A POTYVIRUS ISOLATED FROM PASSION FRUIT (*PASSIFLORA EDULIS F. FLAVICARPA*)[1]

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ABSTRACT

Passion fruit ringspot virus (PRV) isolated from *Passiflora edulis f. flavicarpa* in the wet zone of Sri Lanka was acquired and inoculated in 5 min feeding time by aphids. It is transmitted by mechanical inoculation of sap to host species of Passifloraceae, Leguminosae, Chenopodiaceae. Solanaceae and Amaranthaceae. Sap from *Passiflora foetida* in which species the virus was propagated was infective after dilution to 10^{-3} (but not to 10^{-4}) at 50°C (but not at 60°C) and after 4 days (but not 5 days) at 20°C. Yields of 0.2—0.5 mg of virus per 100 g of leaf tissue were consistently obtained from *P. foetida*. Virus preparations had UV absorption spectra typical of nucleoprotein containing about 6% nucleic acid and numerous non-aggregated particles about 772×13 nm in size. PRV is strongly immunogenic and antisera with titres of 1:8192 have been obtained. It contained single species of double-stranded ribonucleic acid (dsRNA) with a molecular weight of 6.9×10^6 daltons and two protein species with molecular weights of 33,681 and 25,379 daltons. PRV (Sri Lanka) is serologically related to passion fruit woodiness virus (Australia), PRV (Ivory Coast) and potato virus Y and is therefore a member of the potyvirus group.

KEY WORDS : Passion fruit, Potyvirus

INTRODUCTION

Passion fruit (*Passiflora edulis f. flavicarpa*) is a tropical fruit crop confronted with many virus diseases. Several virus-like diseases have been reported in Sri Lanka by Senanayake (1972), Seneviratne and Wickremasingha (1972; 1973; 1974) and Dassanayake (1989). However,

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none of these descriptions indicate a virus with chlorotic ringspot, spotting and mottling. Nevertheless, similar symptoms in *Passiflora* have been reported in passion fruit woodiness virus from Australia (Taylor and Kimble, 1964) and passion fruit ringspot virus (PRV) from Ivory Coast (De Wijs, 1974). The most characteristic feature of the 'new' virus was 3.5 mm sized chlorotic ringspots on intermediate and older leaves especially where these were shaded (Plate 1). The prominent ringspot symptom was persistent throughout the year, causing a reduction in both growth and yield. Some infected fruits showed green and yellow mottling on the pericarp and occasionally green rings were also present on the skin. These symptoms had been found in several other passion fruit cultivations in Kalutara, Galle and Colombo districts (Dassanayake, 1989). In this paper we describe some of the properties of the virus isolated from passion fruit which we have designated as PRV of Sri Lanka.

MATERIALS AND METHODS

Source material and virus isolation

Virus infected passion fruit (*Passiflora edulis f. flavicarpa*) leaf sample was collected from a field at Dodangoda in the Kalutara district and grown in an insect protected glasshouse at University of Bath, England with a temperature range of 15—20 °C in winter and 15—35°C in summer. Test plants were grown in a separate glasshouse and were shaded for 24—48 h before inoculation. Inocula were prepared by grinding infected leaves of *P. edulis f. flavicarpa* in 0.02 M tris-HCI buffer (pH 7.8) containing 1g/l Na_2SO_3. Celite was used as an abrasive powder. Inoculum was applied with a finger over the whole surface of the test leaf. When several inocula were involved, different fingers were used for each inoculum. Inoculated plants were rinsed briefly with tap water to remove inhibitory deposits (Yarwood and Fulton, 1967) and were covered with damp newspaper for 16—18 h to reduce mechanical damage. All the *Passiflora* species were inoculated at 4—6 leaf stage, and other test plants as described by Noordam (1973). Plants not showing symptoms after 3—4 weeks were back inoculated to *Chenopodium amaranticolor*. The virus isolate was transferred

through 3 single lesion serial passages in *C. amaranticolor* and pure culture was maintained in *Passiflora foetida* in aphid-proof glasshouse for subsequent virus identification.

Different transmission methods

Aphid transmission

These studies were based on Noordam (1973) for non-persistent viruses and were carried out in the insectory at University of Bath, England and Central Agricultural Research Institute, at Gannoruwa. Aphids were given 2 h starvation period prior to acquisition feeds of 5 and 30 min on infected detached passion fruit leaves. Fifteen to twenty aphids were then transferred to healthy passion fruit seedlings (5—10 plants) and allowed inoculation access periods of 5 and 30 min.

Graft transmission

This study was made by wedge grafting shoots of virus infected passion fruit to *P. edulis f. flavicarpa* and *P. quadrangularis*. Infection was confirmed by back inoculation to *C. amaranticolor*.

Pollen transmission

This was tested by grinding pollen obtained from infected plants with one or two drops of 0.02 M tris-HCl buffer (pH 7.8) containing 1g/l Na_2SO_3. Infection was confirmed by back inoculation to *C. amaranticolor*.

Seed transmission

Seed transmission was studied using seed from virus-infected *P. edulis f. flavicarpa* and *Cassia occidentalis*. This was done as follows: (a) Twenty-five seeds were soaked in water overnight and inoculum was prepared by homogenizing seed in 0.02 M tris-HCl buffer (pH 7.8) containing 1g/l Na_2SO_3 (1 : 10 W/V) which was assayed on *C. amaranticolor*; (b) Fifty seeds from a fruit in a virus-infected plant selected at random, were grown in an aphid-proof nursery and seedling leaves were used to prepare inoculum as described under (a) and assayed on *C. amaranticolor*.

Properties *in vitro*

All these tests were carried out following a standard procedure (Noordam, 1973). Infective sap was prepared from leaves of systemically-infected *P. foetida* by grinding in distilled water at a ratio of 0.5:1 W/V. These tests were repeated at least three times.

Virus purification

Virus was purified from systemically-infected *P. foetida* according to Hammond and Lawson (1988). This method involved clarification by Triton X—100, concentration by polyethylene glycol and isopycnic centrifugation in caesium sulphate. The specific virus zone was removed with a hypodermic syringe. The UV absorption spectrum of purified virus was determined in a Shimadzu UV visible recording spectrophotometer.

Electron microscopy

Both purified and crude sap virus preparations were examined in JEOL 1200 EX 11 electron microscope after staining with 20 g/l phosphotungstic acid (pH 6.5). The size of particles was estimated by comparison with negatively stained polystyrene latex spheres of 234 nm diameter (BioRed Ltd., U. S. A).

Serology

Antiserum was prepared in rabbits using a schedule of one intravenous injection (0.5 mg virus in 0.85% saline) followed by 3 intramuscular injections (0.3—0.5 mg virus emulsified with equal volume of Freunds incomplete adjuvant–Difco Ltd., U. K.) given at weekly intervals. Serological investigations were made by microprecipitin tests (Van Slogteren, 1955) and indirect and direct enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977; Mowat and Dawson, 1987). Investigations were done to study the cross-reaction to known potyviruses in indirect ELISA. Purified antigens were used at a concentration of 1 μg/ml. Antisera dilutions of 1:1000, 1:1500 and 1: 10,000 were used for these tests.

Double-strandard ribonucleic acid (dsRNA)

The dsRNA molecular weight was estimated as described by Morris and Dodds (1979). In these tests, electrophoresis was carried out on cylindrical 5% v/v polyacrylamide gels (7.5 cm×6 mm) in electrophoresis buffer for 16 h at 2 mA/tube or 8 h at 6 mA/tube. The lambda-DNA cut with HIND 111 (Boeringer Cooperation Ltd., Germany) restriction enzyme was used as a molecular weight marker. These tests were repeated four times.

Capsid protein molecular weight

Estimation of capsid protein molecular weight (mw) was done according to Webber and Osborn (1969) using 100 g/l polyacrylamide gels containing 10 g/l sodium dodecyl sulphate (SDS). Ribonuclease (mw 13,700 daltons), Chymotrypsinogen (mw 25,000 daltons), Ovalbumin (mw 43,000 daltons) and Bovine serum albumin (mw 66,000 daltons) were used as marker proteins. Molecular weight estimations were repeated at least three times using different preparations of purified virus.

Return inoculation

Return inoculation was done by inoculating single lesion cultures to original host (*P. edulis f. flavicarpa*) to confirm the pathogenecity test.

RESULTS

Host range and symptom expression

Symptoms produced in indicator plants are shown in Table 1. Symptomatology of selected host plants are described below:

Cassia occidentalis

The first symptom of infection was vein-clearing in newly developed leaves after 2—3 weeks of inoculation. These leaves showed slight curling. With the spread of infection chlorotic rings and mottling were produced in mature leaves 4-6 weeks after inoculation.

Passiflora foetida

Young systemically-infected and some inoculated leaves developed vein yellowing 7-10 days after inoculation. Secondary symptoms of chlorotic spots and mosaic appeared in mature fully-expanded leaves. This species was highly susceptible to infection.

Passiflora quadrangularis

Test plants produced chlorotic lesions 1-2 mm in diameter on inoculated leaves 2-3 weeks after inoculation. These spots, later turned necrotic. Systemically-infected young leaves showed vein-yellowing, mottling, mosaic and slight leaf deformation.

Chenopodium amaranticolor

The first symptom of infection was the appearance of small circular yellowish non-necrotic spots measuring about 1—1.5 mm in diameter on inoculated leaves, 10—15 days after inoculation. Three weeks later, these lesions enlarged to about 3 mm in diameter and the yellowish spots became reddish-pink.

Aphid transmission

PRV was transmitted by *Aphis gossypii, A. spiraecola* and *A. craccivora* after acquisition and test feeding periods of 5 to 30 min. Inoculation feeds of 5 or 30 min were sufficient to produce 50—75% levels of infection in the test plants.

Graft transmission

PRV was transmitted through wedge-grafting when diseased *P. edulis f. flavicarpa* was wedge-grafted on *P. edulis f. flavicarpa or P. quadrangularis*. Infection was confirmed by back testing to *C. amaranticolor*.

Seed and pollen transmission

Seed and pollen transmission was not observed in inoculated plants which was confirmed by back inoculation.

Properties *in vitro*

Expressed sap from *P. foetida* was infective after 10 min at 50°C (but not at 60°C), after dilution to 10^{-3} (but not to 10^{-4}) and after 4 days (but not 5 days) at 20°C.

Purification

After caesium sulphate density gradient centrifugation, one strong light scattering band 4.5 cm below the meniscus was observed. Virus concentration of 0.2—0.5 mg/100 g leaves was routinely obtained from *P. foetida*. In these tests extinction coefficient of 2.5 was used to calculate the virus yield.

UV absorption

The absorption spectrum of purified virus with a maximum at 260 nm, a minimum at 246 nm and a slight shoulder at 290 nm (Fig. 1) was typical of a virus with a low nucleic acid content. The 260 : 280 absorption ratio, uncorrected for light scattering varied between 1.07—1.11, which indicated a nucleic acid content of about 6% (Layne, 1957).

Electron microscopy

Crude sap of *P. edulis f. flavicarpa* and *P. foetida* purified virus preparations (Plate 2), showed many slightly flexous filamentous virus particles. The particle length of the virus under study was determined using leaf squash preparations of *P. foetida*. More than 140 particles were measured using electron micrograph negatives. A mean maximum of 700- 850 nm which contained over 82% of the particles was used to calculate the normal length (Fig. 2). Fifty particles were measured to calculate their width. The normal particle was 772 nm ± 3.8 long and 13 nm ± 0.17 wide.

Serology

Homologous titre of 1 : 8192 was obtained in microprecipitin test. In cross-reaction studies PRV (Sri Lanka) is closely related to passion fruit mottle virus, PFMV (SDI 0.78) and *P. caerulea* yellow fleck virus, PCYFV (SDI 1.45) according to these reciprocal serological differentiation values (Dassanayake, 1989). PRV (Sri Lanka) was tested against antisera to PRV (Ivory Coast), Passionfruit woodiness virus, PWV (Australia) and Potato virus Y, PVY (England). Positive reactions were obtained in indirect ELISA for the antisera tested.

Double-stranded RNA analysis

The detectable amount of dsRNA was very low and barely visible even when samples from 20—30 g of *P. foetida* were loaded onto one tube gel. A single high molecular weight species of dsRNA was found in each of the two experiments. This had an approximate molecular weight of 6.9×10^6 daltons.

Capsid protein molecular weight

Most preparations showed high and low molecular weight components with mobilities indicating molecular weights of 33,681 ($\pm$518) and 25,379 ($\pm$757) daltons.

Return inoculation of virus to *P. edulis f. flavicarpa*

Mechanical inoculation of *P. edulis f. flavicarpa* with the single lesion isolate of PRV (Sri Lanka), which was uncontaminated with other viruses produced the typical chlorotic ringspot symptoms as observed originally in the field. However, these symptoms were present only for about 4—6 weeks and disappeared during summer. This may be due to high temperature which would have suppressed this particular symptom. This is similar to what was observed in the field where chlorotic ringspots were more prominent on shaded leaves. Other symptoms produced were mottling and chlorotic spotting.

DISCUSSION

PRV (Sri Lanka) had moderate to narrow host range and was similar to potyviruses infecting *C. amaranticolor, C. quinoa, C. murale, Gomphrena globosa, Nicotiana clevelandii, Petunia hybrida, Phaseolus vulgaris* cv. Prince (Hollings *et al.*, 1980). *Aphis spiraecola, A. craccivora* and *A. gossipii* transmitted the virus in a non-persistant manner. Hollings and Brunt (1981) reported that many viruses were transmitted by aphids in a non-persistant manner.

PRV (Sri Lanka) could be considered as a strain or a pathotype of PFMV and PCYFV (Dassanayake, 1989) on the basis of its symptom expression, physical properties, particle morphology, aphid transmission, dsRNA molecular weight, capsid protein molecular weight and serological properties. PRV (Sri Lanka) appeared to produce similar symptoms

to the virus causing ringspot (Ivory Coast) in *P. edulis f. flavicarpa* but differred in infecting *N. clevelandii* and not infecting *P. suberosa* which was susceptible to PRV (Ivory Coast). Physical properties showed that the virus was moderately stable. The particle length range was consistent with the potyvirus group classification (Matthews, 1979). Protein preparation of the virus migrated as two bands in sodium dodecyl sulphate gel electrophoresis which are in the range of potyvirus classification (Hiebert and McDonald, 1973; Hill *et al.*, 1973). A major dsRNA in a similar size range (6.9×10^6 daltons) was reported for the potyviruses such as bean mosaic virus, potato Y virus, tobacco etch and turnip mosaic virus (Valverde *et al.*, 1986). In cross-reaction studies, PRV (Sri Lanka) reacted with antisera to definitive potyviruses.

It is well known that the extent of differences in disease symptoms, host range and physical properties are now, not considered to be of any great diagnostic value to study the relationship between viruses (Walkey, 1985; Hamilton *et al*,, 1981). Moreover in this study, the degree of serological relationship to PWV (Australia) and PRV (Ivory Coast) could not be determined, since homologous antigens were not available for comparison. Therefore, a more critical assessment of the serological relationship of PRV (Sri Lanka), to PRV (Ivory Coast) and PWV (Australia) will require the production of several antisera and a range of monoclonal antibodies to each virus.

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Table 1. Symptoms produced on herbaceous and woody hosts by passion fruit ringspot virus

Plant species	*Local reaction*	*Systemtc reaction*
Amaranthaceae		
Gomphrena globosa (Globe amaranth)	NS	—
Chenopodiaceae		
Chenopodium album	CS	--
C. amaranticolor	CS	—
C. foetidum	CS	—
C. quinoa	CS	—
Leguminosae		
Cassia occidentalis	CMO	VV, LC, RS, CMO
C. tora	NS	—
Crotalaria usuramonensis	CM	CM
Phaseolus vulgaris (cv. Top Crop)	NL	--
P. vulgaris (cv. The Prince)	NS	—
Vigna unquiculata (cv. Bushita mae)	—	—
Passifloraceae		
Passiflora edulis f. flavicarpa	CM, CMO	VY, CMO, CS, (RS)
P. foetida	CMO, CM, VY	VY, CS, CMO
P. mollissima	CMO	CS, CMO
P. quadrangularis	CL, CMO	VY, CMO, LD
Solanaceae		
Nicotiana clevelandii	SL	—
Petunia hybrida	SL	—

CM — Chlorotic Mosaic
CMO — Chlorotic Mottle
CS — Chlorotic Spots
NS — Necrotic Spots
LC — Leaf Curl
— — No Infection
RS — Ringspot
VY — Vein Yellowing
LD — Leaf Distortion
SL — Symptomless Local
( ) — Occasional Symptoms

The following species were not infected : *Arachis hypogaea*, *Centrosema pubescens*, *Cucumis sativus* cv. (Marketer, *Datura stramonium*, cv.) Arboetea, *Glycine max*, cv. Local, *Nicotiana megalosiphon*, *N. tabacum*, cvs. White Burley and Xanthi, *N. rustica*, *Passiflora suberosa*, *Pisum sativum* cv. Metor, *Pueraria phaseoloides*, *Vigna unquiculata* cv. Local.

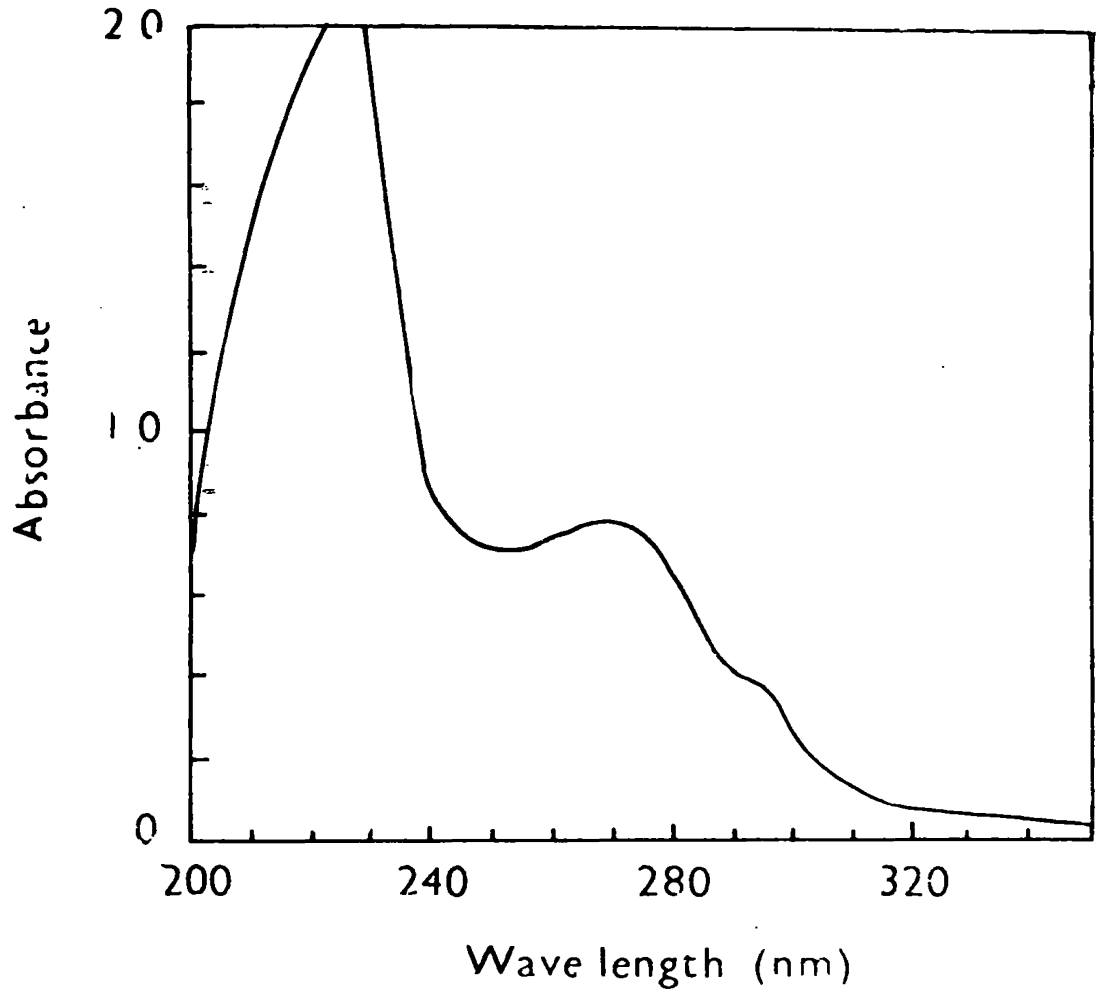

Fig. 1. UV absorption spectrum of purified preparations of passion fruit ringspot virus

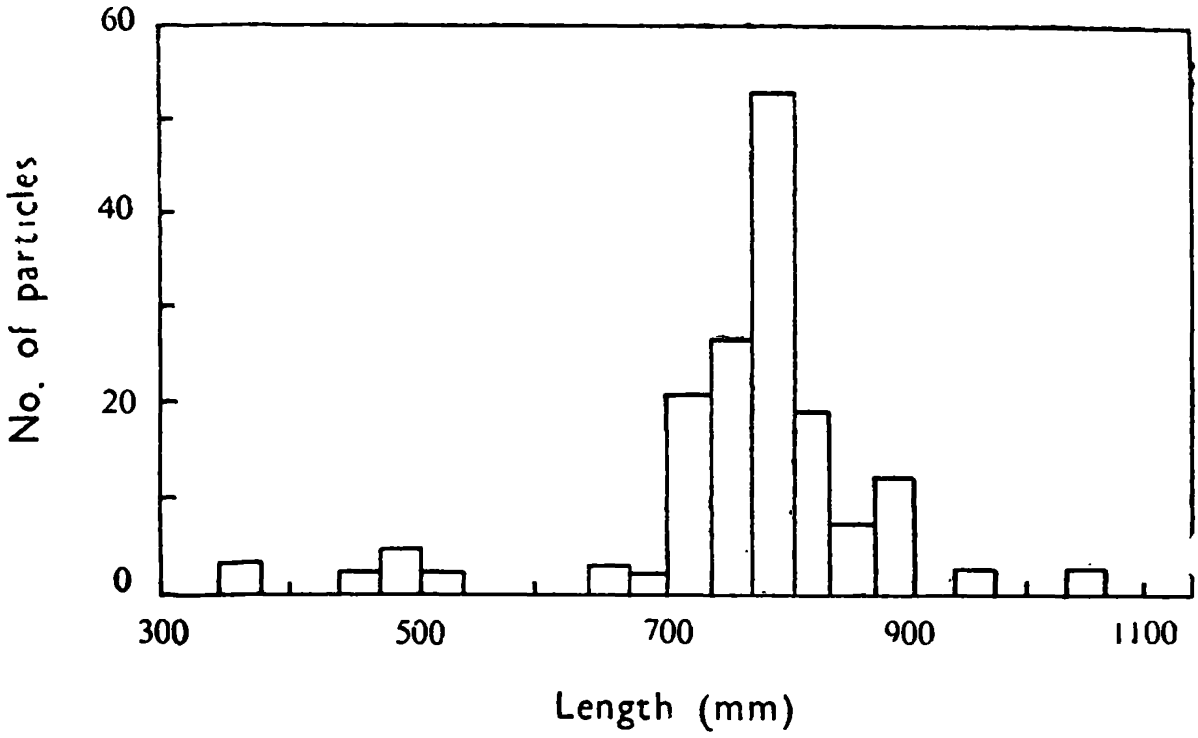

Fig. 2. Histogram of length of virus particles in a leaf squash homogenate (Class interval 1:33.3 nm)

Plate 1 (a). Mottling and chlorotic ringspotting on leaves of *Passiflora edulis f. flavicarpa*

Plate 1 (b). Close up of ringspot symptom

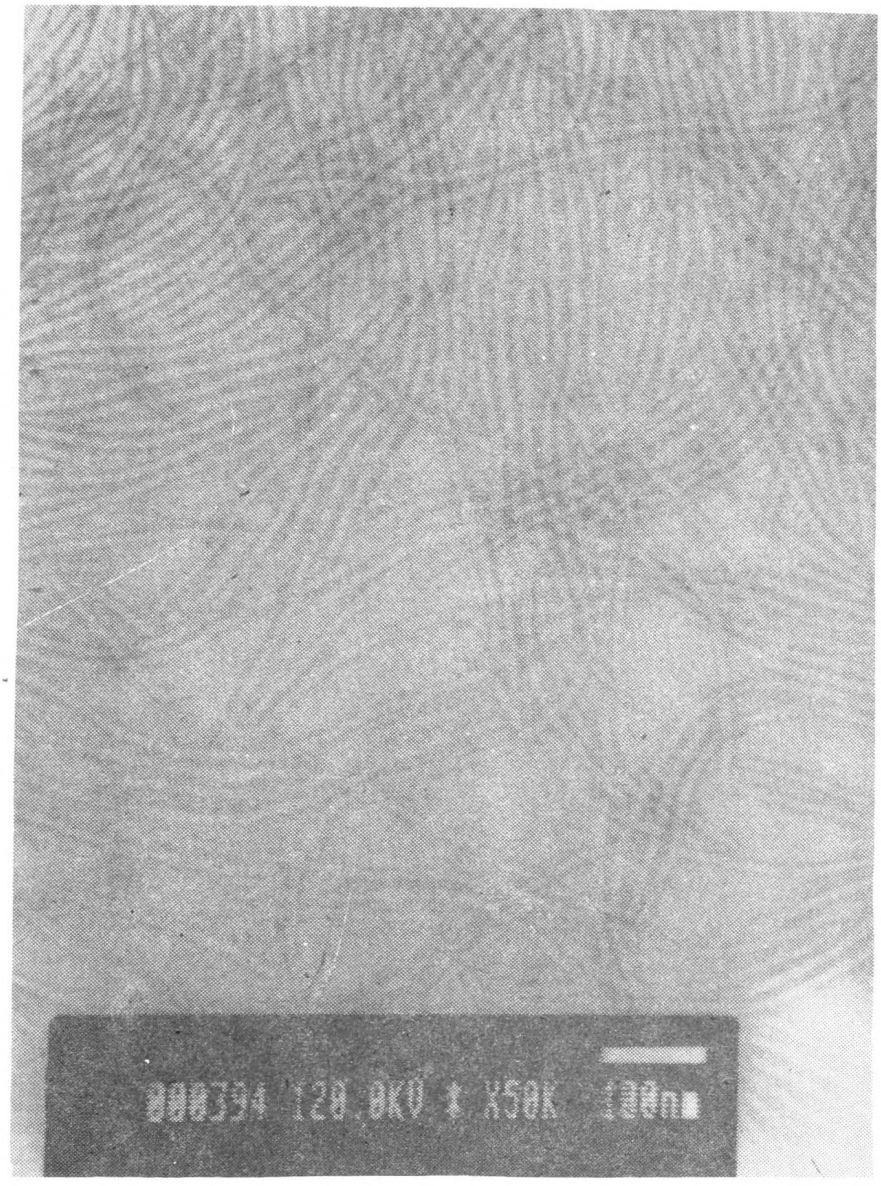

Plate 2. **Electron micrograph of purified virus from *Passiflora foetida* after caesium sulphate density gradient centrifugation, stained in 20 g/1 PTA, pH 6.5. Bar represents 100 nm**