COWPEA MILD MOTTLE VIRUS OCCURRING ON SOYBEAN AND PEANUT IN SOUTHEAST ASIAN COUNTRIES[1]

Mitsuro IWAKI[2], Pornpod THONGMEEARKOM[3], Yohachiro HONDA[4], Mitree PROMMIN[5], Nualchan DEEMA[5], Tadaaki HIBI[6], Norio IIZUKA[7], Ching Ang ONG[8], and Nasir SALEH[9]

Abstract

A rod-shaped virus was isolated from naturally infected soybean (*Glycine max* (L.) Merr.) plants collected at Phitsanulok, northern Thailand, in 1979. The virus caused mild mottle symptoms in soybean and was transmitted by whiteflies (*Bemisia tabaci* Genn.), by sap inoculation, and through soybean seed, but not by the aphids, *Aphis craccivora* Koch and *A. glycines* Matsumura. Virus particles consisted of flexuous rods 10–15 × 650–700 nm in size. In tube precipitin tests, the virus reacted with antiserum to cowpea mild mottle virus (CMMV). Infectivity of the virus was retained in sap diluted to 10^{-5} but not 10^{-6}, heated for 10 min at 70°C but not 75°C, or stored at 20°C for 21 days but not 28 days. The virus contained single stranded RNA and was identified as CMMV on the basis of host range, symptomatology, particle morphology, and serological relationships.

1. Introduction

During surveys of virus diseases of soybean (*Glycine max* (L.) Merr.) in the major growing areas in Thailand in 1978–1981, we collected many plants with virus-like diseases. Among these plants, a virus was isolated mechanically from soybean plants showing crinkle leaf, rugose mosaic, mosaic, yellow mottle symptoms, etc.

The virus was flexuous and rod-shaped and whitefly-borne. The same virus was isolated from soybean and peanut in Malaysia and Indonesia.

This paper describes some properties and whitefly transmission of the virus.

2. Materials and methods

1) Virus The virus used in this experiment was isolated from naturally infected soybean plants collected at Phitsanulok, Northern Thailand, in 1979.

Sap inoculation was performed by rubbing the Carborundum (600 mesh)-dusted leaf

---

1) Part of the results on some properties and whitefly transmission of CMMV on soybean in Thailand was reproduced from Plant Disease 66 : 365-368, 1982 with the permission of the American Phytopathological Society (September 13, 1983).

2) National Institute of Agro-Environmental Sciences, Kannondai, Yatabe, Tsukuba, Ibaraki, 305 Japan.
3) Department of Agriculture, Banghen, Bangkok 10900, Thailand, present address : Thai Monsanto, Bangkok, Thailand.
4) National Agriculture Research Center, Kannondai, Yatabe, Tsukuba, Ibaraki, 305 Japan,
5) Department of Agriculture, Bangkhen, Bangkok 10900, Thailand.
6) National Institute of Agrobiological Resources, Kannondai, Yatabe, Tsukuba, Ibaraki, 305 Japan.
7) Hokkaido National Agricultural Experiment Station, Toyohira, Sapporo, Japan.
8) Malaysian Agriculture Research and Development Institute, Selangor, Serdang, Malaysia.
9) Bogor Research Institute of Food Crops, Bogor, Indonesia.

surface of test plants grown in glasshouse with the juice of leaves macerated with 0.05 M phosphate buffer, pH 7.0, containing 0.01 M sodium diethyldithiocarbamate and 1 mM L-cysteine.

All the test plants were grown in a glasshouse.

2) Host range Plants representing 26 plant species in nine families were mechanically inoculated with sap from soybean plants infected via whiteflies. Symptomless plants were assayed by back inoculation to Kintoki bean, using sap extracted from inoculated leaves 7–10 days after inoculation and from newly emerged leaves about 21 days after inoculation. All the plant species were tested at least twice in different seasons.

3) Stability in crude sap Dilution end point (DEP), thermal inactivation point (TIP), and longevity in vitro (LIV) for the virus were determined using Kintoki bean as the assay host. Crude sap was prepared by macerating 1 g of infected soybean leaves with 10 ml of 0.05 M potassium and sodium phosphate buffer, pH 7.0, and squeezing through cheesecloth. This crude sap was used for TIP and LIV, and also as 10^{-1} of DEP.

4) Purification and serology Frozen infected leaves of Shirotsurunoko soybean or Kintoki bean were homogenized with 3 volumes of 0.2 M K-phosphate buffer, pH 8.0, containing 0.1% 2-mercaptoethanol (2-ME) and 0.01 M sodium ethylenediaminetetraacetic acid (EDTA). Sap was expressed through cheesecloth and mixed with 1/2 volume of a chloroform : n-butanol mixture (1 : 1 ; v/v), and the emulsion was broken by high speed centrifugation (9,000 g, 15 min). The aqueous phase was recovered, mixed with 0.5% Triton X-100, and centrifuged at 100,000 g for 70 min. Pellets were resuspended in 0.2 M K-phosphate buffer, pH 8.0, containing 0.1% 2-ME and 2 mM Na-EDTA, and centrifuged at 9,000 g for 10 min. The supernatant was subjected to one cycle of differential centrifugation as above, and the resuspended pellets were layered on 10–40% linear sucrose density gradients. Gradients were centrifuged for 120 min at 60,000 g using a Hitachi RPS-25 swinging-bucket rotor. The opaque, virus-containing zone was removed with a syringe and concentrated by centrifugation at 100,000 g for 70 min (Fig. 1).

A rabbit was immunized by two intravenous injections of the virus and two intramuscular injections of the virus mixed with Freund's complete adjuvant. Antiserum obtained 10 days after the final injection reacted with purified virus in tube precipitin tests at a titer of 1/2048. The serological relationship of the virus with cowpea mild mottle virus was determined in tube precipitin tests.

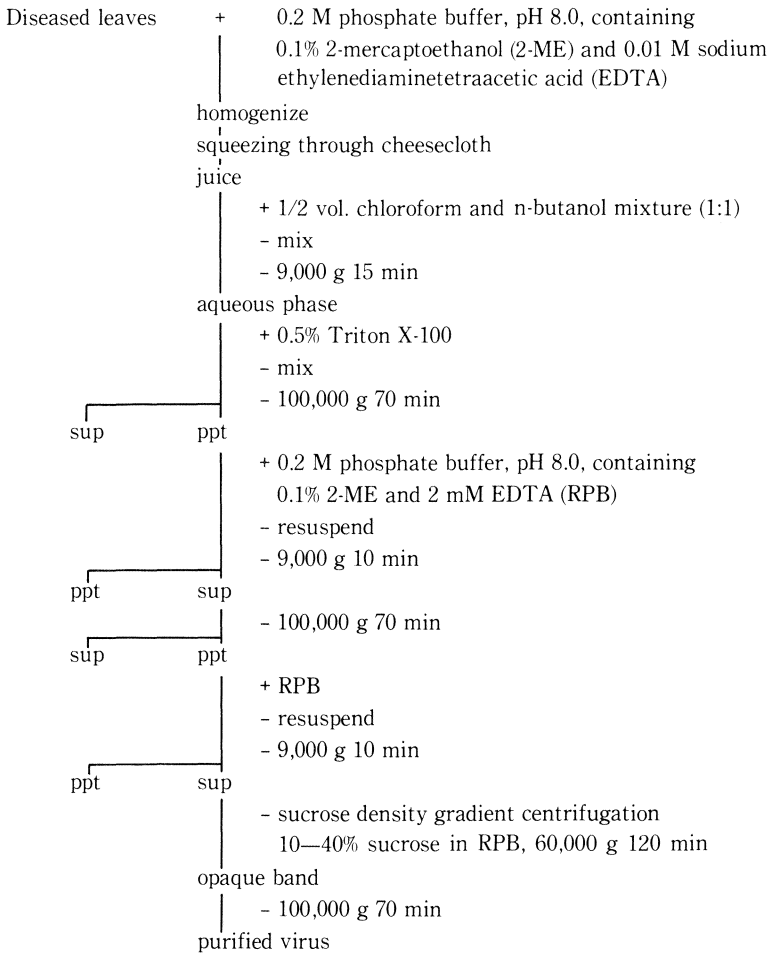

Fig. 1. Purification procedure of cowpea mild mottle virus.

5) Transmission and virus-vector relationships Virus-free aphids, *Aphis craccivora* Koch and *A. glycines* Matsumura, were reared on healthy broad bean and soybean, respectively. Virus-free whiteflies, *Bemisia tabaci* Genn., were reared on healthy hibiscus (*Hibiscus* sp.) or tobacco (*Nicotiana tabacum* L.). Whiteflies were retained in small plastic cages.

Aphid transmission tests were conducted in two types of transmission, namely, in non-persistent and persistent ones. In the non-persistent type transmission, aphids were allowed to fast for 1 hr in a glass beaker before an acquisition access period of 15 min on the diseased plants. After the acquisition access, 10 aphids were transferred to each healthy soybean plant for a first inoculation access period of 1 or 48 hr, then again these aphids were transferred to soybean plants for a second inoculation access period of 24 or 48 hr. In the tests for the persistent type of transmission, aphids were allowed an acquisition access of 24 hr on diseased plants, then 10 or 15 aphids were

transferred to each healthy plant for an inoculation access period of 24 hr. These aphids were removed by spraying with insecticides after the inoculation access period was completed.

Transmission efficiency using single adult whiteflies was determined by first allowing an acquisition access period of 1 day on infected soybean plants. After the acquisition, each whitefly was transferred to a healthy soybean plant at the primary leaf stage and allowed an inoculation access period of 1 day.

For tests on minimum acquisition access period, groups of adult whiteflies were allowed an acquisition access period of 1/6, 1/2, 1, 3, or 24 hr on infected soybean plants. After the acquisition access, 10 or 40 whiteflies were allowed an inoculation access of 1 day on each test plant. To determine the minimum period for inoculation access period, groups of whiteflies were allowed an acquisition access of 1 day on infected soybean plants. Immediately after the acquisition, 10 whiteflies were allowed an inoculation access period of 1/6, 1/2, 1, 3, 6, or 24 hr on each healthy soybean plant.

Virus retention was determined by first allowing whiteflies an acquisition access period of 1 day on infected soybean plants, then 10 whiteflies were transferred daily until 18 days to each healthy soybean test plant. In another test, each group of 10 whiteflies which had been given an acquisition access on infected soybean for 1 day was allowed an inoculation access period of 1/6, 1/2, 1, 3, 6, 12, or 24 hr on a healthy soybean test plant. Immediately after each inoculation access period, these whiteflies were transferred to additional healthy test plants for an inoculation access of 1 day. The number of whiteflies per plant decreased with the increase in the transmission time since some insects died during the transfers.

For the tests on the latent period in whitefly, groups of 15 whiteflies were allowed an acquisition access period of 5 or 10 min on infected soybean plants. Immediately after the acquisition access, each of the 15 whiteflies was allowed an inoculation access of 1/12, 1/6, 1/2, 1, 3, 6, or 24 hr on healthy soybean plants.

Whitefly transmissibility of purified virus was determined by the membrane feeding method (10) using parafilm. Sucrose (20% w/v) was added to the purified virus preparation and groups of 10 whiteflies were allowed 1 or 2 days for acquisition and inoculation access.

Test plants in whitefly transmission tests were observed for symptom appearance for about 2 wk after inoculation, and then checked for the presence of particles by the leaf dip technique.

6) Electron microscopy Dip preparations for electron microscopy were prepared by dipping a piece of infected soybean leaf into a small drop of 2% potassium phosphotungstate, pH 6.5, on a carbon-stabilized, Formvar-coated grid.

For ultrathin sectioning, small pieces of diseases soybean leaves were fixed with 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0, for 1 hr and then post-fixed with 1% osmium tetroxide in the same buffer for 2 hr. After dehydration in an acetone series, these samples were embedded in Epoxy resin. Ultrathin sections were cut with glass knives mounted on a Porter-Blum MT-2B ultramicrotome. Sections were stained with uranyl acetate and lead citrate.

These samples were examined under a Hitachi Model H-300 or H-500 electron microscope operating at 75 Kv.

7) Partial characterization of nucleic acid. Nucleic acid was extracted from partially purified virus by the SDS-phenol method (11). Isopycnic ultra centrifugation

was performed according to the method of Szybalski (13) by mixing the nucleic acid (final concentration ; A_{260} 0.1/ml) with Cs_2SO_4 (final density ; 1.618 g/cm^3 or 1.640 g/cm^3) in 0.01 M Tris-HCl buffer (pH 8.0) and centrifuging for 42 hr at 38,000 g at 25°C with MSE Centriscan 75 analytical ultracentrifuge using ultraviolet optics.

Reaction of the nucleic acid with formaldehyde was analyzed as described by Miura et al. (9).

3. Results

1) Host range and symptoms

The virus infected 14 plant species in 5 families among the 26 species in 9 families inoculated (Table 1).

The virus infected systemically with visible symptoms peanut, *Arachis hypogaea,* soybean, *Glycine max,* bean, *Phaseolus vulgaris.*

Infected peanut plants showed vein clearing and mild mottle symptoms which later

Table 1. Host range of cowpea mild mottle virus

Plant	Symptoms	
	Inoculated leaves	Non-inoculated leaves
Chenopodium amaranticolor	L	—
C. quinoa	—	—
Spinacia oleracea	—	—
Gomphrena globosa	1	—
Tetragonia expansa	—	—
Brassica rapa	—	—
Arachis hypogaea	1	VC, M
Glycine max 'Shirotsurunoko'	1	M, Mo
〃 'Okuharawase'	1	s
〃 'SJ4'	1	M, Mo
〃 'Toyosuzu'	1	M, VN
Phaseolus vulgaris 'Tsurunashi Kintoki'	1	LC, Mo
〃 'Top Crop'	1	s
〃 'Yamashiro Kurosando'	1	s
P. angularis	1	s
Pisum sativum	1	s
Trifolium pratense	—	—
T. repens	—	—
Vicia faba	1	—
Vigna mungo	1	s
V. radiata	1	s
V. sesquipedalis	1	s
V. unguiculata	1	s
Datura stramonium	—	—
Nicotiana clevelandii	1	s
N. glutinosa	—	—
N. tabacum	—	—
Petunia hybrida	—	—
Sesamum indicum	—	—
Cucumis sativus	1	—
Zinnia elegans	—	—

L: local lesion, VC: vein clearing, M: mosaic, Mo: mottle, LC: leaf curling, VN: vein necrosis, 1 and s: symptomless infection on inoculated and non-inoculated leaves, respectively, —: no infection.

became less distinct.

Symptoms on infected soybean plants varied with the cultivar. Cultivars, Shirotsurunoko (Fig. 2), Okuharawase, and SJ 4 showed slight vein-clearing and leaf malformation, either downward curling or upward cupping. Cultivar, Toyosuzu

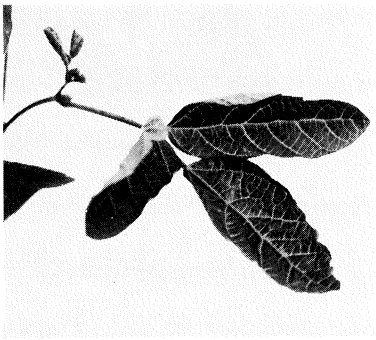

Fig. 2. Vein-clearing and leaf-rolling symptoms on Shirotsurunoko soybean infected with cowpea mild mottle virus.

showed distinct mosaic, vein necrosis and top necrosis symptoms (Fig. 3).

Kintoki bean plants showed leaf malformation, mild mottle, and stunting (Fig. 4).

Fig. 3. Mosaic and vein-necrosis symptoms on Toyosuzu soybean infected with cowpea mild mottle virus.

Fig. 4. Mosaic and stunt symptoms on Kintoki bean infected with cowpea mild mottle virus.

Phaseolus vulgaris cvs. Top Crop and Yamashiro Kurosando, *P. angularis, Pisum sativum, Vigna mungo, V. radiata, V. sesquipedalis, V. unguiculata* and *Nicotiana clevelandii* did not develop symptoms. However, back-inoculation tests indicated that these plants were infected systemically with the virus.

Inoculated leaves of *Chenopodium amaranticolor* showed poorly defined local lesions 10–14 days after inoculation.

Gomphrena globosa, Vicia faba and *Cucumis sativus* did not develop symptoms. However, back inoculation tests indicated that the inoculated leaves of these plants contained the virus.

The virus did not infect *Brassica rapa, Chenopodium quinoa, Datura stramonium, Nicotiana glutinosa, N. tabacum, Petunia hybrida, Sesamum indicum, Spinacia oleracea, Trifolium pratense, T. repens,* and *Zinnia elegans.*

2) Stability in crude sap

Crude sap of infected soybean leaves remained infective after dilution to 10^{-5}, but not 10^{-6}, heating at 70°C for 10 min, but not 75°C, and storage for 21 days at 20°C, but not 28 days.

3) Purification and serology

In the purification of the virus, a single opaque band typical of a virus-containing zone was observed in sucrose density gradients. Purified virus preparations had an ultraviolet absorption spectrum typical of that of a nucleoprotein with maximum absorbance at 260 nm and minimum at 244 nm. The A_{260}/A_{280} ratio was 1.37 and $A_{max\ (260)}/A_{min\ (244)}$ was 1.16 (Fig. 5). Soybean plants inoculated with the purified virus showed symptoms similar to those described above. In membrane feeding tests, whiteflies transmitted the purified virus to 10 out of 21 soybean plants.

The purified virus reacted with antiserum to cowpea mild mottle virus (homologous titer, 1 : 4,096), diluted up to 1 : 4096.

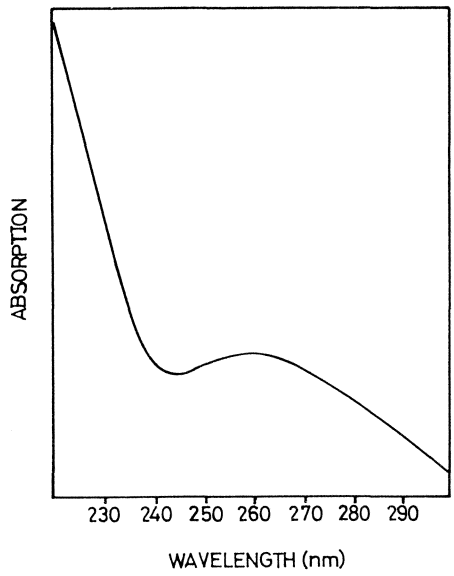

Fig. 5. Ultraviolet absorption spectrum of purified cowpea mild mottle virus.

4) Transmission and virus-vector relationships

Aphid transmission. *Aphis craccivora* and *A. glycines* failed to transmit the virus from bean or soybean to bean or soybean in a non-persistent and persistent manners (Table 2).

Whitefly transmission. Whitefly, *Bemisia tabaci,* transmitted the virus from soybean to soybean at a rate of up to 100% when groups of about 40 whiteflies were

Table 2. Aphid transmission tests of cowpea mild mottle virus

Test	Aphid	Fasting period	Acquisition access period	On first test plant			On second test plant		
				Inoculation access period	No. of insects per plant	No. of infected /inoculated plants	Inoculation access period	No. of insects per plant	No. of infected /inoculated plants
1	*Aphis craccivora*	0	24 hr (bean)	24 hr (bean)	10	0/6	—	—	—
2	*Aphis glycines*	0	24 hr (soybean)	24 hr (soybean)	15	0/6	—	—	—
3	*Aphis glycines*	1 hr	15 min (soybean)	48 hr (soybean)	10	0/9	48 hr (soybean)	10	0/3
4	*Aphis glycines*	1 hr	15 min (soybean)	1 hr (soybean)	10	0/10	24 hr (soybean)	10	0/5

allowed 1 day acquisition and inoculation access periods.

In transmission tests using one whitefly per plant, the virus was transmitted to 10 of the 58 Shirotsurunoko soybean plants tested.

The minimum period for virus acquisition and inoculation by whiteflies was not experimentally determined because whiteflies transmitted the virus in the shortest time tested (10 min) for each access period (Table 3, 4). Percentage of transmission increased with the increase in acquisition and inoculation access periods.

In serial transfer tests, whiteflies retained the virus only for one day. In another serial transfer test, whiteflies transmitted the virus to the second test plants when allowed an inoculation access period of 1 hr or less on the first test plant, but not of 3 hr or longer. These results indicate that the virus retention period was about 1 hr (Table 5).

In the tests of latent period, 15 whiteflies that were allowed 5-10 min of acquisition access transmitted the virus within 5-10 min of inoculation access. These results indicate that there is probably no latent period for the virus in the whitefly vector.

Table 3. Effects of acquisition access period on transmission of cowpea mild mottle virus by *Bemisia tabaci*

Test	Acquisition access period[a]					
	10 min	30 min	1 hr	3 hr	6 hr	24 hr
I[b]	12/12[d]	9/9	8/8	13/13	2/2	21/21
II[c]	9/30	19/29	15/29	15/17	30/30	29/29

Test plant: *Glycine max* cv. Shirotsurunoko,
a): Followed a 1 day inoculation access period,
b): Fourty insects were transferred to each test plant,
c): Ten insects were transferred to each test plant,
d): Number of infected plant/number of inoculated plants.

Table 4. Effects of inoculation access period on transmission of cowpea mild mottle virus by *Bemisia tabaci*

Test	Inoculation access period[a]					
	10 min	30 min	1 hr	3 hr	6 hr	24 hr
I[b]	17/27[c]	14/25	21/25	22/27	23/26	19/22
II[b]	16/30	19/29	25/30	23/30	28/28	23/27

Test plant: *Glycine max* cv. Shirotsurunoko,
a): Following a 1 day acquisition access period,
b): Ten insects were transferred to each test plant,
c): Number of infected plants/number of inoculated plants.

Table 5. Serial transmission tests of cowpea mild mottle virus by *Bemisia tabaci*

Test	First inoculation access[a]		Second inoculation access[b]
I[c]	10 min	2/5[e]	5/6
	30 min	3/5	4/6
	1 hr	6/6	5/6
	3 hr	5/6	0/5
	6 hr	6/6	0/5
	12 hr	4/5	0/4
	24 hr	5/6	0/6
II[d]	10 min	1/6	1/6
	30 min	4/6	0/6
	1 hr	5/6	1/6
	3 hr	5/6	0/6
	6 hr	3/6	0/6
	12 hr	6/6	0/6
	24 hr	2/6	0/6

a): Following a 1 day acquisition access period,
b): Access period : 24 hr,
c): test plant : Shirotsurunoko soybean,
d): test plant : Toyosuzu soybean,
e): Number of infected plant/number of inoculated plants.

5) Electron microscopy

Negatively stained preparations from infected soybean leaves showed the presence of slightly flexuous, rod-shaped particles (Fig. 6), most of which were 10-15 nm wide and 650-700 nm long (Fig. 7).

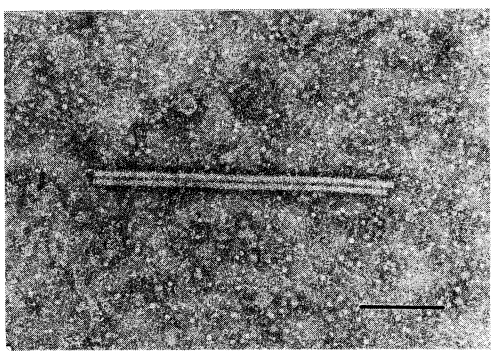

Fig. 6. Particles of cowpea mild mottle virus in direct negative staining preparation. Bar = 200 nm.

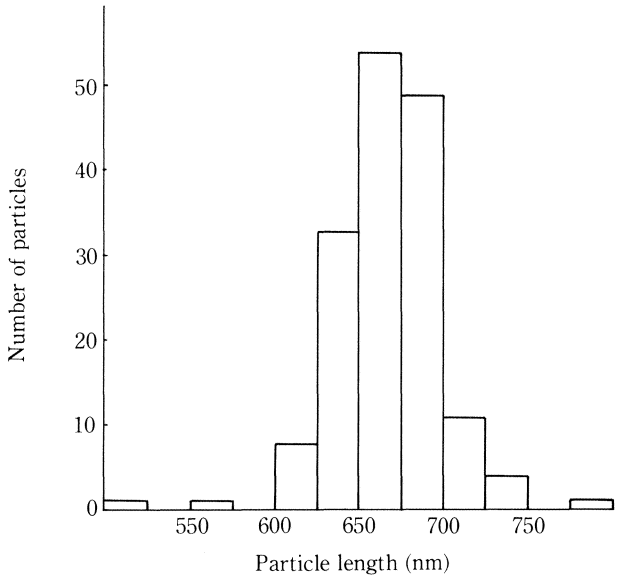

Fig. 7. Histogram showing length distribution of cowpea mild mottle virus.

In ultrathin sections of soybean leaves, feather-like and bundle-type inclusion bodies were observed in the cytoplasm (Fig. 8).

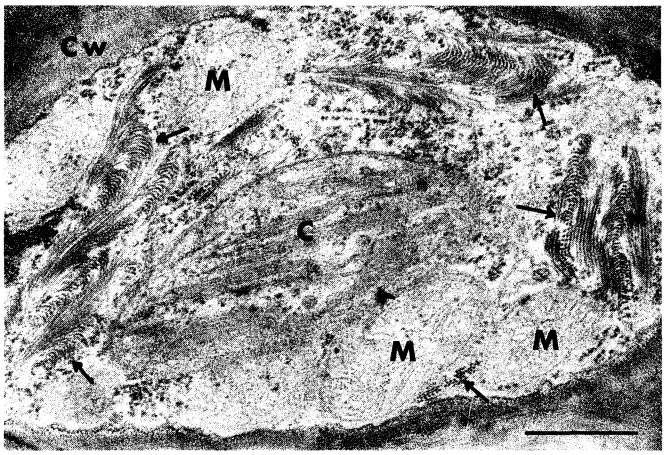

Fig. 8. Feather-like structures and virus particles (arrows) in the cytoplasm of cowpea mild mottle virus-infected sieve element of soybean. Bar = 500 nm.

6) Properties of nucleic acid

Nucleic acid preparations from the virus showed a ultraviolet spectrum with A_{260}/A_{233} and A_{260}/A_{280} ratios of about 1.93 and 1.96, respectively.

Buoyant density of the nucleic acid in Cs_2SO_4 was 1.636 g/cm^3 (Fig. 9), which

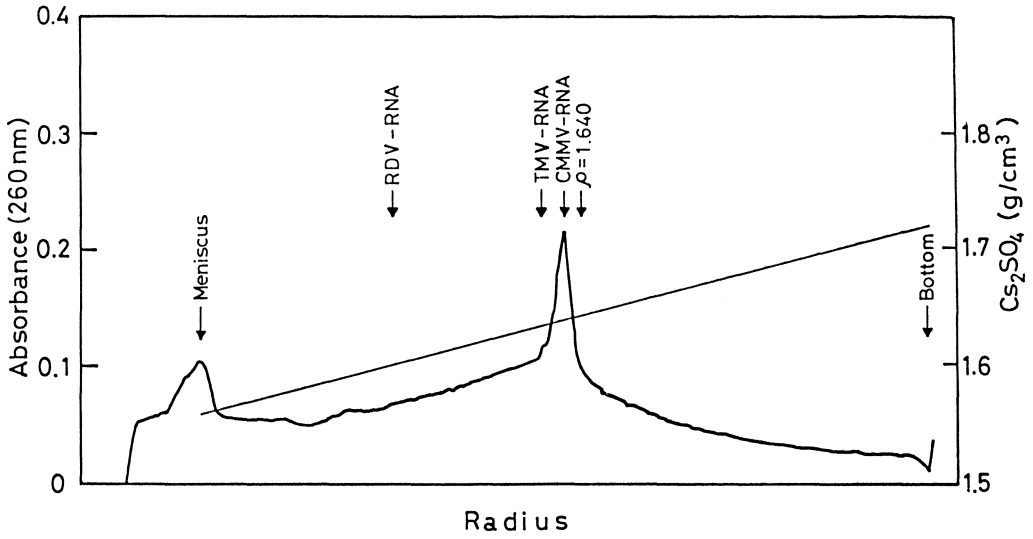

Fig. 9 Isopycnic ultracentrifugation of cowpea mild mottle virus ribonucleic acid (RNA) in cesium sulfate (Cs_2SO_4). RNA was mixed with Cs_2SO_4 in 0.01 M tris-HCl buffer (pH8.0) to produce a solution of density 1.640 g/cm³. The sample was centrifuged at 88,000 g for 42 hr at 25°C with MSE Centriscan 75. Heavy line = absorbancy, light line = density. CMMV = cowpea mild mottle virus, RDV = rice dwarf virus, TMV = tobacco mosaic virus.

corresponded to the values of the other viral single-stranded RNA molecules (13). Single-stranded RNA of TMV and double-stranded RNA of rice dwarf virus banded at a buoyant density of 1.632 g/cm³ and 1.599 g/cm³, respectively, under the same conditions. After formaldehyde treatment, the nucleic acid exhibited a hyperchromicity of 22% and a shift of 2-3 nm of longer wavelength in the ultraviolet absorption spectrum after 22 hr incubation (Fig. 10).

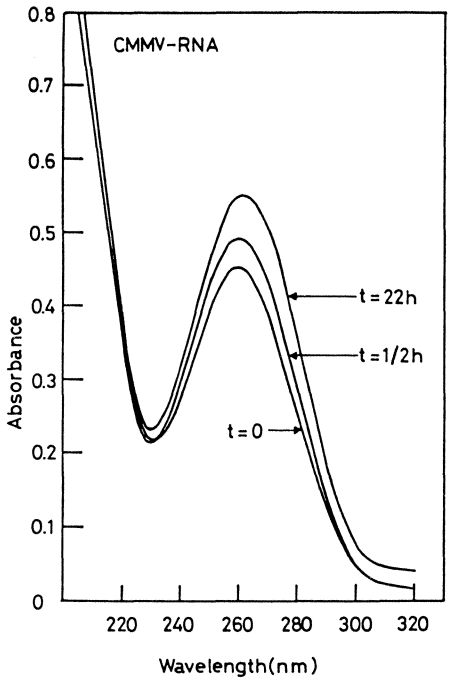

Fig. 10. Reaction of cowpea mild mottle virus ribonucleic acid (RNA) with formaldehyde. The ultraviolet spectra of RNA in 0.1 M sodium chloride were examined after 0. 0.5, and 22 hr of incubation at 37°C in the presence of 1.8% formaldehyde.

4. Discussion

The virus appeared to be a strain of CMMV on the basis of host range, symptomatology, particle morphology and serological relationships. However, whitefly transmissibility of CMMV had not been reported previously.

The whitefly-borne viruses have paired or geminate particles (1, 4), which are known as geminivirus (6), or filamentous particles 740–800 nm long (cucumber vein-yellowing virus) (5, 12), 950 nm long (sweet potato mild mottle virus) (7) and 1000 nm long (cucumber yellows virus) (14). CMMV which has a particle length of 650–700 nm is considered to be morphologically different from these viruses.

CMMV described in this paper displayed some of the carlavirus characteristics. However, most of the carlaviruses are transmitted by aphids in a non-persistent manner at various rates. Some viruses like CMMV from Africa were not transmitted by aphids (2, 8). Our results raise the possibility that some members of the carlaviruses that are not transmitted by aphids may be transmitted by whiteflies. We have found that the type strain (2) and a typical isolate (3) of CMMV (supplied by A.A. Brunt) are transmitted by whitefly (Table 6).

CMMV was isolated from soybean plants showing rugose mosaic symptoms and peanut showing vein-clearing and mild wrinkles in Malaysia and from soybean and peanut showing mosaic symptoms in Indonesia. CMMV appeared to be widely distributed in tropical countries.

Table 6. Transmission tests of type strain and typical isolate of cowpea mild mottle virus by *Bemisia tabaci*

Virus	No. of insects per test plant	No. of infected /inoculated plant
Type strain	20—30	9/9
Typical isolate	15	9/9

Virus source plant: *Phaseolus vulgaris* cv. Tsurunashi Kintoki,
Test plant: *Glycine max* cv. Shirotsurunoko,
Acquisition and inoculation access period: each 1 day.

Feather-like inclusion bodies observed in ultrathin sections of infected soybean leaves had not been reported in ultrathin sections of plants infected with carlaviruses. These inclusions are characteristic of those observed in cowpea mild mottle viruses.

Literature cited

1. Bird, J., and Maramorosch, K. (1978). Viruses and virus diseases associated with whiteflies. Adv. Virus Res. 22 : 55-110.
2. Brunt, A.A., and Kenten, R.H. (1973). Cowpea mild mottle, a newly recognized virus infecting cowpea (*Vigna unguiculata*) in Ghana. Ann. Appl. Biol. 74 : 67-74.
3. Brunt, A.A., and Sue Phillips. (1981). 'Fuzzy vein', a disease of tomato (*Lycopersicon esculentum*) in Western Nigeria induced by cowpea mild mottle virus. Tropical Agriculture 58 : 177-180.
4. Goodman, R.M., Bird, J., and Thongmeearkom, P. (1977). An unusual virus-like particle associated with golden yellow mosaic of beans. Phytopathology 67 : 37-42.
5. Harpaz, I., and Cohen, S. (1965). Semipersistent relationship between cucumber vein yellowing virus (CYVV) and its vector, the tobacco whitefly (*Bemisia tabaci* Gennadius). Phytopath. Z. 54 : 240-248.
6. Harrison, B.D., Barker, H., Bock, K.R., Guthrie, E.J. Meredith, G. and Atkinson, M. (1977). Plant viruses with circular single-stranded DNA. Nature 270 : 760-762.
7. Hollings, M., and Stone, O.M. (1976). Purification and properties of sweet potato mild mottle, a whitefly-borne virus from sweet potato (*Ipomoea batata*) in East Africa. Ann. Appl. Biol. 82 : 511-528.
8. Mathews, R.E.F. (1979). Classification and nomenclature of viruses. Intervirology 12: 129-296.
9. Miura, M., Kimura, I., and Suzuki, N. (1966). Double-stranded ribonucleic acid from rice dwarf virus. Virology 28 : 571-579.
10. Pirone, T.P. (1964). Aphid transmission of a purified stylet-borne virus acquired through a membrane. Virology 23 : 107-108.
11. Reddy, D.V.R., and Black, L.M. (1973). Electrophoretic separation of all components of the double-stranded RNA of wound tumor virus. Virology 54 : 557-562.
12. Sela, I., Assouline, I., Tanne, E., Cohen, S., and Macro, S. (1980). Isolation and characterization of a rod-shaped, whitefly-transmissible, DNA-containing plant virus. Phytopathology 70 : 226-228.

13. Szybalski, W. (1968). Use of cesium sulfate for equilibrium density gradient centrifugation. Methods in Enzymology 12B : 330-360.
14. Yamashita, S., Doi, Y., Yora, K., and Yoshino, M. (1979). Cucumber yellows virus : its transmission by the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), and the yellowing disease of cucumber and muskmelon caused by the virus. Ann. Phytopath. Soc. Japan 45 : 484-496.