then assaying for infectivity. The details of the procedure are given with the results of these comparisons.

Infectivity assay.—No local lesion host has been identified for this virus. Thus a relative measure of infectivity (infectivity index) was used to compare the different, partially purified preparations. The infectivity index was determined by making dilutions (10^{-1} to 10^{-3}) of the sample to be tested and inoculating the cotyledons of three *I. nil* seedlings with each dilution. The number of infected plants at each dilution was multiplied by the negative logarithm of that dilution. The products for each sample were summed to give an infectivity index ranging from zero (no plants infected) to 30 (all plants infected). An additional estimate of virus yield was made by measuring the ultraviolet absorbance at λ=260 nm, correcting for light scattering, and expressing concentration as absorbance units per milliliter (A_{260}/ml). Measurements were made using a Beckman DB-G spectrophotometer with a 1-cm light path.

Particle-length determination.—Virus for particle length determination and antiserum production was prepared by layering 1 ml of the partially purified preparation on a linear density gradient column consisting of 10-40% sucrose (w/v) in 0.05 M borate buffer pH 8.0. The columns were centrifuged in an SW 25.1 Beckman rotor at 23,000 rpm for 3 hr. Ultraviolet (254 nm)-absorbing regions of the column were identified and fractionated using a Model 640 ISCO density gradient fractionator. The region containing virus was detected by infectivity tests and electron microscopic examination of each fraction. Virus particle measurements were made by examining preparations from the density gradient column on Formvar coated grids negatively stained with 2% phosphotungstic acid, pH 7.0. Electron micrographs were taken with a Hitachi Model HS-8-1 electron microscope and magnifications determined using a carbon replica grating with 54,800 lines per inch (E. F. Fullam, Inc., Schenectady, NY 12301). Measurements were made by enlarging the negatives with an overhead projector and comparing particle lengths with the replica grating using an Alvin 1116 map measure.

Serology.—Virus for antiserum production was collected from the density gradient columns. The rabbits were subcutaneously injected monthly with approximately 0.5 mg of virus emulsified with an equal amount of complete Freund's adjuvant. Microprecipitin and agar double-diffusion tests were conducted as previously described (1, 7) using a virus concentration of

0.4 mg/ml which had been estimated using an extinction coefficient of $E^{0.1\%}_{260} = 2.5$.

RESULTS

Transmission.—Initial studies to find an effective diluent for mechanical transmission of SPFMV between *I. nil* plants demonstrated that potassium phosphate buffer, pH 7.2, provided the greatest transmission efficiency (80%). Other diluents tested include: distilled water, pH 7.2 (23%); sodium citrate, pH 5.0 and 6.0 (8%); sodium borate, pH 8.0 (37%); and sodium borate, pH 9.0 (56%).

Aphid-transmissibility of the virus was demonstrated when 12 of 20 indicator plants became infected following exposure to *A. gossypii* which had previously been confined for 5 min on excised leaves of infected *I. nil*. Of the other aphid species tested as vectors in a similar manner, *M. persicae* and *A. craccivora* transmitted virus to 10 of 20 and 7 of 20 indicator plants, respectively. In these initial tests, no transmission occurred with *L. erysimi*, *R. padi*, or *R. maidis*. In subsequent tests designed to ensure that those nontransmitting species actually probed the virus source plant, only *L. erysimi* transmitted the virus, to one of 10 indicator plants. Neither *R. padi* nor *R. maidis* transmitted the virus in any test. In all tests, infectivity of the virus sources were verified by mechanically indexing them to *I. nil* seedlings. In all tests control plants remained symptomless.

Data on the efficiency of transmission by *A. gossypii* and *M. persicae* at different acquisition and inoculation probing times are presented in Table 1. Although the minimum acquisition probing time was 3 sec for both aphid species, transmission was most efficient with acquisition times of more than 6 sec and less than 40 sec. The maximum acquisition times observed resulting in successful transmission were 1,027 and 308 sec for *A. gossypii* and *M. persicae*, respectively. When the aphids were given a standardized virus acquisition probe of 10-15 sec, transmission appeared most efficient when the duration of the inoculation probe exceeded 6 sec. The minimum inoculation probing times resulting in successful transmission by *A. gossypii* and *M. persicae* were 4 and 7 sec, respectively.

A chi-square (χ^2) test for independence on the pooled transmission data from the acquisition and inoculation time studies showed that for the aphid biotypes tested, *A. gossypii* was a more efficient vector of SPFMV than *M. persicae* ($\chi^2 = 65.74$, df = 1, $P \leq 0.005$). *Aphis gossypii* transmitted to a total of 208 of 527 indicator plants (39%), whereas *M. persicae* transmitted to 134 of 721 indicator plants (19%).

In vitro properties.—Tests to determine the stability of SPFMV in *I. nil* sap showed that infectivity is lost in less than 6 hr at room temperature. Sap mixed with buffer or buffer plus DIECA retained infectivity for 6 but not 12 hr. At 4 C, sap remained infectious for 6 hr, but not for 12 hr. The addition of buffer and DIECA did not lengthen longevity.

The dilution end point of SPFMV in sap extracted from *I. nil* plants that had been inoculated for 14 days was between 10^{-3} (4/30 infected) and 10^{-4} (0/30 infected). The inhibitory effect of *I. nil* sap on mechanical transmission was decreased in the 10^{-1} dilution (27/30) over nondiluted sap (8/30).

Initial attempts to determine the thermal inactivation point of the virus in nondiluted *I. nil* sap were inconsistent. Only one to two plants of ten inoculated became infected in the control and inactivation occurred at several temperatures. When 10^{-1} dilutions of sap in 0.05 M phosphate buffer were used, infectivity was retained for the duration of the experiment and inactivation consistently occurred between 60 C and 65 C in three trials.

Host Range.—Infectivity studies with selected species of the Convolvulaceae and other plants commonly used for virus assay and diagnosis demonstrated that the SPFMV host range is limited to *Ipomoea* spp. Convolvulaceae species tested include: *Calonyction aculeatum* L. *Convolvulus sepium* L., *Ipomoea crissicaulis* Robinson, *Ipomoea fistulosa* Martius, *Ipomoea alba* (L.) House 'Moonflower', *Ipomoea nil* (L.) Roth 'Scarlet O'Hara', *Ipomoea setosa* Ker., *Ipomoea tricolor* Ca. 'Heavenly Blue', *Ipomoea lacunosa* L., *Ipomoea hederaceae* (L.) Jacquin, *Ipomoea purpurea* (L.) Roth, *Ipomoea wrightii* (House) Alain, and *Ipomoea batatas* 'Georgia Red' and 'Jersey'. Susceptible species and symptoms are summarized in Table 2.

Ipomoea nil exhibited symptoms 5-7 days following inoculation. The first true leaf exhibited vein clearing, interveinal areas became darker green than normal. This was followed by epinasty (Fig. 1), which resulted in a crinkling of the leaf. Veinbanding, limited to primary and secondary veins, developed on subsequent leaves resulting in severely stunted plants. Plants with one or more true leaves when inoculated recover following initial symptom expression. Symptoms on *I. setosa* plants appear first as vein-clearing which developed into a general chlorotic mottle of that leaf (Fig. 2-B). Vein-clearing and chlorotic veinbanding on the next set of

TABLE 1. Transmission efficiency of sweet potato feathery mottle virus by *Aphis gossypii* and *Myzus persicae* at different virus acquisition and inoculation probe times

	Virus transmission[a]	
Duration (sec)	*A. gossypii*	*M. persicae*
Acquisition probe[b]		
1 - 5	2/13	3/22
6 - 10	19/57	20/77
11 - 20	68/124	47/195
21 - 30	15/38	13/46
31 - 40	9/16	3/11
40	3/17	16/73
Inoculation probe[c]		
1 - 5	3/14	0/15
6 - 10	25/77	7/48
11 - 20	48/131	12/122
21 - 30	7/22	4/36
31 - 40	4/4	2/23
40	5/14	7/53

[a]No. of transmissions per no. of plants tested, one aphid per plant.

[b]Inoculation access period = 2 hr.

[c]Virus acquisition via single probe of 10-15 sec on infected tissue.

leaves are limited to primary and secondary veins (Fig. 2-C) and on subsequent leaves veinbanding is confined to the midrib and is accompanied by mild distortion (Fig. 2-D). Subsequent leaves may be symptomless or have faint chlorotic spots. *Ipomoea tricolor* became severely wilted and frequently died. Surviving plants exhibited vein-clearing and banding. *Ipomoea purpurea* remained symptomless; however, virus was recovered from true leaves that had not been inoculated. The sweet potato cultivar Georgia Red developed chlorotic spotting and veinbanding (Fig. 3) approximately 30 days after inoculation. Jersey sweet potato plants exhibited mild chlorotic mottling and chlorotic banding of the midrib. Other susceptible *Ipomoea* spp. also exhibited vein-clearing, banding, and stunting (Table 2).

Mechanical inoculation of selected test species outside the Convolvulaceae resulted in no infection as determined by symptomatology and indexing on *I. nil* seedlings. These species include AMARANTHACEAE; *Gomphrena globosa* L.; CHENOPODIACEAE, *Beta vulgaris* L., *Chenopodium amaranticolor* Coste & Acyn., *Chenopodium quinoa* Willd.; CRUCIFERAE; *Brassica juncea*, *Raphanus sativus*; CUCURBITACEAE; *Cucumis sativis* L. 'National Pickling', *Cucumis melo* L.; LEGUMINOSAE; *Phaseolus vulgaris* L. 'Bountiful', *Vigna unguiculata* (L.) Walp. 'Early Ramshorn'; SOLANACEAE; *Datura stramonium* L., *Lycopersicon esculentum* Mill., *Nicotiana tabacum* L. 'Burley 21', *Nicotiana glutinosa* L.

Purification.—Buffers that were found most favorable for mechanical transmission were compared in initial purification trials using a scheme proposed for several potyviruses (12). Treatments in each comparison were conducted concurrently using infected tissue from a common source. Although the absolute amount of

TABLE 2. Host range and symptoms in *Ipomoea* spp. susceptible to sweet potato feathery mottle virus

Ipomoea spp. and cultivars	Symptoms[a]
Ipomoea nil 'Scarlet O'Hara'	VC, C, CS, S, R
Ipomoea setosa	VC, M, VB, R
Ipomoea tricolor 'Heavenly Blue'	W, VC, VB, R
Ipomoea lacunosa	VC, VB, M, D, S
Ipomoea hederaceae	VC, VB, S, M
Ipomoea purpurea	Symptomless
Ipomoea wrightii	VB, D, M
Ipomoea batatas 'Georgia Red'	CS, VB
Ipomoea batatas 'Jersey'	M, VB

[a]Abbreviations: C, crinkling; Cs, chlorotic spots; D, distortion; M, chlorotic mottle; R, recovery; S, stunting; VC, vein-clearing; VB, veinbanding; W, wilting.

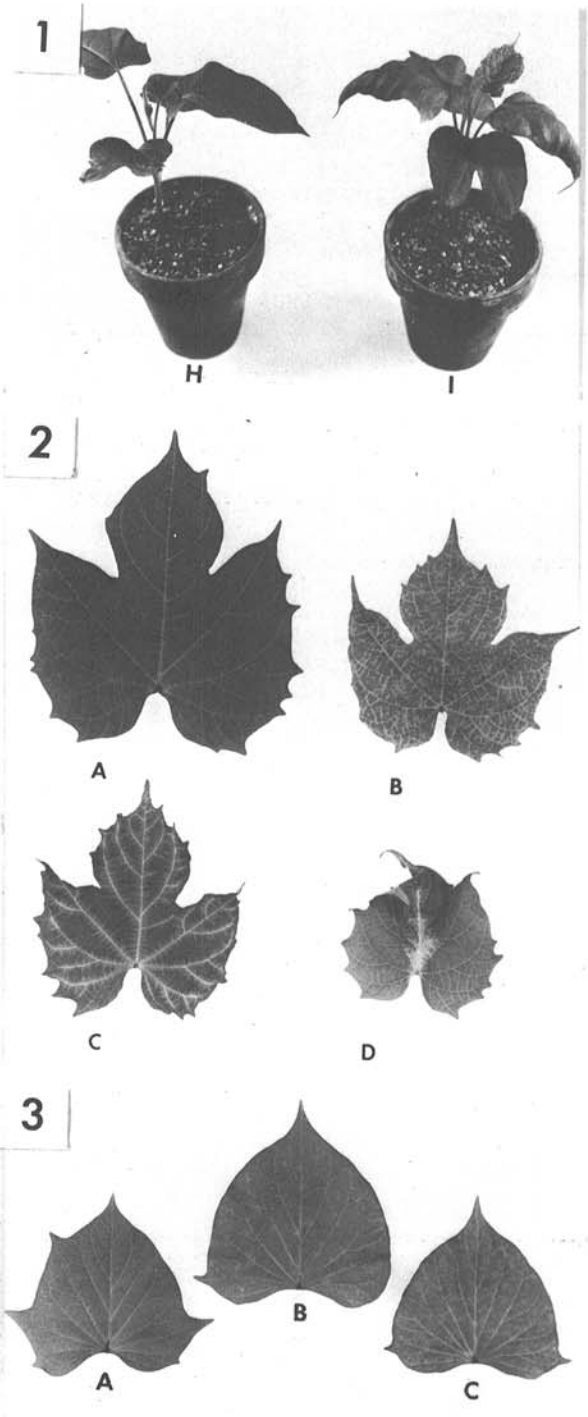

Fig. 1, 2-(A to D), and 3-(A to C). Symptom expression of sweet potato feathery mottle virus in three *Ipomoea* spp. **1)** A healthy (H) and infected (I) *I. nil* plant expressing initial symptoms following mechanical inoculation of cotyledons. **2)** Symptom progression in *I. setosa* following mechanical inoculation of cotyledons: **A)** Healthy *I. setosa* leaf; **B)** Vein-clearing and banding in initial symptomatic leaf; **C)** Chlorotic banding of primary and secondary veins in younger leaves; **D)** Distortion and veinbanding of midrib in youngest leaves. **3)** Healthy (**A**) and infected (**B** and **C**) leaves of *Ipomoea batatas* 'Georgia Red'. Symptoms include chlorotic spotting and vein mottling which appeared approximately 30 days after graft inoculation.

infectivity varied between repetitions of each comparison, the relationship between treatments was consistent (Table 3). The greatest infectivity of partially purified preparations was attained using 0.05 M sodium borate buffer, pH 8.0 (Table 3). The addition of the chelating agent DIECA (0.01 M) to the homogenizing buffer gave more infectious preparations in both trials than did the reducing agent thioglycollic acid (0.1%) (Table 3). Absorbance measurements (λ=260 nm) also were greater in the DIECA preparations.

Clarification by homogenization with a chloroform-carbon tetrachloride-buffer mixture (1:1:2) was compared with dropwise addition of butanol (8 ml/100 ml buffer) to the homogenate. In three trials, butanol resulted in preparations with greater infectivity than did the chloroform-carbon tetrachloride mixture. Absorbance of the butanol preparations also was greater; however, this may have been due in part to the greener color of the butanol preparations. The poor correlation between absorbance and relative infectivity suggested that considerable host material remained in partially purified preparations. This observation was supported by the wide ultraviolet (UV) absorbing region observed in preliminary sucrose density gradient columns (Fig. 4-A).

To determine if virus incubation time in PEG influenced yield and purity, 4% PEG and 1.75% NaCl were added simultaneously to aliquots of clarified sap. One aliquot was incubated for 1 hr and the precipitate was partially purified as before. The supernatant liquid was retained and incubated concurrently with the second aliquot for a total of 2 hr. Density gradient centrifugation of the three preparations demonstrated that 1 hr of incubation was insufficient for complete precipitation of the virus (Fig. 4-A, B). Furthermore, most of the remaining host material could be removed by preliminary centrifugation followed by continued incubation of the supernatant liquid (Fig. 4-C). Thirty minutes was sufficient time for precipitation of the host material. Remaining virus was associated with the UV absorbing region in fractions 7-12 (Fig. 5) as indicated by infectivity and electron microscopy.

The purification procedure for this virus is as follows: All steps are conducted at 4 C. Systemically infected *I. nil* tissue is homogenized in 0.5 M sodium borate buffer pH

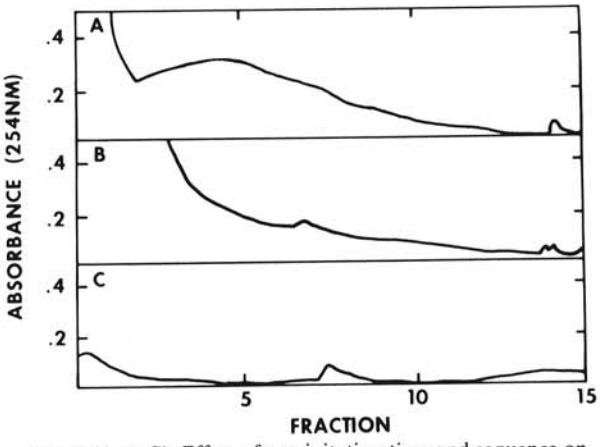

Fig. 4-(A to C). Effect of precipitation time and sequence on the sucrose density gradient profile of partially purified sweet potato feathery mottle virus. **A)** Two cycles of polyethylene glycol 6000 (PEG) precipitation, 1 hr each. **B)** Two cycles of PEG precipitation, 2 hr each. **C)** The supernatant from the first 1 hr PEG precipitation given an additional 1 hr incubation time followed by a second 2 hr PEG precipitation. Precipitate collected by centrifugation 10 min at 10,000 *g*.

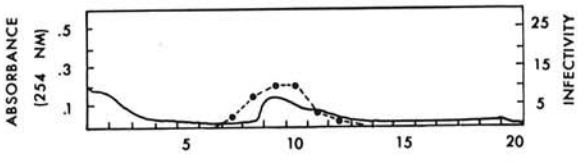

Fig. 5. Ultraviolet absorption profile(—) and infectivity assay (·- -·) of a sucrose density gradient column layered with partially purified virus following centrifugation (Beckman Type 25.1 rotor, 23,000 rpm for 3 hr).

TABLE 3. Effect of buffers, clarifying solvents, and stabilizing agents used during purification, on the infectivity of sweet potato feathery mottle virus

Comparison	Treatment	Relative infectivity[a]	Absorbance[b]
Buffer[c]	.05 M Phosphate pH 7.2	1	
	.05 M Borate pH 8.0	15	
	.05 M Borate pH 9.0	2	
Stabilizing agent[d]			
	0.1% Thioglycollic acid	18	0.56
	0.1 M DIECA[e]	27	4.4
Clarification[f]			
	$CHCl_3$ - CCl_4	17	5.2
	n-Butanol	25	10.8

[a]Infectivity can be compared within but not between groups. Treatments within each group were performed concurrently.
[b]Absorbance measured as A_{260} units per ml, corrected for light scattering.
[c]Thioglycollic acid and $CHCl_3$ - CCl_4 used as stabilizing agent and clarifying agent, respectively.
[d]0.05 M Borate buffer, pH 8.0 and $CHCl_3$ - CCl_4 used as clarifying agent.
[e]DIECA = sodium diethyldithiocarbamate.
[f]0.05 M Borate buffer, pH 8.0 and DIECA used as stabilizing agent.

8.0 containing 0.01 M DIECA (1:3, w/v). N-butanol is added dropwise to the homgenate (7.5 ml/100 ml buffer), stirred for 15 min and centrifuged (8,000 *g*, 10 min). PEG 6000 (4 g/100 ml) and NaCl (1.75 g/100 ml) are dissolved in the supernatant and allowed to stand for 30 min. The solution is centrifuged (8,000 *g*, 10 min) and the remaining supernatant is allowed to stand an additional 1.5 hr. The precipitate is collected (10,000 *g*, 10 min) and resuspended for 2 hr in 0.05 M, pH 8.0, borate buffer (1/5 original volume), by gentle agitation. Insoluble material is removed by centrifugation (8,000 *g*, 10 min) and the precipitation step is repeated, omitting the preliminary centrifugation. The precipitate is collected and resuspended as before. The resulting supernatant is given one cycle of differential centrifugation (Beckman Type 30 rotor, 28,000 rpm, 1.5 hr) followed by resuspension of the pellet in 0.05 M borate buffer pH 8.0 containing 0.005 M EDTA. Insoluble debris is removed by centrifugation (8,000 *g*, 10 min) and the virus is further purified by centrifugation through sucrose density gradient columns as previously described. This procedure gives yields of 8-10 mg virus/kg infected tissue.

Electron micrographs of negatively stained virus taken from density gradients revealed long flexuous rods similar to those present in leaf dip preparations (Fig. 6). Measurements of 218 virus particles from purified preparations indicated a normal length of 830-850 nm, however, many fragmented particles were present. The 260/280 absorbance ratio of the purified virus was 1.18.

Microprecipitin tests indicated an antiserum titer of 1:1,024 against 0.4 mg/ml virus; no precipitate was observed when the antiserum was tested against dilutions of a concentrated healthy plant extract. Precipitin bands were observed in double-diffusion tests at an antiserum dilution of 1:16. Again, no precipitate was observed when tested against healthy extracts.

DISCUSSION

Three sweet potato viruses can be distinguished on the basis of properties reported in the literature. Two viruses, sweet potato vein mosaic virus (SPVMV) and SPFMV are mechanically and nonpersistently aphid transmitted and have a host range limited to the convolvulaceae (3, 14). They can be separated on the basis of particle morphology (15); SPVMV has a normal length of 767 nm and SPFMV 844 nm. The third virus, sweet potato mild mottle virus (SPMMV) is also a long flexuous rod (800-900 nm), however, it is transmitted mechanically and by the whitefly *Bemesia tabaci* (Gennadius) and has a relatively extensive host range which includes many common virus indicators (10).

The virus reported on here is most like SPFMV as previously described (3, 11). It is transmitted mechanically with difficulty to *I. setosa* and more easily to *I. nil* (3); further, it is nonpersistently aphid transmitted as previously reported for other isolates (3, 11). Symptoms observed on *I. nil*, *I. setosa*, and *I. tricolor* also agree closely with those reported for SPFMV (3). Inoculations of Jersey cultivar plants further support identification as SPFMV as foliar symptoms were observed without external root necrosis, a characteristic of russet crack.

The dilution end point tests suggest the presence of a natural inhibitor(s) in the sap and that transmission can be increased by diluting the sap tenfold with buffer. Dilution also was found to be necessary for thermal inactivation point determination as the virus is relatively labile in nondiluted sap. The purification procedure reported here will be useful in obtaining virus for further characterization of its physical and chemical properties.

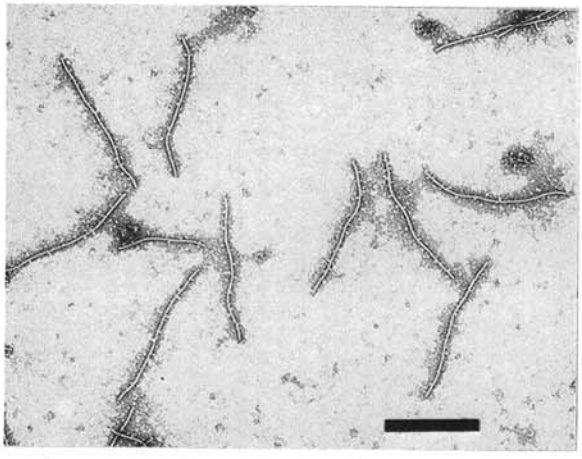

Fig. 6. Electron micrograph of negatively stained sweet potato feathery mottle virus particles obtained from the ultraviolet absorbing region of the sucrose gradient columns (bar equals 1.0 μm).

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