Technical Bulletin 8
December, 1975

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Tech. Bull.
TARC
No. 8

1975
Tropical Agriculture Research Center
Ministry of Agriculture and Forestry
Kitanakazuma, Yatabe-cho, Tsukuba-gun,
Ibaraki, Japan

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Japan

Prinited in Tokyo by Okumura Printing Co., Ltd.

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I. Introduction

In Sri Lanka, cultivation of the chilli plant has recently been increased considerably, especially in the dry zone such as Jaffna, Puttalam, Vavuniya, and Anuradhapura Districts. However, the development of virus disease has become a major problem.

The present research was conducted during the period from October 1969 to November 1971, as a joint endeavour between Plant Pathology Division, Department of Agriculture, Sri Lanka, and Tropical Agriculture Research Center, Ministry of Agriculture and Forestry, Japan. The work consisted mainly of a survey of chilli virus diseases in the fields, characterization of chilli viruses, and field control trials using insecticides.

The author* was grateful to Mr. P. T. Jinendradasa, Former Director of Agriculture, Dr. J. W. L. Peiris, Former Deputy Director of Agriculture Research, Dr. D. V. W. Abeygunawardena, Former Assistant Deputy Director of Agriculture Research, for usefull suggestions and encouragement; also other members of the Central Agricultural Research Institute at Peradeniya and the Thinnavely Farm, Jaffna, who helped me in the field trials, especially, Mr. J. S. Selvaratnam.

I also wish to express my gratitude to Dr. N. Yamada, Former Director of Tropical Agriculture Research Center, Ministry of Agriculture and Forestry, Japan, Dr. K. Murakami, Director of the Center, Mr. K. Nagai, Former Head of Research Division, Mr. S. Hatta, Assistant Director (Planning), and all the staff members of the Center for giving me the opportunity to make a research of chilli virus diseases in Sri Lanka.

I am wish to thank Dr. T. T. Iida, Former Director of Institute for Plant Virus Research and Dr. Y. Komuro, Chief of Second Research Division, Chiba, Japan, for advice and guidance. Dr. Y. Komuro visited Sri Lanka and worked with me from June to September 1971 at Central Agricultural Research Institute and a part of this report was obtained by co-work with him. I am also grateful to Dr. M. Iwaki and Dr. H. Hibino of the same Institute for providing electron microscopic photographs of virus particles and ultrathinsections.

II. A survey of chilli virus diseases in fields in the major chilli producing areas

The major chilli producing areas in Sri Lanka extend from the northern part to the central part of the island, which is called the dry zone. A preiminary survey was carried out to determine the incidence of chilli virus diseases in the major chilli producing areas from October 1969 to March 1970. The following four districts were chosen: Jaffna, Vavuniya, Puttalam, and Anuradhapura. In these places, chilli plants are mostly cultivated once a year during the Maha season, except for rare cases in Jaffna, Vavuniya, and Anuradhapura as shown in Table 1. In areas where irrigation is available, groundnut, tobacco, tomato, potato, corn, and onion are planted as rotation

* On leaves from Institute for Plant Virus Research, Chiba, Japan from October 1969 to November 1971.

Table 1. Cultivation of chilli plants in the major producing areas of Sri Lanka

	Puttalam	Anuradhapura	Vavuniya	Jaffna
Cultivated vareites	1. Navakkadu	1. M. I. H.	1. M. I. H.	1. Myllidy
	2. Heen-Miris	2. Thinnavelly		2. Karuppan
	3. M. I. H.	3. Karuppan		3. M. I. H.
Time of sowing				
Maha season	none	early October or January	early December	early December
Yala season	middle April	late April	none	middle April to early May
Time of transplanting	4 weeks after sowing	4 weeks after sowing	4 weeks after sowing	4 weeks after sowing
Time of picking	8 weeks after transplanting	9 weeks after transplanting	8 weeks after transplanting	8–9 weeks after transplanting
No. times of picking	8–10 times	8–10 times	8–10 times	8–10 times
Rotation crops	chilli to chilli	chilli to chilli or groundnut	chilli to chilli or groundnut	chilli to chilli, tobacco or potato

crops, and chilli is cultivated either intensively or in chenas. The most common variety of chilli cultivated in these districts is Maha-Illuppallama hybrid (M.I.H.) which is fairly resistant to mosaic virus diseases and has a long flowering period. The period between sowing and harvesting is about six month. Virus diseases that occur in chilli fields are mainly of mosaic type and leaf curl type, of which the mosaic type appears in five forms: vein-clearing, vein-banding, vein-yellowing, systemic chlorotic spots, and mottling or ordinary mosaic symptoms.

Results of survey on the incidence of chilli virus diseases in Puttalam district are summarised in Table 2. The percentage of the mosaic type disease was higher than that of the leaf curl disease. More than 80% of the cultivation was found to be infected with mosaic in all the fields that were surveyed near Palakkudha. In this area, almost all the chilli is cultivated under coconut trees that are in their early stages. Cultivating methods are primitive, and farmers pour insecticides such as Sumithion over the chilli plants, instead of spraying.

Table 2. Survey of chilli virus diseases in fields in Puttalam district
(Maha season, 1969)

No. of field	Variety	Days after transplanting	No. of plants	Percentage of mosaic disease	Percentage of leaf curl disease
1	Navakkadu	190	576	97	2
2	Navakkadu	60	474	85	10
3	Navakkadu	70	522	82	10
4	Heen-Miris	60	510	84	15
5	Heen-Miris	60	588	84	9

The survey in Jaffna was carried out two localities in the Maha season of 1969, one being the Experiment Station field at Thinnavelly, and the other private fields.

Results are summarized in Table 3. Fields 1 to 5 belong to the Thinnavelly Station and 6 to 9 are private fields. The percentage of mosaic type disease in the private fields

Table 3. Survey of chilli virus diseases in fields in Jaffna district
(Maha season, 1969)

No. of field	Variety	Days after transplanting	No. of plants	Percentage of mosaic disease	Percentage of leaf curl disease
1	M. I. H.	45	1,548	4	10
2	〃	45	1,326	1	9
3	〃	45	1,707	2	6
4	〃	45	1,521	3	7
5	Santaka	60	702	36	0
6	M. I. H.	80	1,176	54	3
7	〃	70	1,275	85	4
8	〃	70	654	61	13
9	〃	100	1,380	65	9

M. I. H.: Maha-Illuppallama hybrid

was higher than that in the Experiment Station fields. There was hardly any difference between these two places in the percentage of leaf curl disease. The Experiment Station fields had been sprayed with insecticides at ten day intervals. In is possible that mosaic diseases were partly controlled by the insecticidal spraying, whereas it was difficult to control by insecticidal spraying the leaf curl disease which is caused by a whitefly borne virus.

The survey in Vavuniya district was made at two locations: one in the fields of the Special Colonisation Project in the northeast coast of the island surrounded by jungle, about two miles from Mulliyavalai village and about ten miles from the seashore, and the second one in private fields around Vavuniya town.

Results of this survey are summarized in Table 4. Fields 1 to 4 showed only leaf curl which was, however, very severe. The cultivation here do not spray any insecticides for the control of insect vectors. Fields of the second location (fields 5 to 7) showed a higher percentage of leaf curl than mosaic. The percentage of mosaic infection was between 10 and 40, and these only occurred about 210 days after transplanting.

Table 4. Survey of chilli virus diseases in fields in Vavuniya district
(Maha season, 1969)

No. of field	Variety	Days after transplanting	No. of plants	Percentage of mosaic disease	Percentage of leaf curl disease
1	M. I. H.	30	360	0	36
2	〃	40	546	0	80
3	〃	40	615	0	100
4	〃	70	765	0	73
5	〃	210	660	13	76
6	〃	210	372	10	67
7	〃	210	534	39	23

M. I. H.: Maha-Illuppallama hybrid

The survey in Anuradhapura district was conducted during the Maha season 1969 in several villages. Results of this survey are shown in Table 5. Fields 1 to 6 belong to

Table 5. Survey of chilli virus diseases in fields in Anuradhapura district
(Maha season, 1969)

No. of field	Veriety	Days after transplanting	No. of plants	Percentage of mosaic disease	Percentage of leaf curl disease
1	M. I. H.	21	375	0	14
2	〃	40	135	0	55
3	〃	40	135	0	30
4	Santaka	40	225	0	47
5	〃	40	264	0	31
6	M. I. H.	30	1, 824	0	2
7	Thinnavelly	65	1, 095	0. 5	10
8	〃	65	918	1	5
9	M. I. H.	53	1, 470	5	7
10	〃	75	1, 560	8	8
11	〃	75	981	4	5
12	〃	75	450	8	41
13	〃	80	1, 107	4	44
14	〃	75	1, 101	3	8
15	〃	70	1, 098	7	8
16	〃	77	1, 893	6	13
17	〃	45	954	12	2
18	〃	79	618	78	9
19	〃	67	915	70	6
20	〃	83	576	56	8

M. I. H.: Maha-Illuppallama hybrid

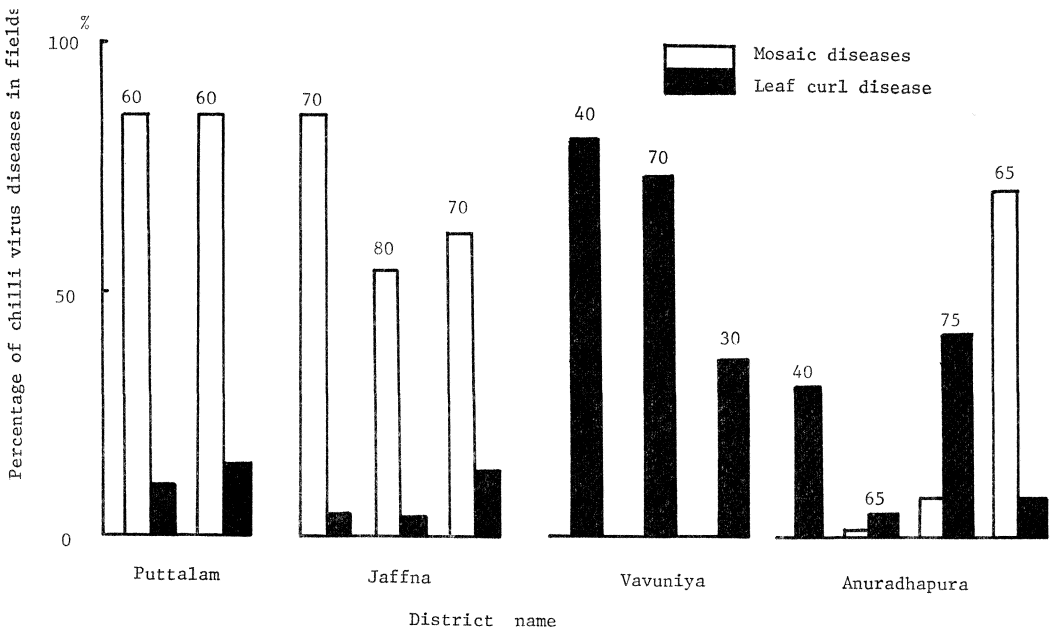

Fig. 1. The incidence of chilli virus diseases in four districts. (Maha 1970/1971)

Maha-Illuppallama Experiment Station and the University Farm, 7 to 11 belong to private cultivators living 5 miles from Anuradhapura town, 12 to 16 belong to the Special Colonisation Project (2 years after reclamation) located 5 to 7 miles from Maragahawewa village, and 17 to 20 belong to private cultivators living around Tirappane village. The incidence of leaf curl disease in this district is higher than that of mosaic diseases. In the fields, leaf curl symptoms appear much earlier than mosaic symptoms.

The incidence of chilli virus diseases in the four districts of the dry zone are compared in Fig. 1.

In Puttalam and Jaffana districts which are located near the seashore, chilli plants are more affected by mosaic diseases than leaf curl disease. Mosaic is most severe and symptoms appear in the fields between the first and the 3rd week after transplanting. In Anuradhapura and Vavuniya districts located inland, leaf curl disease is more prevalent than mosaic diseases. Leaf curl symptoms appear in the fields between 15 to 30 days after transplanting, and the disease spreads vigorously from 60 to 80 days after transplanting. The mosaic diseases in these districts are not severe in early stages, and symptoms appear in the fields between one to two weeks after transplanting. In Jaffna district, leaf curl disease is not so severe in early stages of the chilli cultivation, and symptoms appear in the fields between 20 to 30 days after transplanting. However, as will be mentioned later, if no insecticide is applied to chilli fields, infestation with mites and thrips may cause damage which resemble leaf curl disease symptoms.

III. Characterization and identification of chilli viruses

1. Preliminary classification

Eighty seven diseased chilli samples were collected from Jaffna, Puttalam, Vavuniya, Anuradhapura, Badulla, and Kandy districts. The plants showed mosaic, systemic chlorotic spots, yellow mottle, leaf curl, or mixture of these symptoms. The number of samples collected were: Puttalam—1, Vavuniya—8, Anuradhapura—20, Jaffna—38, Badulla—5, and Kandy—15. In the original diseased chilli plants, there were 20 plants which showed typical leaf curl symptoms.

From 78 samples, virus was transmitted by sap inoculation to *Capsicum annuum* cv. M.I.H. or cv. Santaka (Japanese variety), *Nicotiana tabacum* cv. Bright Yellow or cv. Samsun, *N. glutinosa, Capsicum frutescens* cv. Honenmidori or cv. California Wonder, *Chenopodium amaranticolor, Phseolus vulgaris, Vigna sinensis,* and *Vicia fabae.*

From 9 samples, no virus was transmitted by sap inoculation to these test plants. The original plants showed only leaf curl symptoms, and these were transmitted by the whitefly, *Bemisia tabaci,* as well as by grafting to healthy chilli plants. However, one isolate from Jaffna showing leaf curl symptoms could not be transmitted either by whitefly or by grafting to healthy chilli seedlings. Generally, in the case of plants having mixed infection of mosaic and leaf curl, the leaf curl symptom is more severe than the mosaic.

In sap-inoculation, there was sometimes no infection except on chilli plants. This suggested the presence of an inhibitor or inhibitors in the sap of chilli plants (McKeen 1956). When *Nicotiana tabacum* cv. Bright Yellow or *N. glutinosa* produced mosaic symptoms as a result of sap-inoculation from young mosaic leaves of chilli plants,

further inoculation to other indicator plants were made by using these tobacco or *N. glutinosa* as the source plant. Carborundum (600 mesh) was sprinkled over the leaves of the test plants before inoculation. Infected leaves were ground in a mortar with 0.1 M phosphate buffer (pH 7.0). The inoculated foliage was washed by spraying with water following inoculation. The results are summarized in Table 6. Six virus groups could tentatively be recognized.

Table 6. Preliminary grouping of chilli viruses obtained from six districts in Sri Lanka

Test plants	Virus group					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Sap transmission	+	+	+	+	−	+
Aphid transmission	±	−	+	+	−	?
Whitefly transmission	−	−	−	−	+	−
Graft transmission					+	
Capsicum annuum cv. M. I. H.	SM	SM	SM	SM	VC, LC	VC, SM
Nicotiana tabacum cv. Bright Yellow	SM CS–NL	SM	SM	Mo, CS CS–NL	LCu	?
N. glutinosa	NL (IL) CS (UL)	NL (IL)	SM	CS (UL) Mo	−	?
P. vulgaris cv. Otebo	NL (IL) −(UL)	NL (IL)	−	−	−	−
V. sinensis cv. Kurodane 16 sasage	−	−	NL (IL)	−	−	−
C. amaranticolor	CS–NL (IL)	NL (IL)	NL (IL)	CS–NL (IL)	−	−
V. fabae	−	−	NL (IL)	−	−	−
Identified virus	tobacco mosaic virus and a strain of potato virus Y	tobacco mosaic virus	cucumber mosaic virus	a strain of potato virus Y	a strain of tobacco leaf curl virus	unidentified virus

+: transmission ±: occasional transmission −: non-transmission SM: systemic mosaic
CS: systemic chlorotic spots VC: vein-clearing Mo: mottling LCu: leaf curvature
NL: necrotic lesions IL: inoculated leaf UL: upper leaf LC: leaf curl

No. 1 virus group: Symptoms on the original chilli plants were in most cases vein-banding with mottle or severe mosaic. This virus group showed complicated reactions on test plants under net-house conditions at Central Agricultural Research Institute. Sometimes virus could be transmitted to *N. tabacum* cv. Bright Yellow, *N. glutinosa*, and *Chenopodium amaranticolor*, other times not. However, in most instances, virus was transmitted to chilli. It was later found that this virus group is not a unique group but rather a double infection with two viruses, No. 2 and No. 4 viruses.

No. 2 virus group: Symptoms on the field-infected chilli plants were in most cases vein-banding and slight mosaic. This virus group was transmitted by sap-inoculation and produced systemic mosaic on chilli and *N. tabacum* cv. Bright Yellow, local necrotic lesions on inoculated leaves of *N. glutinosa*, *Phaseolus vulgaris* cv. Otebo, and *C. amaranticolor*. No reaction was obtained on *Vigna sinensis* cv. Black Eye and Kurodane 16-sasage. This virus group was later identified as tobacco mosaic virus.

No. 3 virus group: Symptoms on the field-infected chilli plants were in most cases vein-clearing on young leaves and slight mosaic over the whole plant. Systemic mosaic symptoms were produced on *Capsicum annuum* cv. M.I.H., *N. tabacum* cv. Bright Yellow, and *N. glutinosa*. Local necrotic lesions were produced on sap-inoculated leaves of *V. sinensis*, *C. amaranticolor*, and *V. fabae*. This virus group was later identified as cucumber mosaic virus.

No. 4 virus group: Symptoms on the original chilli plants were vein-banding or vein-yellowing, and severe mosaic, or sometimes slight mosaic similar to that shown by No. 1 virus group. This virus group was transmitted by sap-inoculation and by aphid, producing systemic symptoms on chilli. On inoculated as well as on upper leaves of tobacco it produced systemic chlorotic spots which later became necrotic; sometimes it also produced vein necrosis and or stem necrosis. *N. glutinosa* showed systemic diffuse chlorotic spots which developed into mottle pattern. *C. amaranticolor* gave local lesions on inoculated leaves when kept for 24 hours in a dark box in the net-house. No reactions were obtained on *P. vulgaris*, *V. sinensis*, and *V. fabae*. This virus group was later identified as a strain of potato virus Y.

No. 5 virus group: The field-infected chilli plants displayed vein-clearing on young leaves at early infection stages, upward or downward curling of young and old leaves, and stunting in most cases. This virus group was transmitted from diseased to healthy chilli plants only by whitefly or by grafting. It could not be transmitted by sap-inoculation. Ten to 15 days after whitefly or grafting transmission, leaf curl symptoms appeared on chilli plants. This virus group was identified as a strain of tobacco leaf curl virus.

No. 6 virus group: Symptoms on the field-infected chilli plants were systemic chlorotic spots, vein-yellowing on old leaves and or yellow mottling, sometimes slight yellow mosaic on upper leaves. There were 16 samples collected from Jaffna and Kandy districts. They were inoculated to test plants such as *Capsicum annuum* cv. M.I.H. *N. glutinosa*, *V. fabae*, *Chenopodium amaranticolor*, *Phaseolus vulgaris*, and *Vigna sinensis*. In most instances the virus produced no distinct systemic or local symptoms on these test plants; in a few cases produced systemic slight yellow mosaic on *Capsicum annuum* cv. M.I.H. This virus has as yet not been identified.

As shown in Table 7, it was generally difficult to correlate symptoms of field-infected chilli plants with presence of specific viruses. Tobacco mosaic virus and potato virus Y were associated in most cases with vein-banding, accompanied with mottle or mosaic. Field-infected chilli plants with tobacco mosaic virus alone showed usually vein-banding and slight mosaic. Chilli plants containing cucumber mosaic virus alone showed vein-

Table 7. Relationships between symptoms on original chilli plants and causal viruses

Virus group	Causal virus	Symptoms on original chilli plants
No. 1 virus group	TMV and PVY	vein-banding, severe mosaic, mottling
No. 2 virus group	TMV	vein-banding, slight mosaic
No. 3 virus group	CMV	slight mosaic, vein-clearing
No. 3 virus group	PVY	vein-banding, vein-yellowing, severe or slight mosaic
No. 5 virus group	CLCV	vein-clearing, upward or downward curling of leaves, stunting
No. 6 virus group	unidentfied virus	chlorotic spots, vein-yellowing, slight yellow mosaic

clearing to slight mosaic symptoms in most cases. Field-infected chilli plants with potato virus Y displayed vein-banding or vein-yellowing similar to that shown by chilli plants from which both tobacco mosaic virus and potato virus Y were isolated. An unidentified virus (No. 6 virus) was associated with chlorotic spots on old leaves and vein-yellowing with slight mosaic on young leaves. However, the identification of chilli viruses from field symptom expressions alone is nearly impossible.

2. Characterization of No. 1 virus

The original chilli plants from which this virus group was isolated showed vein-banding with mottle and severe mosaic symptoms. On inoculation, definite symptoms of systemic infection were shown in the following species: *Capsicum annuum* cv. M.I.H. and cv. Santaka, *C. frutescens* cv. Tabasco, cv. Honenmidori, and cv. California Wonder, *Petunia hybrida*, and *Physalis floridana*. Sometimes, systemic infection was also shown in *Nicotiana tabacum* cv. Bright Yellow and cv. Samsun, and *N. glutinosa*. Local infection was obtained on *C. frutescens* cv. Tabasco, *N. tabacum* cv. Bright Yellow, and *Chenopodium amaranticolor*.

The results were thus erratic. Comparison tests were, therefore, carried out between a virus isolated from a local lesion on an inoculated leaf of *N. glutinosa* (tenta-

Table 8. Comparison tests between No. 1-A virus* and No. 1-B virus*

Test plants	Symptoms caused by	
	A virus	B virus
Solanaceae		
Nicotiana tabacum cv. Bright Yellow	SM	CS, NL, Mo
cv. Samsun	SM	CS, NL, Mo
N. glutinosa	NL	slight mottle
Petunia hybrida	SM	SM
Solanum nodiflorum	slight mosaic	severe mosaic
Physalis florridana	slight mosaic	severe mosaic
Capsicum annuum cv. M. I. H.	SM	SM
C. frutescens cv. Tabasco	SM	severe mosaic, necrosis (D)
cv. Honenmidori	SM	SM
Solanum tuberosum cv. Arka	—	NL (D)
Chenopodiaceae		
C. amaranticolor	CS-NL (small lesions) (fast)	CS-NL (large lesions) (delay)
Cucurbitaceae		
Cucumis sativus cv. Ochiai-fushinari	—	—
Leguminosae		
Phaseolus vulgaris cv. Otebo	NL or none	—
Vigna sinensis cv. Black Eye	—	—
Vicia fabae	—	—
Aizoaceae		
Tetragonia expanda	—	—

* cf. text

SM: systemic mosaic CS: systemic chlorotic spots NL: necrotic lesion Mo: mottle D: died

tively called No. 1–A virus) which was obtained by sap-inoculation from original chilli plant to *N. glutinosa,* and a virus isolated from the original chilli plant by aphid transmission to a healthy chilli seedling (tentatively called No. 1–B virus). Results of the inoculation tests are shown in Table 8.

(1) No. 1–A virus (Tobacco mosaic virus)

Table 8 shows that there was local lesion reaction on inoculated leaves of *N. gultinosa* and *C. amaranticolor.* Systemic reaction was obtained on *N. tabacum* cv. Bright Yellow and cv. Samsun, *Petunia hybrida, Solanum nodiflorum, Physalis floridana, C. frutescens* cv. Tabasco and cv. Honenmidori, *C. annuum* cv. M.I.H., and *Tetragonia expanda.*

(2) No. 1–B virus (A strain of Potato virus Y)

(a) Host range

Systemic symptoms were produced by this virus in the following species: *N. tabacum* cv. Bright Yellow and cv. Samsun, *N. glutinosa, Petunia hybrida, Solanum nodiflorum, P. floridana, C. frutescens* cv. Tabasco and cv. Honenmidori, and *C. annuum* cv. M.I.H. *N. tobacum* cv. Bright Yellow or cv. Samsun and *N. glutinosa* showed systemic chlorotic spots or necrotic lesions on inoculated leaves or sometimes on upper leaves, and developed a few or no systemic slight mottle on upper leaves. Local lesions were produced on *C. amaranticolor.* There were more local lesions when test plants were kept in a dark box for 24 hours after inoculation. The same tendency was observed in *C. frutescens* cv. Tabasco and *Solanum tuberosum* cv. Arka.

(b) Aphid transmission

Green peach aphid, *Myzus persicae,* and cotton aphid, *Aphis gossypii,* were used for transmission tests. All aphid transfers were made by mean of a fine writing brush. The insects were starved for 3 hours in petri-dishes at room temperature, and acquisition feeding for 10 to 60 min. was allowed on detached infected leaves. Inoculation feeding on healthy chilli seedlings lasted for 15 to 18 hours at room temperature. Inoculated plants were sprayed with DDVP before being placed in a net-house.

Results of transmission tests are summarized in Table 9. As shown in Table 9, this virus can be transmitted to test plants by both aphids, the transmission rate being higher in green peach aphid, *Myzus persicae,* than in cotton aphid, *Aphis gossypii.*

Table 9. Transmission of No. 1–B virus from infected chilli to healthy chilli seedlings by green peach aphid and cotton aphid

Aphid species	Acquisition feeding time	No. of test plants	No. of insects per plant	No. of infected plants
Green peach aphid (*Myzus persicae*)	10 min.	10	4	2
	30 min.	16	4	5
	60 min.	24	4	11
Cotton aphid (*Aphis gossypii*)	30 min.	12	4	1
	60 min.	24	4	6

(c) Virus particles of No. 1–A and No. 1–B viruses

Upper leaves of a chilli plant field-infected with No. 1 virus group was homogenized with 0.1 M phosphate buffer, pH 7.0, centrifuged at 7,000 rpm for 20 minutes, and the supernatant was inoculated to four or five leaf stage plants of *Capsicum frutescens* cv.

Honenmidori. Young leaves of the inoculated plants showing systemic mosaic were harvested 7 to 14 days after inoculation and homogenized with the same buffer and inoculated to healthy *N. glutinosa* plants. Local lesions appeared on inoculated leaves of *N. glutinosa* 4 to 5 days after inoculation; also systemic chlorotic mottle developed on upper leaves 10 to 14 days after inoculation.

Sub-inoculation was made from a single isolated local lesion produced on *N. glutinosa* and this isolate (No. 1–A virus) was increased and maintained in *C. frutescens* cv. Honenmidori and *N. tabacum* cv. Bright Yellow plants.

Another sub-inoculation was made from young leaves of *N. glutinosa* showing system chlorotic mottle, and this isolate (No. 1–B virus) was propagated in *C. frutescens* cv. Honenmidori.

Young leaves of *C. frutescens* cv. Honenmidori showing systemic mosaic with No. 1–B virus were harvested 14 days after inculation, and used for observation of virus particles. The cut edge of a leaf was dipped in a drop of distilled water placed on Formvar-coated grids and dried in a desiccator; then washed twice in distilled water and dried again. The preparations were shadowed with palladium and viewed on a Hitachi Model HU-11 electron microscope. Filamentous particles ranging from 700 to 750 nm in length were observed, as shown in Plate 8.

Young infected leaves of *C. frutescens* cv. Honenmidori with No. 1–A virus were harvested two to three weeks after inoculation. Ten grams of these leaves were homogenized with 50 ml of 0.1 M phosphate buffer, pH 7.0. Differential centrifugation was used for partial purification of this virus. High-speed centrifugation was done for 1 hour at 40,000 rpm in No. 40 rotor of Hitachi Model 55P ultracentrifuge. Low-speed centrifugation for clarification of homogenate or resuspended pellets was done for 30 minutes at 10,000 rpm in RPH rotor of a Hitachi refrigerated centrifuge. Differential centrifguation was done twice and the final pellets were dissolved in 0.01 M phosphate buffer, pH 7.0. The partially purified preparations of No. 1–A virus showed rod shaped particles about 300 nm in length, as shown in Plate 7.

From the results of the host range tests, the aphid transmission tests, and morphology of virus particles, it was concluded that No. 1–A virus is a common strain of tobacco mosaic virus (No. 2 virus group) and No. 1–B virus is a strain of potato virus Y (No. 4 virus group).

3. Characterization of No. 2 virus (Tobacco mosaic virus)

(a) Host Range

This virus group produced mosaic on *Capsicum annuum* cv. M.I.H. and cv. Santaka, *C. frutescens* cv. Tabasco, cv. Honenmidori, and cv. California Wonder, *N. tabacum* cv. Bright Yellow, cv. White Burley, cv. Turkish, cv. Samsun, and a local variety of Sri Lanka, *Petunia hybrida, N. rustica, N. sylvestris, Lycopersicum esculentum* cv. Fukuju, cv. Ponterosa, and a local variety of Sri Lanka, *Solanum melongena, S. nodiflorum, S. nigrum, Physalis floridana,* and *Zinnia elegans.*

N. glutinosa, Datura stramonium, Phaseolus vulgaris cv. Otebo, *Chenopodium amaranticolor,* and *C. quinoa* showed local lesions on inoculated leaves. *Commelina* sp., *Cucumis sativus* cv. Ochiai-fushinari and a local variety of Sri Lanka, *Cucurbita maxima, C. pepo, Vicia fabae, Vigna sinensis* cv. Black Eye and Kurodane 16-sasage, *Cassia tora, Brassica campestris rapa, B. campestris pekinensis, B. oleracea, Raphanus sativus,* and *Zea mays* produced no reaction.

(b) Physical properties

In the determination of certain physical properties of this virus, *N. tabacum* cv. Bright Yellow was utilized as the source plant and *N. glutinosa* as the test plant. The thermal inactivation point was between 85° and 90°C. The dilution end point appeared to be more than 1 : 1,000,000. Undiluted juice of infected tobacco plant stored in test tubes at room temperature (25°–32°C.) retained infectivity after one month.

(c) Serological reaction

Serological relationship of this virus was determined by reactions with antisera against a common strain of tobacco mosaic virus from Japan. Young leaves of *C. annuum* cv. M.I.H. showing mosaic with No. 2 virus were harvested 14 days after inoculation. Ten grams of these leaves were ground with 40 ml of 0.1 M phosphate buffer, pH 7.0 in a mortar and filtrated with chessecloth. The filtrated sap was clarified by centrifugation at 5,000 rpm for 30 min. at room temperature. The clarified sap was used for precipitin reaction tests against the antisera. Precipitin reaction in small test tubes showed a distinct positive band even at 1 : 3,000 dilution of the clarified sap in 24 hours at room temperature.

From the results of the host range tests, physical properties, and the serological reactions, it was conclude that No. 2 virus is a common strain of tobacco mosaic virus.

4. Characterization of No. 3 virus (Cucumber mosaic virus)

(a) Host range

This virus group produced local lesions on *C. amaranticolor, V. sinensis* cv. Black Eye and cv. Kurodane 16-sasage, and *V. fabae*; mosaic on *C. annuum* cv. M.I.H. and cv. Santaka, *C. frutescens* cv. Tabasco, cv. Honenmidori, and cv. California Wonder, *N. tabacum* cv. Bright Yellow, cv. Samsun, cv. White Burley, cv. KY-57, cv. Turkish, and a local variety of Sri Lanka, and *N. glutinosa*. Local lesions were produced also on *C. quinoa* and *C. murale*.

Systemic infection was shown in *N. rustica, N. sylvestris, Petunia hybrida, Lycopersicum esculentum* cv. Fukuju and a local variety of Sri Lanka, *Solanum nodiflorum, S. nigrum, Physalis floridana, Datura stramonium, Beta vulgaris, Spinacia oleracea, Zinnia elegans, Calendula officinalis, Trifolium incarnatum,* and *Vinca rosea*.

Lycopersicum esculentum cv. Fukuju and a local variety of Sri Lanka, *Solanum melongena,* and *Cucumis sativus* cv. Ochiai-fushinari showed slight mosaic or remained symptomless. A local variety of cucumer could not be infected. The virus was recovered from inoculated leaves of *Callistephus chinensis* and *Gomphrena globosa*.

(b) Physical properties

In the determination of certain physical properties of the virus, *N. tabacum* cv. Bright Yellow was utilized as the source plant and *Vigna sinensis* cv. Black Eye or Kurodane 16-sasage as the test plant. Repeated trials of four to five plants per treatment were made.

The thermal inactivation point was between 50° to 55°C. The dilution end point appeared to lie between 1 : 2,000 and 1 : 3,000. Undiluted juice of infected tobacco plants obtained 10 days after inoculation was stored in test tubes (2 ml each) at room temperature for various period. Virus infectivity was lost between 2 and 3 days. When stored in test tubes in a refrigerator at 5°C, infectivity was lost between 5 and 6 days.

(c) Aphid transmission

Green peach aphid, *Myzus persicae,* were reared on caged plants of *C. frutescens*

cv. Honenmidori, and cotton aphid, *Aphis gossypii*, were reared on *N. tabacum* cv. Brght Yellow. All aphid transfers were made by means of a fine writing brush. Non-alate adults were starved for 3 hours in petri-dishes and acquisition feeding of 5 min. to 2 hours was given on detached infected chilli leaves. The aphids were then transferred to healthy chilli seedlings for inoculation feeding for 24 hours. These tests were carried out at room temperature ranging from 25° to 30°C. Plants were sprayed with DDVP before being placed in a net-house and also routinely sprayed to control insects.

The results are summerized in Table 10. As is evident from this table, the virus is transmitted by both aphids, green peach aphid, *Myzus persicae*, and cotton aphid, *Aphis gossypii*, although there appears to be a slight difference in the ability of transmission between both aphids.

Table 10. Transmission of No. 3 virus from infected chilli by cotton aphid, *Aphis gossypii*, and green peach aphid, *Myzus persicae*

Aphid species	Acquisition feeding time	No. of inoculated plants	No. of insects per plant	No. of infected plants
Cotton aphid (*Aphis gossypii*)	10 min.	9	4	1
	30 min.	12	4	3
	60 min.	18	4	10
	120 min.	11	4	7
Green peach aphid (*Myzus persicae*)	5 min.	15	2	2
	10 min.	10	3	4
	30 min.	14	4	8
	60 min.	20	4	16
	120 min.	10	4	8

5. Characterization of No. 4 virus (A strain of Potato virus Y)

(a) Host range

Sixteen isolates of this virus were obtained from field chilli plants in Jaffna and Puttalam districts. This virus was most prevalent in Jaffna. In Jaffna and Matale districts, it was also isolated from tobacco plants (personal communication from Dr. Y. Komuro, 1971.).

Symptoms on certain plant species are summarized in Table 11. *N. tabacum* cv. Bright Yellow, cv. KY-57, cv. White Burley, cv. Samsun, cv. Turkish, and a local variety of Sri Lanka, *N. rustica*, and *N. sylvestris* showed broad systemic chlorotic spots, necrotic spots, necrotic lesions, vein necrosis, vein net yellowing, stem necrosis, and/or stunting. On *petunia hybrida*, *Lycopersicum esculentum* cv. Fukuju, cv. Ponterosa, and a local variety of Sri Lanka, *Solanum nodiflorum*, *Physalis floridana*, *Capsicum annuum* cv. M.I.H. and cv. Santaka, *C. frutescens* cv. Honenmidori and cv. California Wonder, systemic mosaic or mottle was observed.

On inoculated leaves of *Solanum tuberosum* cv. Arka, *Chenopodium amaranticolor*, *C. frutescens* cv. Tabasco, local lesion reaction was obtained only when kept in a dark box for 24 hours after inoculation.

Inoculated leaves of *C. murale* and *Amaranthus viridis* showed chlorosis. The virus was recovered from inoculated leaves of *Pisum sativum*, *Trifolium incarnatum*, *Physalis* sp. of Sri Lanka 14 days after inoculation. Vein necrosis on inoculated leaves and stem

Table 11. Symptoms caused by No. 4 virus in certain plant species

Family	Species	Symptoms caused by No. 4 virus	Virus recovery
Aizoaceae	*Tetragonia expanda*	—	—
Amaranthaceae	*Amarathus viridis*	—	+ (IL) — (UL)
	A. tricolor	—	—
	Gomphrena globosa	—	—
Apocynaceae	*Vinca rosea*	—	—
Chenopodiaceae	*Beta vulgaris*	—	—
	Chenopodium amaranticolor	NL (IL)	— (UL)
	C. murale	CL–D (IL)	— (UL)
	Spinacia oleracea	—	—
Commelinaceae	*Commelina* sp. (Sri Lanka)	—	—
Compositae	*Calendula officinalis*	—	—
	Callistephes chinensis	—	—
	Zinnia elegans	SM (mild)	+ (UL)
	Helianthus annuus	—	—
Cucurbitaceae	*Cucumis sativus* cv. Ochiai-fushinari	—	—
	a local variety of Sri Lanka	—	—
	Cucurbita maxima	—	—
Cruciferae	*Brassica campestris rapa*	—	—
	B. campestris pekinensis	—	—
	B. oleracea	—	—
	Raphanus sativus	—	—
Gramineae	*Zea mays*	—	—
Leguminosae	*Phaseolus vulgaris* cv. Otebo	—	—
	P. mungo	—	—
	Pisum sativum	—	+ (IL) — (UL)
	Vicia fabae	—	—
	Vigna sinensis cv. Black Eye	—	—
	cv. Kurodane 16-sasage	—	—
	Trifolium incarnatum	—	+ (IL) — (UL)
	Cassia tora	—	—
Pedaliaceae	*Sesamum indicum*	VNe, St–Ne	+ (UL)
Solanaceae	*Nicotiana glutinosa*	DSCS, Ne, Mo	+
	N. rustica	SCS–NL (IL) Mo, ST (UL)	+
	N. sylvestris	Ne, VNe, ST	+
	N. tabacum cv. Bright Yellow	SCS, VY, VNe, St–Ne, ST	+
	cv. White Burley	〃	+
	cv. KY–57	〃	+
	cv. Samsun	〃	+
	a local variety of Sri Lanka	〃	+
	cv. Turkish	SCS, NL (mild)	+

Table 11. (Continued)

Family	Species	Symptoms caused by No. 4 virus	Virus recovery
Solanaceae	*Petunia hybrida*	SM, ST	+
	Physalis floridana	SM (severe)	+
	P. peruviana	CL (mild)	+
	Physalis sp. (Sri Lanka)	VC, SM	+
	Datura stramonium	—	—
	Lycopersicum esculentum	VC, SM (mild)	+
	Solanum melongena	—	—
	S. nodiflorum	VC, SM	+
	Capsicum fultescens cv. Tabasco	NL (IL) SM (UL) death (sometimes)	+
	cv. Honenmidori	SM	+
	C. annuum cv. M. I. H.	SM	+
	cv. Santaka	SM	+

—: no reaction or unrecovered +: recovered IL: inoculated leaves UL: upper leaves
NL: necrotic lesion CL: chlorosis D: death SM: systemic mosaic VNe: vein necrosis
St-Ne: stem necrosis SCS: systemic chlorotic spots VY: vein yellowing VC: vein clearing
Mo: mottle ST: stunt Ne: necrosis DSCS: diffuse systemic chlorotic spots

necrosis were shown by *Sesamum indicum* 7 to 10 days after inoculation.

(b) Physical properties

In the determination of certain physical properties of this virus, *N. tabacum* cv. Bright Yellow was utilized as the source and test plants. Repeated trials using 5 to 6 plants per treatment were made. In the determination of the thermal inactivation point, test tubes containing 3 ml of fresh sap were heated in a hot water bath for 10 minutes at 45°, 50°, 55°, 60°, 65°, 70°, and 75°C. The results showed that the thermal inactivation point of the virus lies between 55° and 60°C. The dilution end point appeared to lie between 1 : 5,000 and 1 : 10,000. Undiluted juice of infected tobacco plants was stored in test tubes at room temperature (25°–30°C) for various periods. Virus infectivity in this case was lost between 3 and 4 days. The same juice was stored in test tubes in a refrigerator at 5°C for various periods. Virus infectivity in this case was lost between 9 and 10 days.

(c) Aphid transmission.

Three to five leaf stage plants of *Capsicum annuum* cv. M.I.H. and *N. tabacum* cv. Bright Yellow were used. Plants that had been infected for 12 to 15 days grown in a net-house were used as inoculum sources. As in the case of cucumber mosaic virus, green peach aphid, *Myzus persicae,* and cotton aphid, *Aphis gossypii,* were used for transmission tests. Results are summarized in Table 12.

As is evident from Table 12, the virus was transmitted by *Myzus persicae* from infected chilli to healthy chilli seedlings as follows: 5 min. acquisition time, 17 percent transmission; 10 min., 22 percent; 30 min., 38 percent; 60 min., 55 percent; and 120 min., 43 percent, respectively. A maximum in virus transmission was shown in 60 min. acquisition time.

In tests on the transmission from infected chilli to healthy tobacco seedlings, the

Table 12. Transmission of No. 4 virus from chilli by cotton aphid, *Aphis gossypii*, and green peach aphid, *Myzus persicae*, to healthy chilli and tobacco seedlings

Test plant	Aphid species	Acquisition feeding time	No. of inoculated plants	Inoculation feeding time	No. of aphids per plant	No. of infected plants	Transmission percentage
chilli	*Aphis gossypii*	10 min.	17	3 hrs.	2	2	12
	〃	20 min.	14	3	2	1	7
	〃	30 min.	10	3	4	3	30
	〃	60 min.	24	18	3	5	21
tobacco	〃	20 min.	10	3	3	1	10
	〃	30 min.	8	3	3	2	25
	〃	60 min.	11	18	4	2	18
chilli	*Myzus persicae*	5 min.	18	3	3	3	17
	〃	10 min.	9	3	2	2	22
	〃	30 min.	16	3	2	6	38
	〃	60 min.	20	18	4	11	55
	〃	120 min.	21	18	4	9	43
tobacco	〃	10 min.	9	3	2	1	11
	〃	30 min.	15	3	2	3	20
	〃	60 min.	10	18	4	3	30

virus was transmitted by the same aphid species as follows: 10 min. acquisition time, 11 percent transmission; 30 min., 20 percent; and 60 min., 30 percent, respectively. The rate of transmission was about 2 : 1 in favor of the chilli plant.

In case of *Aphis gossypii*, the virus was transmitted from infected chilli to healthy chilli seedlings as follows: 10 min. acquisition time, 12 percent transmission; 20 min., 7 percent; 30 min., 30 percent; and 60 min., 21 percent, respectively. In tests on the transmission from infected chilli to healthy tobacco seedlings, the virus was transmitted by the same aphid as follows: 20 min. acquisition time, 10 percent; 30 min., 25 percent; and 60 min., 18 percent, respectively. The rate of transmission was about equal in chilli and tobacco plants.

It was concluded that No. 4 virus can be transmitted by both species of aphids from infected chilli to chilli or to tobacco and from infected tobacco to tobacco or to chilli.

(d) Host range tests of the virus transmitted by aphids

The virus transmitted by *Myzus persicae* was inoculated mechanically to some tests plants from infected *Capsicum annuum* cv. M.I.H., *Nicotiana tabacum* cv. Bright Yellow and cv. Samsun, *N. glutinosa, N. rustica, Petunia hybrida, Lycopersicum esculentum, Physalis floridana, Datura stramonium, Capsicum frutescens* cv. Tabasco, *C. annuum* cv. M.I.H., *Chenopodium amaranticolor, Solanum tuberosum* cv. Arka, *Phaseolus vulgaris, Vigna sinensis* cv. Black Eye, and *Vicia fabae* were used as the test plants.

Results of these inoculation tests were the same as those of direct inoculations of the original virus from *Capsicum annuum* cv. M.I.H., *N. tabacum* cv. Bright Yellow and cv. Samsun and *N. rustica* developed relatively broad systemic chlorotic spots, necrotic lesions, vein necrosis, vein yellowing, stem necrosis, and/or stunting. Mosaic reaction was obtained on *Petunia hybrida, Physalis floridana,* and *C. annuum* cv. M.I.H., while *L. esculentum* showed slight mosaic symptoms. Diffuse chlorotic spots to mottle

occurred on upper leaves of *N. glutinosa*.

On inoculated leaves of *C. amaranticolor, C. frutescens* cv. Tabasco and *Solanum tuberosum* cv. Arka local lesion reaction was obtained when kept in a dark box for 24 hours after inoculation.

Datura stramonium, Phaseolus vulgaris, Vigna sinensis, and *Vicia fabae* showed no reaction.

(e) Cross-protection

It was found from electron microscopic study and host range tests that No. 1 virus is not a single virus, but a composite of tobacco mosaic virus (No. 1–A virus) and a virus resembling potato virus Y (No. 1–B virus). Cross-protection tests were therefore made to determine the degree of relationship between No. 4 virus and No. 1–B virus, and also between No. 4 virus or No. 1–B virus and authenticated potato virus Y of Japan (PVY-J) by Tomaru and Udagawa.

Nicotiana tabacum cv. Bright Yellow previously infected with No. 1–B virus (first virus) was inoculated with No. 4 virus (second virus). Similar tests were carried out between PVY-J and No. 4 virus and also between PVY-J and No. 1–B virus. At least five plants of *N. tabacum* cv. Bright Yellow were inoculated with the second virus on upper leaves 10 days after inoculation with the first virus. At the same time, five unifected *N. tabacum* cv. Bright Yellow plants were inoculated only with the second virus as the control. Local lesions appeared 7 days after inoculation on leaves inoculated with the second virus. The number of local lesions was counted 7 and 14 days after the second virus inoculation.

Table 13. Cross-protection tests among No. 4 virus, No. 1–B virus, and potato virus Y of Japan

Plants species	First virus	Second virus	Days after inoculation with the second virus	No. of tested plants	No. of lesions per leaf
N. tabacum cv. Bright Yellow	PVY–J	No. 4 virus	7	5	28
〃	none	No. 4 virus	7	5	95
〃	PVY–J	No. 4 virus	14	5	28
〃	none	No. 4 virus	14	5	117
〃	No. 1–B virus	No. 4 virus	7	5	21
〃	none	No. 4 virus	7	5	103
〃	No. 1–B virus	No. 4 virus	14	5	12
〃	none	No. 4 virus	14	5	124
〃	PVY–J	No. 1–B virus	14	5	24
〃	none	No. 1–B virus	14	5	108

Results of the cross-protection tests are summerized in Table 13. Average numbers of local lesions per leaf were: followed by No. 4 virus 7 days after inocualtion, 28 lesions; control No. 4 virus alone, 95 lesions; same treatment 14 days after inoculation, 28 lesions; control, 117 lesions; No. 1–B virus followed by No. 4 virus 7 days after inoculation, 21 lesions; No. 4 virus alone, 103 lesions; same treatment 14 days after inoculation, 12 lesions; control, 124 lesions; PVY-J followed by No. 1–B virus 14 days after inoculation, 24 lesions; No. 1–B virus alone, 108 lesions.

These results clearly showed that No. 4 virus and No. 1–B virus are identical or very close, and that both viruses are also closely related with potato virus Y. Therefore, both viruses were identified as a strain of potato virus Y.

(f) Virus particles

Young leaves of *Capsicum frutescens* cv. Honenmidori showing systemic mosaic with No. 4 virus was harvested 14 days after inoculation, and used for observation of virus particles. The cut edge of a leaf was dipped in a drop of distilled water placed on Formvar-coated grids and dried in a disiccator; then washed twice in distilled water, dried again, shadowed with palladium, and viewed on a Hitachi Model HU-11 electron microscope.

Particle lengths varied from about 500 to 850 nm, as shown in Fig. 2 and Plate 9. Normal length was estimated from 700 to 750 nm, the range comprising 42% of the particle population.

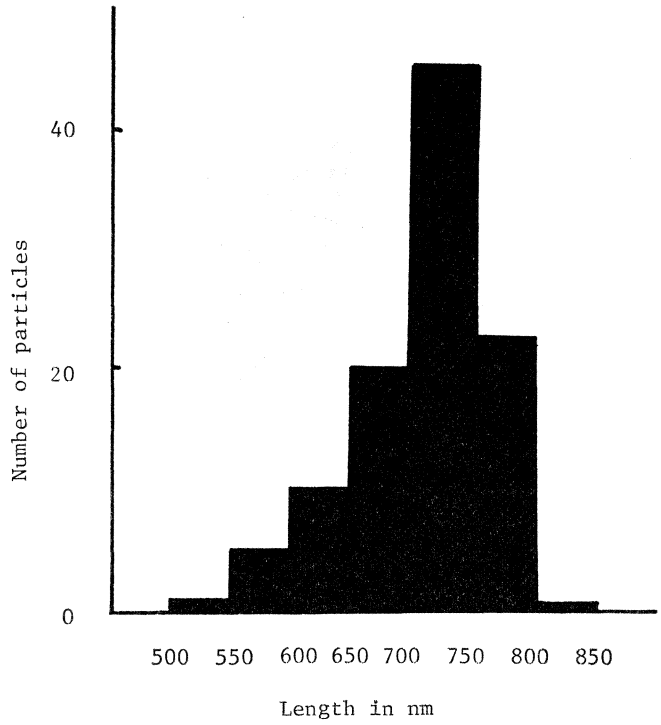

Fig. 2. Distribution of particle lengths of No. 4 virus. (dipping method)

(g) Ultrathin sections of chilli leaves infected with No. 4 virus

Plants of *Capsicum annuum* cv. M.I.H. were inoculated with No. 4 virus, and left for 14 to 20 days, when systemic symptoms were obvious. Samples, 2 to 3 mm^2, were taken from systemically infected leaves, fixed in 5% glutaraldehyde in phosphate buffer, pH 7.2 for 3 days (because these samples were taken back from Sri Lanka to Japan) and rinsed with distilled water once before and three times after postfixation for 1 hour with OsO_4 solution, pH 7.2. After dehydration in graded dilutions of ethanol, they were treated with propylene oxide, and embedded in Epon 128. Ultrathin sections

were cut with glass knives from the blocks by a Porter-Blum Ultra-Microtome MT-2. The sections were stained with uranyl acetate and lead citrate, and electron micrographs were taken with Hitachi Model HU-11 electron microscope.

Plate 10, 11 and 13 show large areas of cytoplasm containing several pinwheels, some in cross-section. Plate 12 shows a portion of a mesophyll cell containing laminated aggregates and circular inclusions, but pinwheels are not present. Many cylindrical inclusions are shown in a portion of a mesophyll cell in Plate 11 and 12. These inclusions appear similar to those induced by several members of the potato virus Y group.

6. Characterization of No. 5 virus (Chilli leaf curl virus)

Chilli leaf curl disease transmitted by the whitefly, *Bemicia tabaci*, has been reported in Sri Lanka by Fernando and Peiris (1957). In this chapter, some additional data on whitefly transmission and host range of this virus are presented. The tests were made mainly to provide information on relationships among chilli leaf curl virus and tomato and tobacco leaf curl viruses, all transmitted by the same *Bemisia tabaci*.

(a) Graft transmission

Chilli plants infected by whitefly transmission from originally infected chilli were used as the source plants. The tests were carried out in a net-house throughout year, using infected chilli as the scion and healthy chilli, tomato, and tobacco seedlings as the stock.

Results of graft transmission tests are summarized in Table 14.

Table 14. Graft transmission tests from infected chilli to healthy chilli, tomato, and tobacco seedlings

Stock	Scion	Longevity of scion in days	No. of test plants	No. of infected plants
Capsicum annuum cv. M. I. H.	Infected *C. annuum* cv. M. I. H.	1- 5	22	0
〃	〃	6-10	5	0
〃	〃	11-15	2	1
〃	〃	16-20	4	1
〃	〃	21-30	18	15
〃	〃	over 31	12	12
Lycopersicum esculentum cv. Fukuju	〃	1- 5	8	0
〃	〃	6-10	3	0
〃	〃	11-20	5	1
〃	〃	over 21	14	6
Nicotiana tabacum cv. Bright Yellow	〃	1- 5	3	0
〃	〃	6-10	4	0
〃	〃	11-20	4	0
〃	〃	21-40	16	0
〃	〃	over 41	21	0

Chilli leaf curl virus was successfully transmitted by grafting from chilli to chilli when the scion lived for more than 11 days on the stock. Hundred percent transmission

was obtained when the scion lived for more than 30 days. When tomato was used as the stock, symptoms of leaf curl appeared on tomato when the scion lived for more than 10 days. The percentage of transmission was less than 50. When tobacco was used as the stock, no symptoms appeared, even if the scion in many cases lived for more than 40 days.

(b) Whitefly transmission

The whiteflies, *Bemisia tabaci*, were caged generally in groups on healthy *Solanum nodiflorum*. Naturally infected chilli plants showing leaf curl were used as the original disease source. The test plants for use in the experiments were raised in a net-house and sprayed at regular intervals to keep resonably free from insects and mites.

(i) Number of insects required for various levels of transmission

Groups of whiteflies were kept on infected chilli plants for 48 hours and then for 48 hours on healthy chilli seedlings for inoculation.

Results of the tests are shown in Table 15.

Table 15. Transmission of chilli leaf curl virus as a function of the number of insects kept on each test plant

No. of insects per plant	Transmission rate*	Percentage
5	11/24	46
10	8/11	73
20	9/12	75
30	14/18	78

* No. of infected plants/No. of inoculated plants

It is clear that transmission to 70 to 80 percent of the test plants were attained when more than 10 whiteflies were caged per test plant.

(ii) Acquisition feeding period

After different acquisition feeding periods on infected plants, groups of 15 to 20 whiteflies were kept for 72 hours on healthy chilli seedlings.

The results are shown in Table 16.

As is evident from Table 16, the virus was transmitted by *Bemisia tabaci* from

Table 16. Transmission of chilli leaf curl virus by whiteflies after different acquisition feeding periods on infected chilli plants

Acquisition feeding period	Transmission rate*	Percentage
10 min.	0/ 25	0
30 min.	1/ 21	5
1 hr.	6/ 20	30
3 hrs	2/ 20	10
1 day	41/104	39
2 days	38/ 51	74
3 days	40/ 61	66
4 days	17/ 25	68
10 days	20/ 20	100

* No. of infected plants/No. of inoculated plants

infected chilli to healthy chilli seedling as follow: 10 min. acquisition feeding period, no transmission; 30 min. acquisition feeding period, 5 percent transmission; 1 hr., 30 percent; 3 hrs., 10 percent; 1 day, 39 percent; 2 days, 74 percent; 3 days, 66 percent; 4 days, 68 percent; and 10 days, 100 percent, respectively. The whiteflies transmitted the virus following a minimum acquisition feeding period of 30 min. on infected plants under net-house conditions. A fairly high rate of transmission was obtained after 48 hours or more of acquisition feeding.

(iii) Inoculation feeding period

Groups of 15 to 20 whiteflies reared on infected chilli plants were kept for different periods on healthy chilli seedlings.

The results are shown in Table 17. Transmissions were observed after an inoculation feeding period of 60 min. or more on the test plants, but none after 10 to 30 minutes.

Table 17. Transmission of chilli leaf curl virus by whiteflies reared on infected chilli plants after different inoculation feeding periods on healthy chilli seedlings

Inoculation feeding period on test plants	Transmission rate*	Percentage
10 min.	0/15	0
20 min.	0/14	0
30 min.	0/10	0
60 min.	2/15	13
180 min.	4/11	36

* No. of infected plants/No. of inoculated plants

(iv) Serial transmission

Caged whiteflies after acquisition feeding periods of from 30 min. to 72 hours on infected chilli plants were transferred to healthy chilli seedlings. The insects were transferred to new test seedlings at 1 or 3 days intervals until their death.

The results of these experiments are shown in Table 18. A single whitefly allowed to feed for 3 hours on virus infected plants was able to transmit the virus within 1 to 3 days after the initiation acquisition feeding, and also they could transmit the virus for 12 to 15 days after acquisition feeding. Sometimes whiteflies could not transmit the virus within 6 days after acquisition. Some failed to get the virus from infected chilli plants under these experimental conditions. For the most part, whiteflies transmitted the virus for 9 days after acquisition feeding. It is clear that the chilli leaf curl virus persists in its vector, but not for the full life span of the insect.

(v) Test for virus transmission to progeny

From the above test (iv), it is recognized that chilli leaf curl virus does not persist throughout the life span of the vector. However, it is necessary to see whether virus transmission to the progeny of viruliferous females might occur through their eggs. Viruliferous whiteflies were allowed to produce eggs on *Gossypium indicum* and *Solanum nodiflorum* which are immune to chilli leaf curl virus, and they were killed by insecticide. Ten adults reared from the eggs were placed in cages on each of 20 chilli plants, and these experiments were repeated three times. None was found to transmit the virus.

Table 18. Serial transmission of chilli leaf curl virus by whiteflies, *Bemisia tabaci*, after different acquisition feeding periods

Acquisition feeding period	No. of insects	Days after acquisition feeding					
		1	3	6	9	12	15
30 min.	1	−	−	−	−	D	
1 hr.	1	−	−	−	−	−	D
3 hrs	1	+	+	+	+	−	−
〃	1	−	−	−	D		
24 hrs	2	+	+	+	+	+	−
〃	2	+	+	+	−	−	D
〃	2	+	+	+	+	D	
〃	2	−	+	+	+	D	
〃	1	+	+	+	+	D	
48 hrs	1		+	−	−	D	
〃	1		+	+	+	D	
〃	1		+	+	+	D	
〃	2		+	+	+	+	D
〃	2		+	−	−	D	
〃	2		+	−	−	D	
72 hrs	1		+	−	D		
〃	1		+	−	D		
〃	1		+	−	D		
〃	1		+	+	−	D	
〃	1		+	+	+	D	
〃	1		+	+	+	D	
〃	1		+	+	−	−	−
〃	2		+	+	−	+	D
〃	2		+	+	+	+	D
〃	2		+	−	D		
〃	2		−	−	−	D	
〃	2		+	+	+	D	
〃	2		+	+	+	D	

+ : Transmission − : Non-transmission D : Death of insect

(c) Host range

Two to three palnts of each species to be tested were inoculated by caging 20 to 30 whiteflies per plant for 72 hours. The insects had been given an acquisition feeding of 48 hours on infected chilli plants.

To determine differences between whitefly feeding injury and virus symptoms, virus-free whiteflies were kept on the same species of test plants for the same period as a control.

Inoculation tests were repeated several times throughout year. One month after inoculation, a group of virus-free whiteflies were fed on some of the inoculated plants for 72 hours and then transferred to healthy chilli seedlings to determine whether the inoculated plants were symptomless carriers; and whether the observed symptoms on some of the test plants were caused by virus infection.

Results of the host range tests are summarized in Table 19. The following species

Table 19. Transmission tests by whiteflies, *Bemisia tabaci*, to determine susceptibility of various plant species to chilli leaf curl virus

Plants tested	No. of inoculated plants	No. of infected plants	Symptom	Virus recovery
Amarantaceae				
Emilia sonchifolia	10	0	—	—
Gomphrena globosa	5	0	—	—
Chenopodiaceae				
Beta vulgaris	5	0	—	—
Chenopodium amaranticolor	6	0	—	—
Spinacia olercea	6	0	—	—
Compositae				
Callistephus chinensis	6	0	—	—
Zinnia elegans	6	0	—	—
Ageratum conyzoides	10	2	VC	+
Synedrella nodiflora	11	0	—	—
Helianthus annuum	5	0	—	—
Eupatrium japonicum	8	0	—	—
Cucurbitaceae				
Cucumis sativus	6	0	—	—
Euphorbiaceae				
Acalypha indica	10	0	—	—
Leguminosae				
Cassia tora	5	0	—	—
Phaseolus vulgaris	5	0	—	—
Trifolium incarnatum	5	0	—	—
T. repens	5	0	—	—
Vicia fabae	5	0	—	—
Vigna sinensis	5	0	—	—
Malvaceae				
Gossypium herbaceum	10	0	—	—
Hibiscus esculentum	15	0	—	—
Pedaliaceae				
Sesamum indicum	6	0	—	—
Solanaceae				
Capsicum annuum cv. M. I. H.	10	10	VC, LC, Ye, St	+
cv. Santaka	10	10	VC, LC, Ye, St	+
C. frutescens cv. Honenmidori	5	3	LC	+
cv. Tabasco	6	4	LC	+
Datura stramonium	8	8	VC, LC	+
Lycopersicum esculentum cv. Fukuju	6	3	Ye, St	+
Nicotiana glutinosa	5	0	—	—
N. rustica	6	0	—	—
N. tabacum cv. Bright Yellow	5	2	LCu (slight)	+
cv. Samsun	6	4	〃	+
Physalis floridana	8	5	VC	+
P. peruviana	5	4	LCu (slight)	+
Solanum melongena	5	0	—	—
S. nodiflorum	8	0	—	—
S. tuberosum cv. Arka	5	0	—	—

VC: vein-clearing LC: leaf curling LCu: leaf curvature Ye: yellowing St: stunting
—: negative +: positive

and varieties were susceptible to the virus and produced clear symptoms of the disease: *Petunia hybrida, Lycopersicum esculentum* cv. Fukuju and cv. Ponterosa, *Physalis floridana, P. peruviana, Ageratum conyzoides,* and *Datura stramonium. Nicotiana tabacum* cv. Samsun and cv. Bright Yellow produced slight leaf curvature and outward curling, but no enation symptoms. *Petunia hybrida* and *Datura stramonium* showed severe leaf curl symptoms and finally died.

The following species and varieties were apparently immune to the virus: *Nicotiana glutinosa, N. rustica, Solanum melongena, S. nodiflorum, S. tuberosum, Beta vulgaris, Chenopodium amaranticolor, Spinacia oleracea, Callistephus chinensis, Zinnia elegans, Synedrella nodiflora, Helianthus annum, Eupatrium japonicum, Cucumis sativus, Phaseolus vulgaris* cv. Black Eye and cv. Kurodane 16-sasage, *Vigna sinensis, Vicia fabae, Trifolium incarnatum, Cassia tora, Gomphrena globosa, Emilia sonchifolia, Sesamum indicum, Acalypha indica, Hibiscus esculentum,* and *Gossypium herbaceum.*

(d) Relationship between chilli, tomato, and tobacco leaf curl diseases

In order to determine the relationship among chilli (CLCV: chilli leaf curl virus), tomato (ToLCV: tomato leaf curl virus), tobacco (TLCV: tobacco leaf curl virus) leaf curl viruses, further experiments were made by graft and whitefly transmissions.

Tobacco leaf curl disease may be divided into two groups according to symptoms on tobacco (Komuro, Y. 1971). The first type, tobacco leaf curl-1 (TLC-1), shows typical tobacco leaf curl symptoms and enations on the lower surface of leaves, while the second type, tobacco leaf curl-2 (TLC-2), shows no enation but only vein curvature and sometimes rugose symptoms. Characteristic symptoms of tomato leaf curl disease in Sri Lanka are vein clearing, pale colour of the upper leaves, stunting, and inward or outward curling of leaf margins. Chilli leaf curl disease is characterized by vein clearing, upward curling of the leaves and severe stunting of the plants; also leaves become pale green in colour.

Thung (1932) differentiated three types of leaf curl (Kroepoek): (1) common kroepoek, in which leaf edges are curled in places towards the dorsal side, and show thickening and outgrowths (enations) of virus; (2) curl disease, characterized by curling of the whole leaf edge towards the dorsal side, with enations on veins, the lamina arching towards the ventral side between finer veins; and (3) transparent kroepoek distinguished by curling of leaves towards the ventral side and clearing of veins, enations being absent. It is possible that more than one leaf curl virus may be concerned.

Results of graft transmission tests on tobacco leaf curl disease to healthy chilli, tobacco, and tomato seedlings are shown in Table 20. As is evident from Table 20, when TLC-1 infected tobacco scion was grafted to tomato seedlings, new shoots from the stock in 8 out of 10 plants grafted showed typical leaf curl symptoms (vein clearing and then severe stunting) two weeks after grafting, while TLC-2 infected tobacco scion on new shoots of tomato stock produced vein clearing, yellowing, and stunting symptoms in 6 out of 8 plants two weeks after grafting.

When TCL-1 infected tobacco scion was grafted to healthy old chilli seedlings, new shoots from the stock did not develop leaf curl symptoms three weeks after grafting, but same infected tobacco scion was grafted to 6 to 8 leaf stage of chilli seedlings, new shoots from the stock showed yellowing in 3 out of 3 plants about two weeks after grafting.

When TLC-1 tobacco scion was grafted to healthy tobacco stock, new shoots from 10 out of 10 stock plants developed typical tobacco leaf curl symptoms with enations,

Table 20. Graft transmission tests of tobacco leaf curl viruses to healthy chilli, tobacco, and tomato seedlings

Species of stock	Species of scion	No. of test plants	No. of infected plants	Symptoms
L. esculentum cv. Ponterosa	*N. tabacum* local variety (TLC-1)	10	8	vein clearing, severe stunting
〃	〃 (TLC-2)	8	6	vein clearing, yellowing, stunting
C. annuum cv. M. I. H.	*N. tabacum* local variety (TLC-1)	3	0	none
〃	〃 (TLC-2)	3	3	yellowing
N. tabacum cv. Bright Yellow	*N. tabacum* local variety (TLC-1)	10	10	vein clearing, leal curling, stunting, enation
〃	〃 (TLC-2)	10	9	vein clearing, leaf curvature, no enation

while TLC-2 infected tobacco scion to healthy tobacco stock, new shoots from 9 out of 10 stock plants showed vein clearing and leaf curvature without enation two weeks after grafting.

Whitefly transmission tests of tobacco and tomato leaf curl viruses to healthy chilli, tomato, and tobacco seedlings were carried out in July, August of 1971, and the results of this experiments are summerrized in Table 21.

Table 21. Whitefly transmission tests of tobacco and tomato leaf curl viruses to healthy chilli, tomato, and tobacco seedlings

Virus	Species of test plant	No. of insects per plant	No. of test plants	No. of infected plants	Symptoms
TLC-1	*Nicotiana tabacum* cv. Samsun	30	8	3	VC, LC, En
〃	*Lycopersicum esculentum* cv. Ponterosa	30	8	5	VC, Ye
〃	*Capsicum annuum* cv. M. I. H.	30	5	0	none
TLC-2	*Nicotiana tabacum* cv. Samsun	30	10	4	VC, LCu
〃	*Lycopersicum esculentum* cv. Ponterosa	30	10	2	VC, Ye
〃	*Capsicum annuum* cv. M. I. H.	30	19	3	VC, LCu
ToLC	*Nicotiana tabacum* cv. Samsun	30	8	1	VC, LCu no enation
〃	*Lycopersicum esculentum* cv. Ponterosa	30	10	3	VC, Ye
〃	*Capsicum annuum* cv. M. I. H.	30	6	2	VC, LC

VC: vein clearing LC: leaf curl En: enation Ye: yellowing LCu: leaf curvature

In these tests, it was possible to recognize vein clearing and leaf curling with enations 15 to 18 days after inoculation with TLC-1 virus to healthy tobacco in 3 out of 8 plants; yellowing without enation two weeks after inoculation with the same virus to healthy tomato in 5 out of 8 plants; but chilli plants did not develop any leaf curl symptoms and yellowing.

Inoculation of TLC-2 virus to the same test plants using whitefly, produced vein clearing and slight leaf curling (leaf curvature) without enaction in 4 out of 10 tobacco plants; 2 out of 10 tomato plants showed vein clearing, yellowing, and stunting; and 3 out of 10 chilli plants produced typical leaf curl symptoms similar to that of chilli leaf curl 15 to 18 days after inoculation.

Inoculation of ToLC (tomato leaf curl virus from Jaffna) to the same test plants using whitefly produced vein clearing and slight leaf curling without enation 15 to 18 days after inoculation in 1 out of 8 tobacco plants; 3 out of 10 tomato seedlings showed vein clearing, yellowing, and severe stunting 15 to 18 days after inoculation; and 2 out of 6 chilli plants showed typical leaf curl symptoms 15 to 18 days after inoculation.

7. Detection of viruses from seedlings in nursery beds

About three hundred chilli seedlings were collected from nurseries in Jaffna, Anuradhapura, and Vavuniya during Maha season 1969/1970 and Yala season 1970. A part of the samples were tested for the presence of viruses, by sap inoculation and whitefly inoculation to test plants, and the rest were kept in pots in a net-house for 40 days for observation any symptom appearance. Test plants used were *N. tabacum* cv. Bright Yellow and cv. Samsun, *N. glutinosa, Chenopodium amaranticolor, Vigna sinensis* cv. Black Eye, *Phaseolus vulgaris* cv. Otebo, *Capsicum annuum* cv. M.I.H., and *Vicia fabae.*

Table 22. Detection of viruses by sap inoculation, in chilli seedlings from nurseries in three districts

District	No. of seedlings tested	Virus isolated					
		TMV	CMV	PVY	Unidentified virus	Total	%
Jaffna	30	1	2	2	0	4	13
Anuradhapura	54	1	2	0	0	3	6
Vavuniya	44	1	3	0	1	5	11
Total	128	3	6	2	1	12	
%		2.3	4.6	1.5	0.8		9

Table 23. Detection of leaf curl virus by whitefly transmission tests, in chilli seedlings from nurseries in three districts

District	No. of seedlings tested	No. of seedlings showing symptoms resembling leaf curl	%	No. of infected plants	Total
Jaffna	10	1	10	0	0
Anuradhapura	14	4	28	0	0
Vavuniya	12	2	17	0	0
Total	36	7	19	0	0

Table 24. Observation of virus symptoms in chilli seedlings collected from nurseries in three districts and kept in a net-house for 40 days

District	No. of seedlings	No. of plants showing symptoms			
		Mosaic	Leaf curl	Total	%
Jaffna	44	5	0	5	11
Anuradhapura	51	5	0	5	10
Vavuniya	36	4	0	4	11
Total	131	14	0	14	11

Results are shown in Table 22, 23, and 24. As is evident from these Tables, chilli seedlings from nurseries in these districts were infected with viruses such as tobacco mosaic virus, cucumber mosaic virus, a strain of potato virus Y, and an unidentified virus. Chilli leaf curl virus was not detected by insect transmission tests, and also seedlings kept under observation in the net-house for 40 days showed no leaf curl symptoms. The seedlings were thus infected with mosaic viruses at the nursery stage, but not with leaf curl virus.

IV. Geographic distribution of chilli viruses

Chilli plants, mostly *Capsicum annuum*, are grown all over Sri Lanka. Although crops can be grown during the entire year, most plantings are made either for Maha or Yala season harvest. Severe virus diseases are quite prevalent. During 1969 to 1971, a geographic survey of the viruses affecting chilli was attempted to provide information for developing control plans. Inoculated indicator plants were kept under observation for approximately three weeks, and virus identifications were made according to combination of symptoms on the indicator plants as mentioned earlier.

Table 25 lists the number of samples in which viruses were identified by sap-inoculation and by insect transmission tests.

Table 25. The geographic distribution of chilli viruses in six districts

District	No. of plants infected with												
	TMV + PVY	TMV	TMV + CMV	CMV	CMV + PVY	PVY	LC	LC+ TMV+ PVY	LC + TMV	LC + CMV	LC + PVY	Uni-dentified virus	Total
Jaffna	0	8	0	3	4	10	2*	0	0	0	1	10	38
Puttalam	0	0	0	0	0	0	0	0	0	0	1	0	1
Anuradhapura	2	1	3	4	0	0	5	1	2	2	0	0	20
Vavuniya	0	0	1	1	0	0	2	3	0	1	0	0	8
Badulla	0	0	0	5	0	0	0	0	0	0	0	0	5
Kandy	2	2	0	5	0	0	0	0	0	0	0	6	15
Total	4	11	4	18	4	10	9	4	2	3	2	16	87

* One diseased plant showed symptoms resembling leaf curl which could not be transmitted by whitefly. Other plants showed leaf curl symptoms which were transmitted by whitefly to healthy chilli seedlings.

Field-grown chilli plants, *Capsicum annuum,* are subject to infection by at least five viruses: tobacco mosaic virus, cucumber mosaic virus, potato virus Y, an unidentified virus, and chilli leaf curl virus. Cucumber mosaic virus as well as chilli leaf curl virus were found in the fields all over the island, the incidence of chilli leaf curl virus being higher in inland areas (Anuradhapura and Vavuniya) than in coastal areas (Jaffna and Puttalam). Tobacco mosaic virus and the unidentified virus were commonly encountered in Jaffna and Kandy districts. Potato virus Y appeared to be endemic and widely spread in Jaffna district. Multiple infections with potato virus Y and other viruses were found in Anuradhapura, Vavuniya and Kandy districts, suggesting that chilli or other crops in these areas are to some extent affected with this virus.

Generally, mosaic diseases were found mainly in coastal areas of the dry zone (Jaffna) and inland areas of the wet zone (Kandy and Badulla districts), and leaf curl disease appeared to be endemic and widely spread in inland areas of the dry zone (Anuradhapura and Vavuniya districts).

V. Insecticidal control of chilli viruses

1. Field spraying trials

Several workers have reported on the use of insecticides in controlling non-persistent aphid-borne viruses (Watson, 1937, Swenson, 1954, and Simons, 1957). Economic control of henbane mosaic through the use of nicotine was reported by Watson, but in other cases usually no appreciable reduction in spread of non-persistent viruses resulted from the use of insecticides. Futhermore, there have been no reports on the control of whitefly-borne virus in chilli.

In order to obtain information on the control of viruses affecting chilli plants in dry and wet zones of Sri Lanka, the following experiments were carried out in Peradeniya (wet zone) and Jaffna (dry zone).

In Peradeniya, experiments were made in the fields of Central Agricultural Research Institue. *Capsicum annuum* cv. M.I.H. was sown on 17th November 1970 (Maha season trial, 1970/1971) and 25th June, 1971 (Yala season trial, 1971), respectively, and allowed to grow for about one month in a net-house at the Division of Plant Pathology, C.A.R.I. to get virus-free chilli seedlings. These chilli seedlings were transplanted to the experimental fields on 18th December, 1970 and 25th July, 1971, respectively. An open area of 12×12 feet (144 feet^2) was separated out into 2 blocks. Each block consisted of 24 hills, spaced two to three feet apart with 2 plants per hill. Fourty eight chilli plants per block were planted in four rows of 12 plants (6 hills) per row. All blocks were separated by 3 feet width of cultivated borders. Irrigation in the fields were applied from canals at intervals of 2 to 3 days when there was no rain; otherwise, usual horticultural practices were employed.

In Jaffna, experiments were made in the fields of Thinnavely Farm of C.A.R.I. Experimental design was almost the same as in Peradeniya. *Capsicum annuum* cv. M.I.H. was sown on 10th December, 1970 (Maha season trial, 1970/1971), and allowed to grow for one month in a net-house at C.A.R.I. in Peradeniya to get virus-free seedlings. In Yala season 1971, *Capsicum annuum* cv. M.I.H. and cv. Santaka (a Japanese variety of red pepper) were sown on 22th April in nurseries. In the nurseries, sprayings of

Sumithion at a dilution of 1 : 1,500 were carried out at 7 days intervals. Additional sprayings were applied at weekly interval until transplanting.

After transplanting a position of each treatments in the fields were selected at randam, 4 blocks per a treatment was selected among all blocks, and these blocks received the following insecticides at 10 day intervals: 1) 18 ml of Sumithion 50% EC (Sumitomo Chemical Co. LTD., Osaka, Japan) diluted with 4 gallons water sprayed until 50 days after transpalnting; and 36 ml of Sumithion 50% EC diluted with 8 gallons of water sprayed over 60 days period on growing chilli field. 2) Cyanox 50% EC (Sumitomo Chemical Co. LTD., Osaka, Japan) diluted and sprayed in the same way as in Sumithion treatments. 3) 18 grams of Padan (Takeda Chemical Co. LTD., Osaka, Japan) dissolved in 4 gallons of water sprayed until 50 days after transplanting; and 36 grams of Padan in 8 gallons of water sprayed over 60 days period on growing chilli fields. 4) 48 grams of Ekatin-TD granular type (Sankyo Co. LTD., Tokyo, Japan) applied to the soil 2 grams per hill at 20 day intervals from transplanting time until 30 days before harvesting. 5) PSP-204 (Hokko Chemical Co. LTD., Tokyo, Japan) applied in the same way as in Ekatin-TD treatments.

Four blocks were kept as the control without any chemical treatments.

The rate of virus disease incidence was determined by visual symptom observation and calculating the percentage of plants affected. Yield figures were given by average kilogram fresh weight per hill.

Effects of spraying Sumithion, Cyanox, and Padan on the incidence of mosaic type virus diseases in the fields in Maha season 1970/1971 at Jaffna and Peradeniya are shown in Figs. 3, 5, and 7; and in Yala season 1971 at both districts are shown in Figs. 4, 6, and 8.

The insecticides were effective. In Jaffna 50 days after transplanting, the percentage of mosaic type diseases in the three sprayed plots was about 10% as compared to about 30% in the control. However, in Yala season, 50 days after transplanting, the percentage of mosaic type diseases in sprayed by Sumithion and Cyanox were increased about 2 times controlled as compared to that of Maha trial in same treatments.

In Peradeniya, in Maha season 1970/1971, Sumithion was found to be more effective than the others. The disease percentage in the Sumithion sprayed plot was only 22% 70 days after transplanting, while non-sprayed control plot showed 100% infection 70 days after transplanting. In Yala season 1971, Sumithion, Cyanox, and Padan were found to be only a little effective compared with the control.

Effects of introducing Ekatin-TD and PSP-204 to the soil on the incidence of mosaic type virus diseases in Maha season 1970/1971 are shown in Figs. 9 and 11; and also in Yala season 1971 are shown in Figs. 10 and 12.

In Jaffna, in Maha season 1971, the percentage of mosaic type virus diseases was 6% with Ekatin-TD, 13% with PSP-204, and 26% in the control, 60 days after transplanting. First symptoms of mosaic type appeared 50 days after transplanting in Ekatin-TD plot and 40 days after transplanting in PSP-204 plot, while 12% of the plants already showed symptoms in the control 20 days after transplanting. In Peradeniya, Ekatin-TD treatment was effective, the percentage of mosaic type virus diseases being 5% 50 days and 43% 90 days after transplanting; control showed 74% 50 days and 100% infection 70 days after transplanting. PSP-204 was a little less effective, the percentage of mosaic type virus diseases showed 18% 50 days and 80% 90 days after transplanting; control showed 72% 50 days and 100% infection 70 days after transplanting. The first

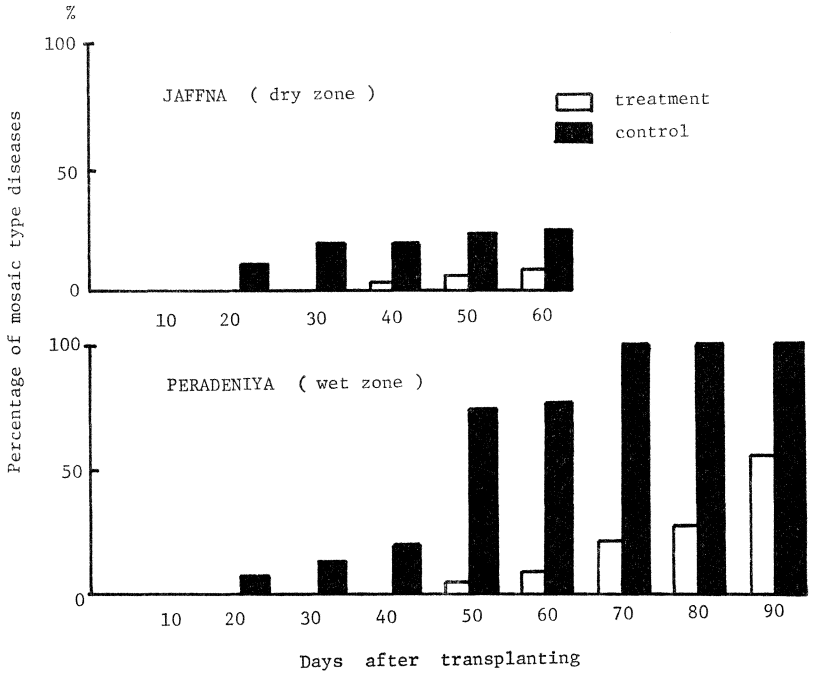

Fig. 3. Effect of spraying Sumithion on the incidence of mosaic type diseases in chilli fields. (Maha 1970/1971)

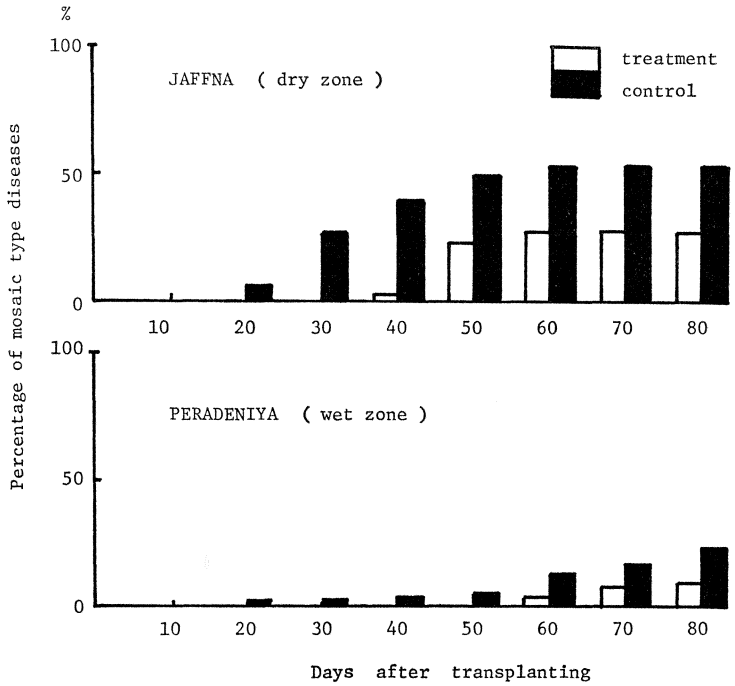

Fig. 4. Effect of spraying Sumithion on the incidence of mosaic type diseases in chilli fields. (Yala 1971)

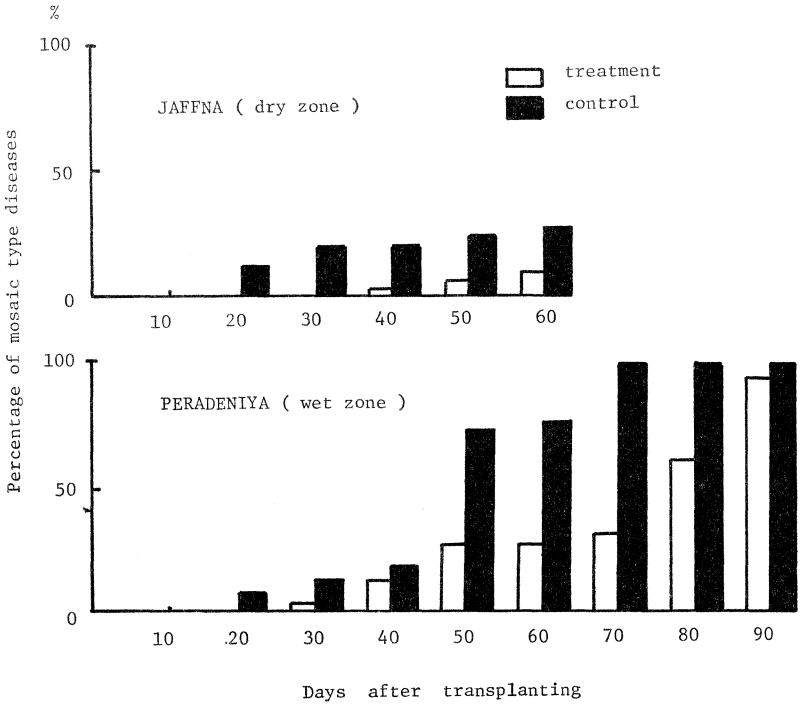

Fig. 5. Effect of spraying Cyanox on the incidence of mosaic type diseases in chilli fields. (Maha 1970/1971)

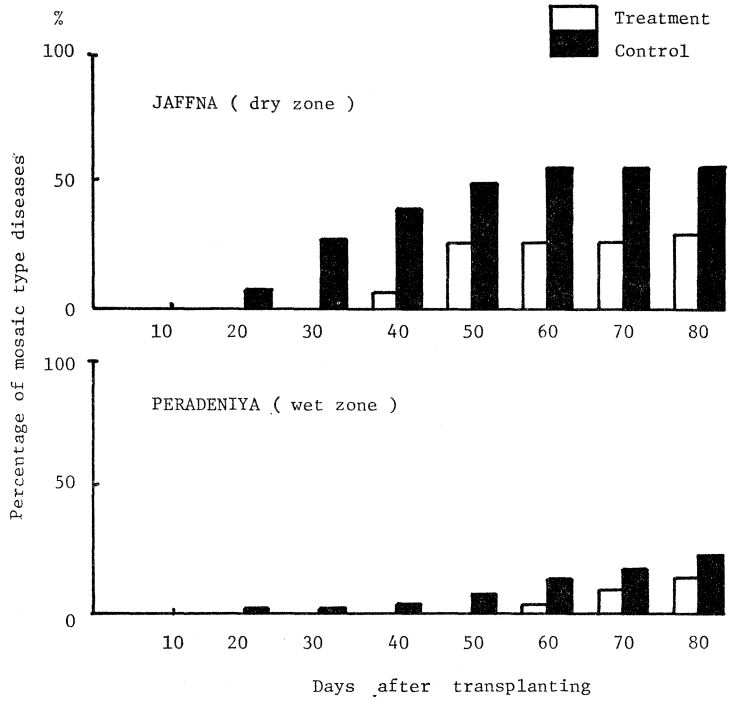

Fig. 6. Effect of spraying Cyanox on the incidence of mosaic type diseases in chilli fields. (Yala 1971)

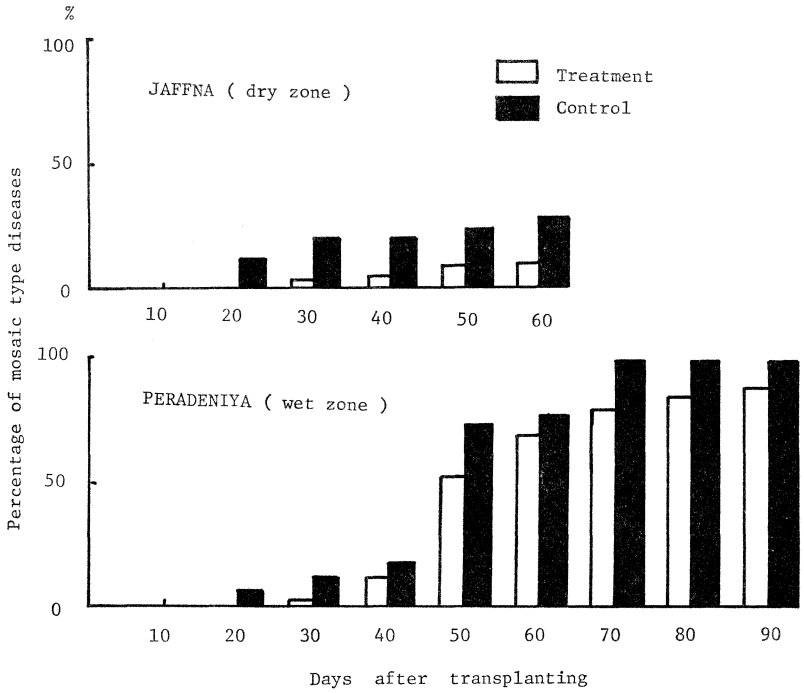

Fig. 7. Effect of spraying Padan on the incidence of mosaic type diseases in chilli fields. (Maha 1970/1971)

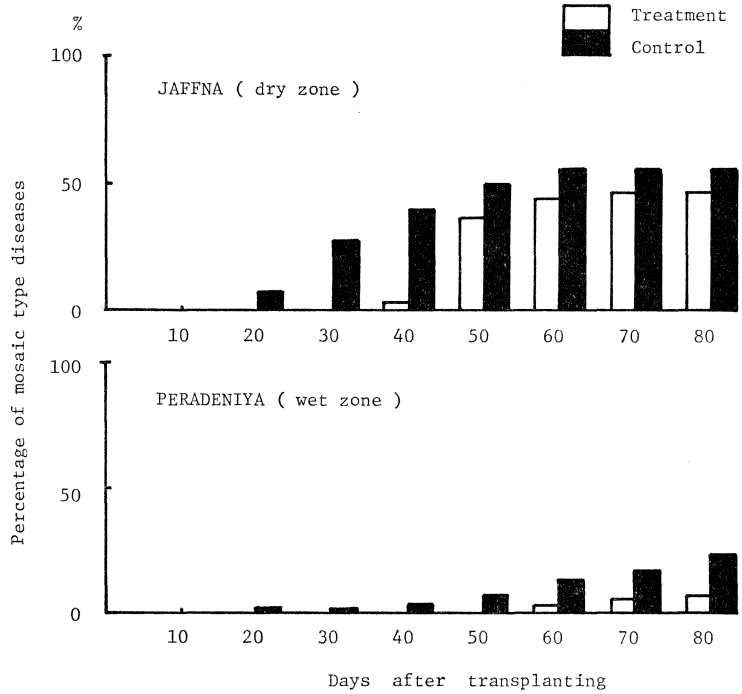

Fig. 8. Effect of spraying Padan on the incidence of mosaic type diseases in chilli fields. (Yala 1971)

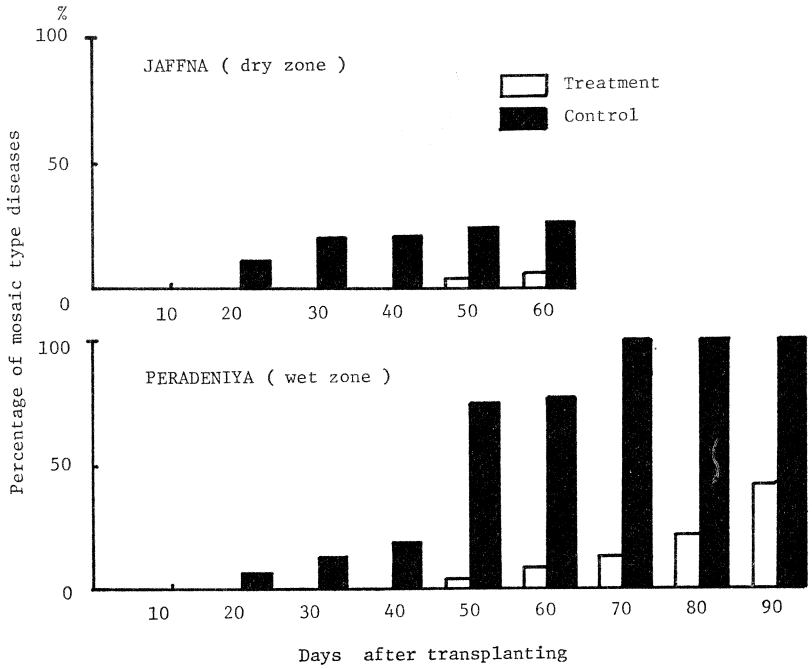

Fig. 9. Effect of Ekatin-TD on the incidence of mosaic type diseases in chilli fields. (Maha 1970/1971)

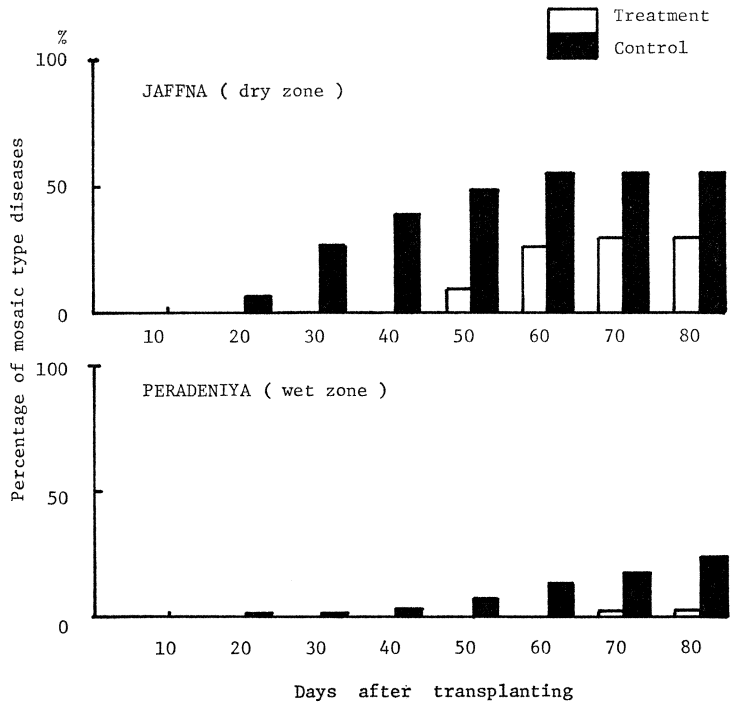

Fig. 10. Effect of Ekatin-TD on the incidence of mosaic type diseases in chilli fields. (Yala 1971)

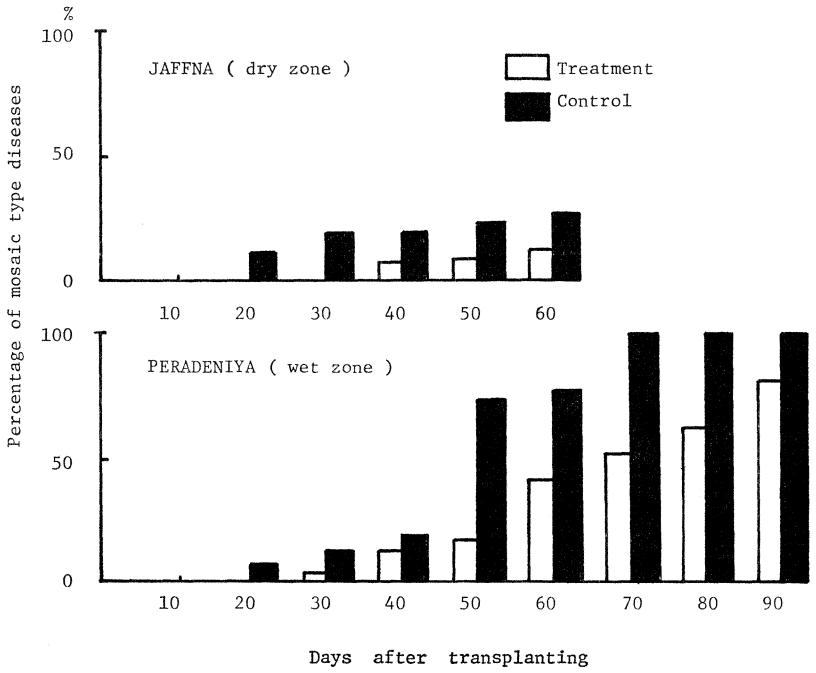

Fig. 11. Effect of PSP-204 on the incidence of mosaic type diseases in chilli fields. (Maha 1970/1971)

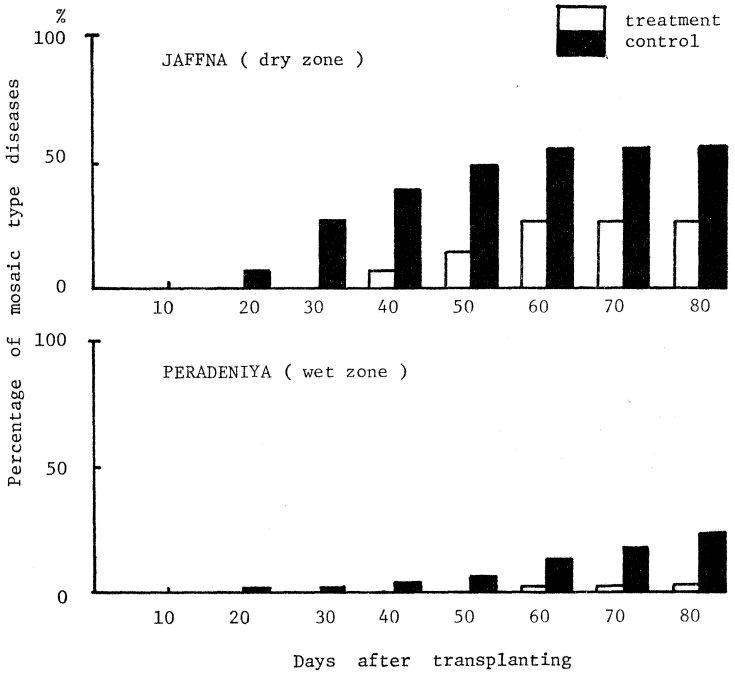

Fig. 12. Effect of PSP-204 on the incidence of mosaic type diseases in chilli fields. (Yala 1971)

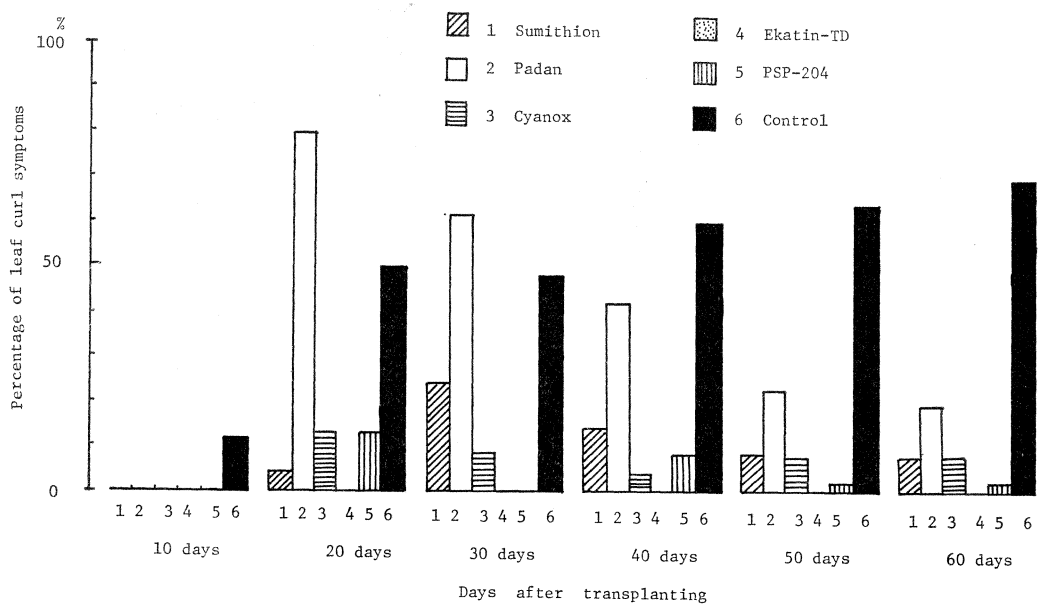

Fig. 13. Effects of certain insecticides on the incidence of leaf curl symptoms in chilli fields in Jaffna. (Maha 1970/1971)

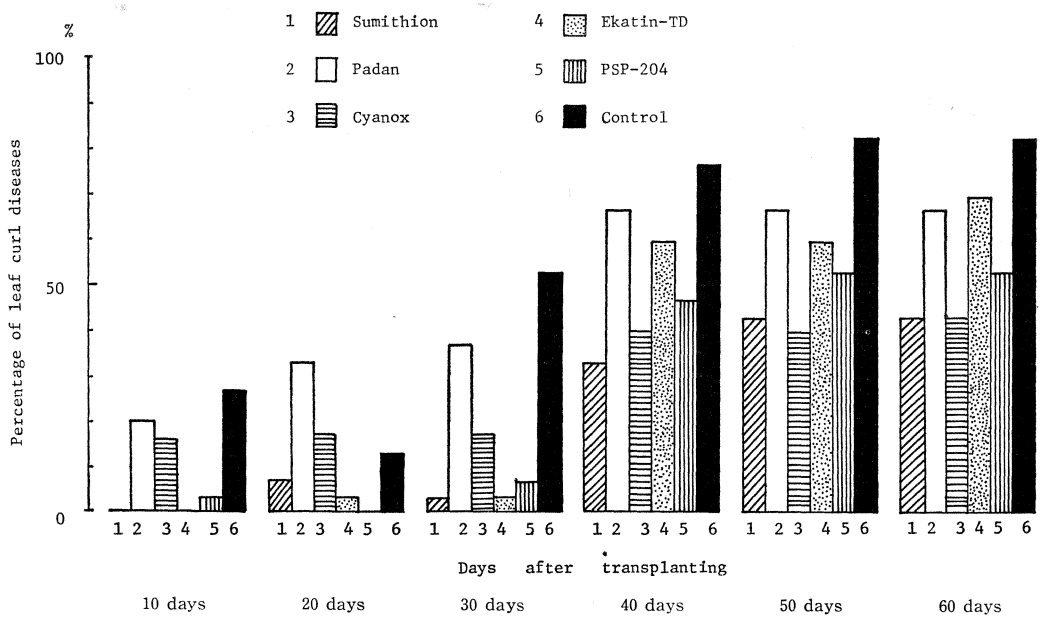

Fig. 14. Effects of certain insecticides on the incidence of leaf curl symptoms in chilli fields in Jaffna. (Yala 1971)

symptoms of mosaic type diseases on leaves appeared 50 days in Ekatin-TD plot and 30 days in PSP-204 plot; 20 days after transplanting in the control.

In Yala season, in Jaffna, 50 days after transplanting, the percentage of mosaic type virus diseases in Ekatin-TD plot was 10% as compared to 50% in the control; 30% in Ekatin-TD plot 80 days after transplanting and 57% in the control. First symptoms appeared in the treatment 50 days after transplanting and in the control 20 days after transplanting. The percentage of mosaic type diseases in PSP-204 plot was 16% 50 days and 26% 80 days after transplanting, respetcively, and in the control 50% 50 days and 57% 80 days after transplanting. In Peradeniya, both Ekatin-TD and PSP-204 treatments were effective, the percentage of mosaic type diseases being only 2% in Ekatin-TD and in PSP-204 plots 70 days after transplanting, and the control showed 22% 80 days after transplanting.

From these results of trials in both seasons, Ekatin-TD, PSP-204, Sumithion, and Cyanox were found to be effective for controlling mosaic type virus diseases in chilli cultivation in both dry and wet zones of Sri Lanka.

Effects of these insecicides on the incidence of leaf curl symptoms in fields in Jaffna are shown in Figs. 13 (Maha season 1970/1971) and 14 (Yale season 1971).

It was difficult to distinguish symptoms of chilli leaf curl disease from leaf curl due to mites and thrips injury hence the data collected from these experiments are complex. Furthermore, in Peradeniya, leaf curl disease was so scarce that the data on leaf curl in these control trials were excluded.

In the Maha season trial in Jaffna, symptoms of leaf curl appeared and increased to 12% within 10 days after transplanting in the control plot. These leaf curl symptoms may be those caused by mites and thrips, because leaf curl disease symptoms usually appear on chilli more than 10 days after inoculation, based on the results of transmission tests using whitefly. Percentage of leaf curl in the control showed 50%, 48%, 60%, and 64%, 20, 30, 40, 50, and 60 days after transplanting, respectively.

Ekatin-TD was highly effective as compared to other insecticides used: symptoms of leaf curl did not appear until 60 days after transplanting. Percentages of leaf curl in Sumithion, Cyanox, and PSP-204 plots 50 days after transplanting were 8%, 8%, and 2%, respectively. On the contrary, percentage of leaf curl in Padan plot reduced from 80% 20 days after transplanting to 20% 60 days after transplanting.

In the Yale season trial, the first symptoms of leaf curl in the control appeared 27% 20 days after transplanting and percentage in the control was 13%, 53%, 77%, 83%, and 83%, 30, 40, 50, 60, and 70 days after transplanting, respectively. Sumithion, Cyanox, Ekatin-TD, and PSP-204 were very effective as compared to the control until 40 days after transplanting. However, the effect of the chemicals decreased from 50 days after transplanting until the end of the experiment.

Systemic insecticides such as Ekatin-TD or PSP-204 are especially effective in Maha season when the amount of rainfall is much higher than in Yala season. It is, however, difficult to draw any definite conclusion from these results. It may be that these systemic insecticides are highly effective in the control of whitefly as well as mites and thrips, but they may as well be only effective in the control of mites and thrips but not whitefly and hence leaf curl virus disease. This problem should be left for a future study.

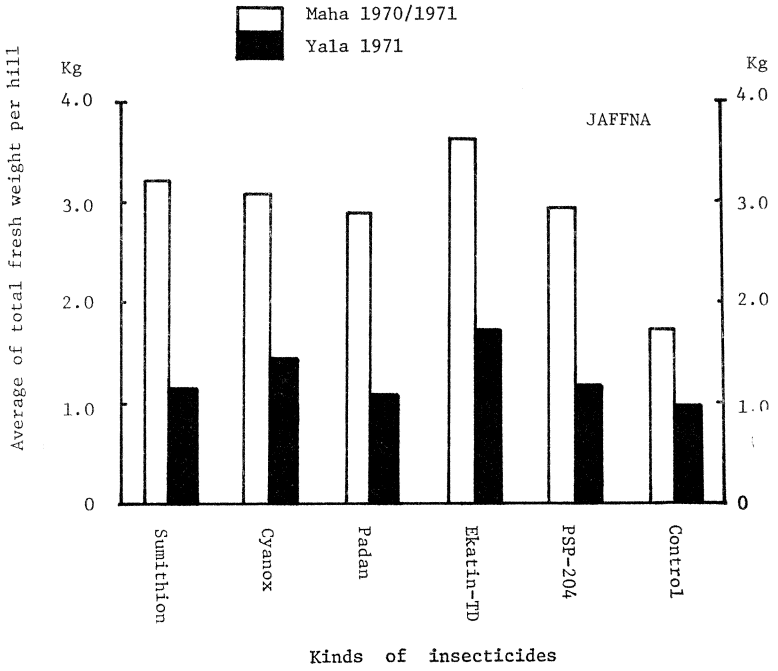

Fig. 15. Yields of green chilli from insecticides sprayed plots and no sprayed plot in Jaffna, Maha 1970/1971 and Yala 1971. (*Capsicum annum* cv. M. I. H.)

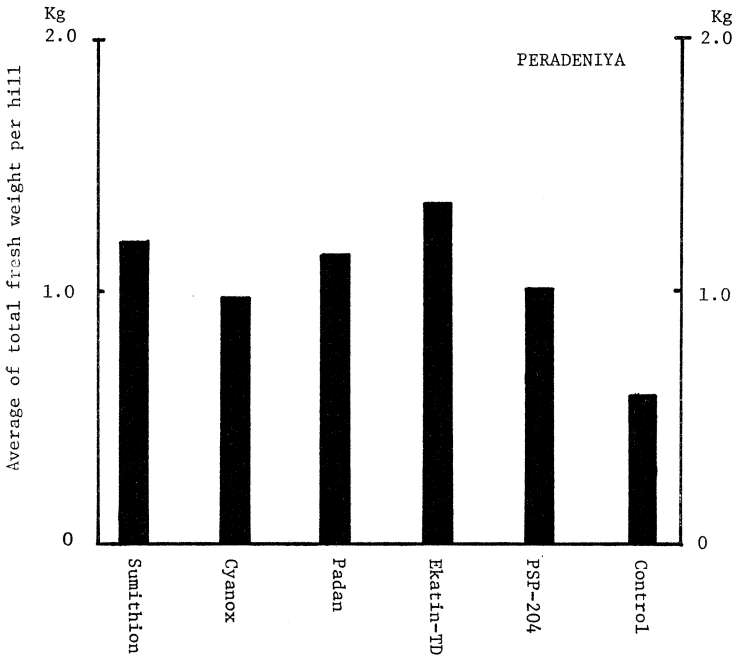

Fig. 16. Yields of green chilli from insecticides sprayed plots and no sprayed plot in Peradeniya, Maha 1970/1971. (*Capsicum annuum* cv. M. I. H.)

2. Yield measurements

Yields of green chilli as fresh weight from these insecticide-sprayed plots and the control plot are shown in Figs. 15 and 16.

Fresh weight of green chilli from each plot was measured at every harvest and cumulative weight for each plot was recaluculated to average yield per hill.

In Jaffna, in Maha season 1970/1971 trial, there was a very significant increase in yield between the Ekatin-TD plot or Smithion plot and the control plot. Average fresh weights of 3.6 kg and 3.2 kg per hill were harvested in the Ekatin-TD and Sumithion sprayed plots, respectively, compared to 1.6 kg per hill in the control. In Peradeniya, 1.3 kg and 1.2 kg were harvested in the Ekatin-TD and Sumithion sprayed plots, respectively, compared to 0.6 kg per hill in the control in Maha season 1970/1971. Marked increases in yield, of more than 2 times, were thus observed in Ekatin-TD treatment, compared to the non-treated control in Maha season 1970/1971. This much difference in yields was not observed in Yala season 1971 trial in Jaffna.

Comparison of yields of green chilli between *Capsicum annuum* cv. M.I.H. and cv. Santaka in Yala season 1971 trial in Jaffna is shown in Fig. 17.

Yield of M.I.H. was more than 2 times that of Santaka in the control plots; and Cyanox, Ekatin-TD, and PSP-204 plots showed similar tendencies.

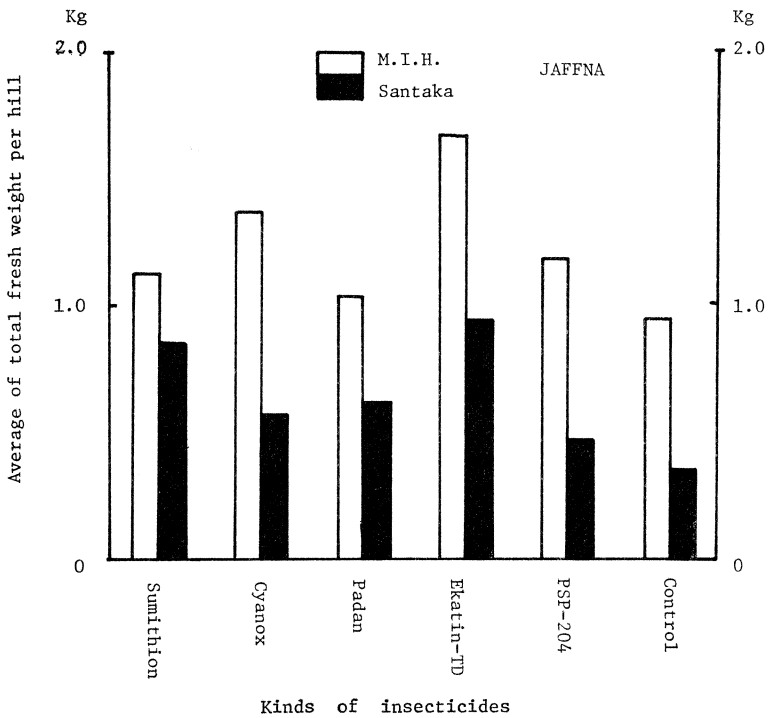

Fig. 17. Comparision of yields between *Capsicum annuum* cv. M. I. H. and cv. Santaka in Jaffna, Yala 1971.

3. Identification of viruses from chilli fields sprayed with insecticides

In the fields sprayed with insecticides at the Thinnavelly Farm, Jaffna, in Maha 1971, chilli plants showing mosaic disease symptoms long after insecticidal treatments were collected for identification of viruses involved. Twelve samples showing systemic symptoms (mosaic and systemic chlorotic spots) were collected as follows: eight from treated plots and four from the control plots. Mechanical inoculations were made with the aid of 600 mesh carborundum and cotton swabs to the following test plants: *Nicotiana tabacum* cv. Bright Yellow and cv. Samsun, *Nicotiana glutinosa, Capsicum annuum* cv. M.I.H., *Phaseolus vulgaris* cv. Otebo, *Vigna sinensis* cv. Black Eye, *Chenopodium amaranticolor,* and *Vicia fabae.* In addition, two samples showing leaf curl symptoms were collected from the control plots. Whitefly transmission tests were carried out on these, as described earlier.

Results of the virus identification tests are summarized in Table 26. Tobacco mosaic virus was isolated from the plots sprayed with Sumithion, Ekatin-TD, PSP-204, Cyanox, and control plots. Six out of twelve isolates were found to be tobacco mosaic virus. Cucumber mosaic virus was detected from only control plots, and potato virus Y from Padan treatments and control plots. An unidentified virus was isolated from plots sprayed with Sumithion, Cyanox, and control plots.

Table 26. Inentification of viruses from treated and untreated fields in Thinnavelly Farm in Jaffna

Sample number	Symptoms on the original chilli plants	Kinds of insecticide	Identified virus
1	slight mosaic	Sumithion	TMV
2	slight mosaic	Ekatin-TD	TMV
3	systemic chlorotic spots, yellow mottle	Sumithion	unidentified virus
4	systemic chlorotic spots, yellow mottle	Cyanox	unidentified virus
5	vein-banding, mosaic	PSP-204	TMV
6	severe mosaic, vein-banding	Padan	PVY
7	slight mosaic	Cyanox	TMV
8	slight mosaic	Ekatin-TD	TMV
9	systemic chlorotic spots, yellow mottle	control	unidentified virus
10	slight mosaic	control	CMV
11	vein-banding, slight mosaic	control	PVY
12	slight mosaic	control	TMV
13	vein-claering, leaf curl	control	CLCV
14	leaf curl	control	—

A leaf curl symptom was transmitted by whiteflies to healthy chilli seedlings, but in another case, it could not be transmitted by whiteflies. The original diseased chilli plants showed no vein-clearing on diseased leaves, although it resembled the leaf curl disease. It is believed that this type of symtom is caused by mites and/or thrips injury.

According to the data obtained, leaf curl symptoms were not observed on treatment with Ekatin-TD 60 days after transplanting. Since leaf curl symptoms in most fields in Jaffna district may be ascribed to mites and thrips injury, it is suggested that soil

treatment with Ekatin-TD would result in effective control of these insects under conditions of the dry zone such as Jaffna. On the other hand, when insecticidal spraying for the control of insect-borne viruses are carried out, tobacco mosaic virus may remain as a major pathogen of chilli in this district.

VI. Detection of tobacco mosaic virus contained in cigarette and beedi tobacco

Major sources of tobacco mosaic virus in the fields are known to be seeds, remains of diseased plants in the soil, cigarettes, contaminated hands, clothes, and farm tools as shown by Taylor et al. (1961), Broadbent (1965a, b), Tsuzuki and Komuro (1967), and Komuro and Iwaki (1968).

The aim of this experiment was to determine the presence of tobacco mosaic virus in cigarettes and beedi tobacco in Sri Lanka.

Six brands of cigarettes and five brands of beedi tobacco were tested. Twenty cigarettes from four packets of each kind were tested. Three to four leaf stage *N. glutinosa* and *N. tabacum* cv. Bright Yellow seedlings were used as test plants. Tobacco from one cigarette homogenized in 5 ml of 0.1 M phosphate buffer, pH 7.0 was inoculated to the test plants.

Results are summerized in Table 27. The data reveals that infection of cigarette tobacco with a common strain of tobacco mosaic virus is 80 to 100 percent. The tomato strain of this virus could not be found from any cigarette in this experiment.

Table 27. Detection of tobacco mosaic virus from cigarettes and beedi tobacco in Sri Lanka

Kinds		No. of cigarettes and beedis tested	No. of cigarettes and beedi from which virus was isolated	Percentage	Strain of TMV
Cigarettes					
Bristol		20	20	100	common
Three roses		20	16	80	common
Capstan		20	20	100	common
Gold leaf		20	18	90	common
Four aces		20	16	80	common
Beedi tobacco					
Janatha	wrapper	20	0	0	
	inside	20	2	10	common
Style	wrapper	20	0	0	
	inside	20	4	20	common
A. C. M.	wrapper	20	0	0	
	inside	20	3	15	common
Visaka	outside	20	0	0	
	inside	20	0	0	
G. K.	outside	20	0	0	
	inside	20	0	0	

On the other hand, the common strain of tobacco mosaic virus infection in beedi tobacco was found to be nil to 20 percent, and it was isolated only from the tobacco inside of the beedi while the wrapper did not show presence of any virus.

VII. Detection of tobacco mosaic virus in chilli and tomato seeds

The transmission of tobacco mosaic virus through seeds of infected tomato, *Lycopersicum esculentum,* and chilli, *Capsicum annuum,* and especially its location in infected or contaminated tomato seeds have been the subject of numerous investigations.

In the present study, seeds of chilli, *C. annuum* cv. M.I.H. were obtained from

Table 28. Tests for the detection of tobacco mosaic virus in chilli seeds

Repeat	Species	Variety	No. of seeds tested	No. of inoculated leaves	Total local lesions
1	*Capsicum annuum*	cv. M. I. H.	10	10	0
2	〃	〃	10	10	0
3	〃	〃	10	10	0
4	〃	〃	10	10	2
5	〃	〃	10	10	0
6	〃	〃	10	10	5
7	〃	〃	10	10	0
8	〃	〃	10	10	0
9	〃	〃	10	10	0
10	〃	〃	10	10	7
11	〃	〃	10	10	0
12	〃	〃	10	10	3
13	〃	〃	10	10	0
14	〃	〃	10	10	0
15	〃	〃	10	10	8
16	〃	〃	10	10	0
17	〃	〃	10	10	0
18	〃	〃	10	10	0
19	〃	〃	10	10	4
20	〃	〃	10	10	0
21	〃	〃	10	10	0
22	〃	〃	10	10	0
23	〃	〃	10	10	9
24	〃	〃	10	10	0
25	〃	〃	10	10	0
26	〃	〃	10	10	0
27	〃	〃	10	10	5
28	〃	〃	10	10	0
29	〃	〃	10	10	15
30	〃	〃	10	10	0

Thinnavelly Farm in Jaffna. Ten seeds were washed for 5 minutes in 2 ml of 0.1 M phosphate buffer, pH 7.0 and each seed was homogenized in 0.5 ml of 0.1 M phosphate buffer, pH 7.0 in a small glass motor. Inoculations were made to one leaf per sample of four or five leaf stage plants of *Nicotiana glutinosa*.

Seeds of tomato, *L. esculentum* cv. Goraka from Teldeniya No. 3, No. 4, and No. 6 and from Madugoda No. 3 and Hybrid 33 MI and Hybrid No. 50 from Gannoruwa, Batu and Marglobe from undetermined sources, and *Lycopersicum pinpinellifolium* were obtained from the Division of Botany, Central Agricultural Research Institute at Gannoruwa, Peradeniya. The same method of inoculation was used.

Results of these tests are summerized in Table 28 and 29. In the test on the detection of tobacco mosaic virus from chilli, *Capsicum annuum*, seeds produced in fields under natural conditions in Jaffna, the virus was detected in 8 cases out of 30 samples. It is clear that chilli seeds are contaminate with tobacco mosaic virus, although the virus

Table 29. Tests for the detection of tobaco mosaic virus in tomato seeds

Repeat	Species	Variety	Production place	No. of seeds tested	No. of inoculated leaves	Total local lesions
1	*Lycopersicum esculentum*	Goraka	Teldeniya No. 3	10	10	0
2		〃	〃	10	10	0
3		〃	〃	10	10	0
1		Goraka	Teldeniya No. 4	10	10	0
2		〃	〃	10	10	0
3		〃	〃	10	10	0
1		Goraka	Teldeniya No. 6	10	10	0
2		〃	〃	10	10	0
3		〃	〃	10	10	0
1		Goraka	Madugoda No. 3	10	10	0
2		〃	〃	10	10	0
3		〃	〃	10	10	0
1		Hybrid 33 MI	Gannoruwa	10	10	0
2		〃	〃	10	10	0
3		〃	〃	10	10	0
1		Hybrid 50	Gannoruwa	10	10	0
2		〃	〃	10	10	0
3		〃	〃	10	10	0
1		Batu	unknown	10	10	0
2		〃	〃	10	10	0
3		〃	〃	10	10	0
1		Marglobe	unknown	10	10	0
2		〃	〃	10	10	0
3		〃	〃	10	10	0
1	*Lycopersicum pinpinellifolium*		Gannoruwa	10	10	0
2			〃	10	10	0
3			〃	10	10	0

concentration may be low. The total number of local lesions on *N. glutinosa* was only 58 lesions on 300 inoculated leaves.

On the other hand, tobacco mosaic virus could not be detected from tomato seeds, *L. esculentum* cv. Goraka, Hybrid 33 MI, Hybrid 50, Batu, Marglobe, and *L. pinpinellifolium* which were produced in Teldeniya, Madugoda, and Gannoruwa. It will be necessary to carry out more testing before concluding whether or not tomato seeds in Sri Lanka are contaminated with tobacco mosaic virus.

VIII. Discussion

Virus diseases occurring in chilli producing areas, especially in the dry zone, constitute a major for the production of chilli in Sri Lanka. Tobacco mosaic virus, cucumber mosaic virus, and chilli leaf curl virus affecting chilli plants in Sri Lanka have been reported by Park and Fernando (1938), Johnpulle (1939), Peiris (1944, 1953), Fernando (1953, 1957), and Abeygunawardena (1969).

In other countries, several viruses naturally occurring in chilli and green pepper plants have been identified: tobacco mosaic virus (Anderson and Corbett 1957, Miller and Thornberry 1958, Murakishi 1960, Nitzany and Edna 1962, Fletcher 1963, Greenleaf, Cook and Heyn 1964, Gracia, Feldman, Pontis and Boninsegna 1968, and Milbrath and Cook 1971), cucumber mosaic virus (Wellmann 1935, Doolittle and Zaumeyer 1953, Anderson and Corbett 1957, Simons 1957, Sutic 1959, Nitzany and Edna 1962, Delevic 1963, Kovachevshi 1965, Anjaneyulu and Apparao 1967), potato virus Y (Anderson and Corbett 1957, Bhargava and Joshi 1961, Cook 1962, Nitzany and Edna 1962, Laird and Dickson 1963, Laird, Desjardins and Dickson 1964, Horvath (1966a, 1966b), Steepy, Lewis, and Varney 1967, Nagai and Smith 1968, Gracia, Feldman, pontis, and Boninsegna 1968, Brunt and Kenten 1971, Milbrath and Cook 1971, Zitter 1972), tobacco etch virus (Doolittle 1946, Greenleaf 1953, Anderson and Corbett 1957, Mclean 1962, Laird and Dickson 1963, Laird, Desjardins, and Dickson 1964, Steepy, Lewis, and Varney 1967, Nagai and Smith 1968, Milbrath and Cook 1971, Zitter 1972), Alfalfa mosaic virus (Berkely 1947, Sutic 1959, Delevic 1963, Gracia, Feldman, pontis, and Boninsega 1968), aster ring-spot virus (Anderson and Corbett 1957), tomato spotted wilt virus (Gracia, Feldman, Pontis, and Boninsega 1968, Milbrath and Cook 1971), potato virus X (Paulus, Kendrick, and Desjardins 1960), tobacco rattle virus (Paulus, Thomason, and Weathers 1963), veinbanding mosaic virus (Simons, 1956), Philippine tomato leaf curl virus (Retuerma, Pableo, and Price 1970), chilli leaf curl virus (Varma 1963, Mishra, Raychaudhuri, and Ashrafi 1963), etc.

A preliminary survey showed that in Jaffna and Puttalam districts chilli plants are affected more by mosaic diseases than by leaf curl disease, in line with the findings by Shivanathan and Abeygunawardena (1968).* In Vavuniya and Anuradhapura districts located inland, the incidence of leaf curl disease was higher than mosaic diseases. These differences in incidences of mosaic diseases and leaf curl disease between inland and coastal aeras may be due to differences in the kind and amount of vectors and of inoculum source plants. Mosaic symptoms usually appear in the fields from the first to the 3rd week after transplanting, and leaf curl symptoms appear in the fields from

* unpublished.

15 to 30 days after transplanting.

Anderson and Corbett (1957) selected five basic indicator plants for use in the survey of virus diseases on peppers: *Datura stramonium, Nicotiana glutinosa, N. tabacum* cv. Turkish or cv. White Burley, and *Capsicum frutescens* cv. California Wonder or cv. Tabasco. In addition to these plants, *Zinnia elegans, C. frutescens* cv. Large Bell Hot, *Vigna sinensis* cv. Black Eye or cv. Sill and Walker, *Cassia tora* were recomended as secondary indicators. Gracia et al. (1968) proposed indicator plants for identification of viruses in tomatoes and peppers as follows: *Capsicum annuum, Chenopodium amaranticolor, Cucumis sativus, Datura metel, D. stramonium, Gomphrena globosa, Lycopersicum esculentum, Nicotiana clevelandii, N. glauca, N. glutinosa, N. rustica, N. tabacum* cv. Samsun, cv. Turkish, cv. White Burley, and cv. Xanthi-nc, *N. sylvestris, Ocimum basilicum, Petunia hybrida, Phaseolus vulgaris, Pisum sativum, Vicia fabae,* and *Vigna sinensis.* Test plants used by Nagai and Smith (1968) were: *N. tabacum* cv. Xanthi-nc, *N. glutinosa, N. glauca, D. stramonium, Nicandra physalodes, Solanum nigrum, Chenopodium amaranticolor, Gomphrena globosa,* and *Vigna sinensis.* Test plants used by Milbrath and Cook (1971) were: *D. stramonium, N. glutinosa, C. frutescens* cv. Tabasco, and *C. annuum* cv. Yolo Y and other varieties.

The basic indicator plants selected for use in the present survey were: *Capsicum annuum* cv. M.I.H. and cv. Santaka, *C. frutescens* cv. Honenmidori and cv. California Wonder, *Nicotiana glutinosa, N. tabacum* cv. Bright Yellow and cv. Samsun, *Chenopodium amaranticolor, Phaseolus vulgaris* cv. Otebo, *Vigna sinensis,* and *Vicia fabae.* As the result, tobacco mosaic virus, cucumber mosaic virus, and chilli leaf curl virus were found to be causing severe chilli losses all over the island. Furthermore, a strain of potato virus Y was found to be commonly affecting chilli in Jaffna district. An unidentified virus was also found to be the cause of virus disease of chilli in both wet and dry zones.

Very often, these viruses were found to be mixed-infecting chilli plants, and it was generally difficult to correlate symptoms on field infected chilli plants with the presence of specific viruses. There are, however, certain tendencies. Vein-banding and slight mosaic symptoms usually indicate the presence of tobacco mosaic virus. Potato vrus Y is associated with vein-banding and mosaic symptoms, and sometimes vein-yellowing. Cucumber mosaic virus causes vein-clearing and slight mosaic symptoms in most cases under the dry zone conditions. An unidentified virus is associated with systemic chlorotic spots or mottling symptoms in Jaffna district.

Spread of potato virus Y from other crops to chilli has not been noted in this survey, but the virus is widely distributed also in tobacco and tomato in Jaffna so that there may be ample occasions for the virus to spread from tobacco or tomato to chilli.

All potato virus Y isolates tested in the present study produced similar reactions in tobacco: inoculated leaves sometimes showed difinite nocrotic lesions, but more often they showed faint, diffuse chlorotic spots. Young actively growing leaves usually showed systemic chlorotic spots and then became chlorotic and slightly mottled, without etch pattern. There was no infection on *Datura stramonium* and *Cassia tora.* Inoculated leaves of tabasco pepper showed local lesion reaction when kept in a dark box for 24 hours after inoculation under conditions prevailing at Peradeniya, but there was no wilt reatcion. In cross protection tests on tobacco, positive reaction was obtained between this virus and an authenticated potato virus Y from Japan. In electron microscopy, normal length of virus particles was estimated from 700 to 750 nm.

Ultrathin sections of infected mesophyll cells showed large areas of cytoplasm con-

taining many pinwheel structures and cylindrical inclusions. The virus was thus identified as a strain of potato virus Y, being first report of potato virus Y from Sri Lanka.

Cucumber mosaic virus from chilli was identified as a common strain. The virus isolates were characterized by their inability to infect local varieties of cucumber, whereas they produced slight mosaic on cucumber, *Cucumis sativus* cv. Ochiai-fushinari from Japan.

According to Costa (1969), whitefly transmitted viruses infect plants from many families, including representatives of Compositae, Convolvulaceae, Cruciferae, Cucurbitaceae, Euphorbiaceae, Geraniaceae, Leguminosae, Linaceae, Malvaceae, Pedaliaceae, Solanaceae, Scrophulariaceae, Urticaceae, Verbenaceae, and others.

Several virus diseases transmitted by whiteflies have been reported mainly tropical areas, but occur also under sub-tropical areas: tobacco leaf curl virus (Thung 1932, Storey 1935, Sharp and Wolf 1948, Morgan 1952, Varma 1963, Yassin and Nour 1965), cotton leaf curl virus (Kirkpatrick 1931, Laird and Dickson 1959, Varma 1963, Yassin and El Nur 1970), tomato leaf curl virus (Vasudeva and Sam Raj 1948, Yassin and Nour 1965, Retuerma, Pobleo, and Price 1970), tomato yellow leaf curl virus (Cohen and Nitzany 1966), chilli leaf curl virus (Fernando 1953, Fernando and Peiris 1957, Mishra, Raychaudhuri, and Ashrafi 1963), cassava mosaic virus (Storey and Nichols 1938, Chant 1958, Menon and Raychaudhuri 1970), *Euphorbia* mosaic virus (Costa and Bennetti 1950, Costa and Carvalho 1960), infectious chlorosis of Malvaceae (Silberschmidt and Tommasi 1956, Silberschmidt, Flores, and Tommasi 1957, Costa and Carvalho 1960), virus diseases of Cucurbits (Cohen and Nitzany 1963, Hariharasubramanian and Badami 1964), cucumber vein-yellowing virus (Harpaz and Cohen 1965), bean double yellow mosaic (Capoor and Varma 1948), *Hibiscus rosa-sinensis* leaf curl (Vasudeva, Varma, and Capoor 1953), pumpkin yellow-vein mosaic (Varma 1955), *Ageratum* yellow-vein mosaic (Gadd and Loos 1941), *Wissadula amplissima* mosaic virus (Schuster 1964), tobacco yellow-net virus (Dhingra and Nariani 1962), sweet potato virus B (Sheffield 1957, 1958), Jatrophas gossypifolia mosaic (Bird 1957), bhendi yellow-vein mosaic (Varma 1952), cotton leaf crumple (Dickson, Johnson, and Laird 1954, Erwin and Meyer 1961), golden mosaic of beans and lima beans (Costa 1965), infectious chlorosis of *Sida carpinifolia* (Bird 1958), beet pseudo-yellow virus (Duffus 1965), etc.

However, the relationship between these virus diseases has not always been well established and it is possible that in some cases they represent diseases from different species caused by the same virus or by variants of the same virus complex that became better adapted to certain host plants.

Chilli leaf curl virus is transmitted by whitefly, *Bemisia tabaci,* and belong to the group of circulative viruses such as *Euphorbia* mosaic virus, tobacco leaf curl virus, cucumber vein-yellowing virus and others.

Whitefly, *Bemisia tabaci,* requires the following minimum acquisition periods to become infective: 3 hrs for cotton leaf crul virus (Kirkpatrick 1931); 5 hrs for tobacco leaf curl virus, but shorter periods were not tried (Pruthi and Samuel 1939); 30 min. for *Euphorbia prunifolia* mosaic virus (Costa and Bennett 1950); 1 hr for the bhendi yellow-vein mosaic virus (Varma 1952); 2 hrs for *Jatrophas gossypifolia* mosaic virus (Bird 1957); 15 min. for the infectious chlorosis of *Sida carpinifolia* (Bird 1958); 4 hrs for cassava mosaic (Chant 1958); 4–8 hrs for cotton leaf-crumple virus (Laird and Dickson 1959); 30 min. for tomato leaf curl virus (Cohen and Nitzany 1966). Beet pseudo-yellows virus is acquired by *Trialeurodes vaporairoum* in 1 hr (Duffus 1965).

Chilli leaf curl virus in the present studies showed a comparable figure of minimum acquisition feeding period for 30 min. but not 10 min.

Whitefly-transmitted viruses have a definite incubation period in the vector. This varies from 4 to 8 hrs for most viruses transmitted by *Bemisia tabaci*; 6 to 8 hrs for yellow-vein mosaic virus of *Abelmoschus* (Varma 1955); 8 hrs for *Ageratum conyzoides* yellow-vein mosaic virus (Varma 1963); 8 hrs for cassava mosaic (Storey and Nichols 1938); 6½ hrs for cotton leaf curl virus (Laird and Dickson 1959); 7 hrs for *Eclipta alba* yellow-vein mosaic virus (Graga 1960); 8 hrs for *Euphorbia prunifolia* mosaic virus (Costa and Bennetti 1950); 8 hrs for *Phaseolus luntatus* yellow mosaic virus (Capoor and Varma 1948); 4 hrs for tobacco leaf curl (Storey 1935); 4 hrs for *Malvastrum coromandelianum* yellow-vein mosaic virus (Costa and Carvalha 1960); less than 6 hrs for the beet pseudo-yellows virus in *Trialeurodes vaporarium* (Duffus 1965). However, Laird and Dickson (1959) suggested that the cotton leaf curl-crumple virus might have an incubation period in *B. tabaci* of 20 hrs or longer. The incubation period was also 21 hrs more for the tomato yellow leaf curl virus in the same whitefly (Cohen and Nitzany 1966). In the present experiments, a single whitefly allowed to feed for 3 hrs on infected plant was able to transmit the virus within 24 hrs after acquisition.

Costa and Bennett (1950) investigated retention of the *Euphorbia* mosaic virus by serial transfer of single, viruliferous *Bemisia tabaci* onto healthy test seedlings. The virus was retained in the vector for 20 days. The retention of the bhendi yellow-vein mosaic virus in *B. tabaci* was studied by Varma (1952). Vectors fed for 12–24 hrs on the virus sources retained infectivity for life. Bird (1957, 1958) studied the retention of the viruses inducing mosaic on *Jatrophas gossypifolia* and infectious chlorosis of *Sida carpinifolia*. Virus was retained in the vector, *B. tabaci*, for 4 and 7 days, respectively. Flores and Silberschmidt (1958) found that abutilon infectious variegation virus (AIVV) from Sida in Brazil could be retained in *Bemisia tabaci* for periods of 20 days. In serial transfer tests, Cohen and Nitzany (1966) determined that the tomato yellow leaf curl virus may be retained in female vector for 20 days, but not for life. Duffus (1965) showed that *Trialeurodes vaporariorum* retains the beet pseudo-yellows virus for 6–7 days. Most insects stopped transmitting after the tenth days. Chilli leaf curl virus in the present experiments was not retained throughout the life span of the whitefly, but maximum length of persistence was obtained more than 12 days.

Chilli leaf curl virus was successfully to chilli and tomato by grafting, but not to tobacco under our experimental conditions. The reason is unknown.

Field symptoms of chilli leaf curl in Sri Lanka, however, comprises two entities: true leaf curl virus disease transmitted by whitefly, *Bemisia tabaci*, and one induced by mites and thrips feeding injury as discribed by Johnpulle (1939) and Peiris (1953). It is difficult to distinguish one from the other in the fields.

According to Costa (1969), virus diseases transmitted by whiteflies may be divided into two main groups: (1) Those recognized by leaf symptoms such as clearing or yellowing of the vein, yellow net, or leaf crinkling and curling resulting from unequal growth of normal and mosaic areas. Mosaic symptoms are of the green or more frequently of the yellow type. (2) The curl type of disease in which mosaic symptoms are not evident, but invaded plants may show stunting, leaves with diffuse yellowing, crinkling, vein clearing or vein thickening, and leaf enations.

Costa (1969) also described that symptoms of tobacco leaf curl on *Petunia hybrida* are reduced size of leaves which are closely grouped on the stems and vein on the lower

surface of leaves become more prominently raised than on the upper surface, although they do not develop into enations. Virus was readily transmitted by grafting the infected petunia scion to tobacco, *N. glutinosa*, tomato, and petunia, Well-marked enations developed on *N. glutinosa* and tomato in this case.

Olivares and San Joan (1966) showed that *Datura stramonium, N. glutinosa, N. tabacum, N. rustica, Physalis peruviana, Lycopersicum esculentum, Ageratum conyzoides, Synedrella nodiflora,* and *Plantago major* were found to be susceptible to tobacco leaf curl virus by whitefly transmission tests in Philippine. *Capsicum annuum, Solanum melongena, S. tuberosum, Cosmos bipinnatus, Phaseolus mungo, P. calcaratus, Hibiscus esculentum, Gossypium herbaceum* were reported as apparently immune.

In whitefly transmission tests of tomato leaf curl virus in Philippine, only *Lycopersicum esculentum* was susceptible. *N. glutinosa, N. tabacum, Datura* sp., *Ageratum conyzoides, Synedrella nodiflora, Elaeis guineensis, Capsicum annuum, Cucumis vulgaris, Gossypium hirsutum, Canna indica,* and *Vinca rosea* were designated apparently immune by Retuerma et al. (1970).

In the present tests, *N. tabacum, Petunia hybrida, Lycopersicum esculentum, Physalis floridana, P. peruviana, Datura stramonium, Capsicum annuum, C. frutescens,* and *Ageratum conyzoides* were susceptible to chilli leaf curl virus, but there developed no enations on these test plants. *N. glutinosa, N. rustica, Solanum nodiflorum, Synedrella nodiflora, Eupatrium japonicum, Phaseolus vulgaris, Emilia sonchifolia, Acalypha indica, Hibiscus esculentum, Gossypium herbaceum* were apparently immune to this virus.

Chilli leaf curl virus was thus not transmissible to *N. glutinosa, N. rustica,* and *Synedrella nodiflora.* On the contrary, tobacco leaf curl virus is not transmissible to *Capsicum annuum,* whereas both chilli leaf curl virus and tobacco leaf curl virus are transmissible to *D. stramonium, N. tabacum, Physalis peruviana, L. esculentum,* and *Ageratum conyzoides.* Important differences between both viruses: failure of chilli leaf curl virus to induce any enation on tobacco and tomato viz. well-marked enations on tobacco and tomato by tobacco leaf curl virus.

It may be conclued that tobacco leaf curl virus in Sri Lanka is not a single virus, but a composite of a leaf curl virus which causes enations on tobacco and is not infectious to *Capsicum annuum* and another leaf curl virus which causes no enation on tobacco and is infectious to *C. annuum.* Then chilli leaf curl virus is identified as this latter leaf curl virus which produces no enation on tobacco. It may be named chilli leaf curl strain of tobacco leaf curl virus.

Chilli seedlings in nurseries in the dry zone were found to be affected by several viruses such as tobacco mosaic virus, cucumber mosaic virus, potato virus Y, and an unidentified virus. The percentage of virus infection in nurseries in Jaffna (13%) was higher than in any other districts (Anuradhapura 6% and Vavuniya 11%).

In trials to control virus diseases by spraying insecticides in both Maha and Yala seasons in 1970 to 1971, in Kandy and in Jaffna, Ekatin-TD, PSP-204, Sumithion, and Cyanox proved to be very effective for the control of mosaic diseases and also leaf curl disease. It was found that the effects of systemic insecticides are more pronounced in dry climate than in wet climate. The data indicated that an appropriate use of effective systemic insecticides (Ekatin-TD or PSP-204) enable an increase in the yield of green chilli by two to three folds in comparison to no insecticide. Insecticidal control, however, will be of limited use, and it should be accompanied by selection and breeding of resistant varieties. Control of tobacco mosai cvirus is another important subject for future study.

IX. Summary

1. A preliminary survey of chilli virus diseases in fields in the major chilli producing areas of Sri Lanka, Jaffna, Puttalam, Vavuniya and Anuradhapura districts, were carried out from 1969 to 1970.

In Jaffna and Puttalam districts which are in the coastal areas, chilli plants are affected more by mosaic type diseases than by leaf curl type disease. In Vavuniya and Anuradhapura districts located inland, the incidence of leaf curl is higher than mosaic type diseases. As mentioned by Peiris (1953), leaf curl symptoms may be caused not only by leaf curl virus which is transmitted by whitefly but also from feeding injury by mites and thrips. The symptoms are almost indistinguishable in the fields. In the case of plants having infection of mosaic and leaf curl, symptom of leaf curl usually prevails over mosaic symptoms.

2. To identify chilli viruses in the major chilli producing areas, eighty seven samples were collected from Jaffna (38), Vavuniya (8), Anuradhapura (20), Puttalam (1), Badulla (5), and Kandy (15) districts. From seventy eight of these samples, viruses were transmitted by sap inoculation to *Capsicum annuum, C. frutescens, Nicotiana tabacum, N. glutinosa, Vicia fabae, Chenopodium amaranticolor, Phaseolus vulgaris,* and *Vigna sinensis*; from nine samples, viruses were not transmissible by sap inoculation but were transmitted by whitefly and by grafting. All virus isolates were inoculated to test plants such as *Capsicum annuum* cv. M.I.H., *Nicotiana tabacum* cv. Bright Yellow, *N. glutinosa, Vicia fabae, Phaseolus vulgaris, Vigna sinensis,* and *Chenopodium amaranticolor.* From the results of host range and cross-protection tests, physical properties of viruses, insect transmission, serological tests, and observation of virus particles by electron microscopy, the following viruses were identified: tobacco mosaic virus, cucumber mosaic virus, a strain of potato virus Y, chilli leaf curl virus and an unidentified virus.

3. Potato virus Y is the most common cause of severe chilli losses in Jaffna and is reported for the first time in Sri Lanka. In Jaffna, Matale, and Kandy districts, it was also isolated from tobacco, tomato, and chilli. The virus was transmissible by the green peach aphid, *Myzus persicae,* and the cotton aphid, *Aphis gossypii,* and by sap inoculation. The physical properties determined for the virus was: thermal inactivation point 55°–60°C; dilution end point between 1 : 5,000 and 1 : 10,000; and virus longevity between 3 and 4 days at room temperature, or between 9 and 10 days when stored at 5°C. Systemic chlorotic spots, necrotic lesions, vein necrosis, vein net yellowing, stem necrosis, and stunting on *Nicotiana tabacum* cv. Bright Yellow and Samsun and *N. rustica* were obtained. On *Petunia hybrida, Physalis floridana,* and *Capsicum annuum* cv. M.I.H., and *C. frutescens* cv. Honenmidori mosaic reaction was obtained, while *Lycopersicum esculentum* cv. Fukuju showed slight mosaic symptoms. Diffuse chlorotic spots to mottle appeared on upper leaves of *N. glutinosa, Chenopodium amaranticolor, Capsicum frutescens* cv. Tabasco developed local lesions on inoculated leaves. In *Solanuum tuberosum* cv. Arka local reaction was obtained when kept in a dark box for 24 hours after inoculation.

Cross-protection tests between this virus and an authenticated potato virus Y from Japan indicated that both viruses are closely related. Virus particles are of length varying from 500 to 800 nm, with estimated normal length of 700 to 750 nm. Ultrathin sections of infected mesophyll cells showed large areas of cytoplasm containing pinwheels,

laminated agregates, and cylindrical inculsions, similar to those induced by several other member of potato virus Y.

4. Chilli leaf curl virus was transmitted by grafting infected chilli to healthy chilli and tomato seedlings, but it could not be transmitted to tobacco. It was also transmitted by whitefly, *Bemisia tabaci*. The whiteflies transmitted the virus following a 30 min. acquisition feeding time on infected plants, but failed to do so after 10 min. acquisition feeding time. Rate of transmission increased more than for 48 hours of acquisition feeding. Transmission was observed after an inoculation feeding time of 60 min. on test plants, but not after 30 min.. A single whitefly allowed to feed for 3 hours on infected plants was able to transmit the virus within 1 day after initiation of acquisition feeding. The whiteflies transmitted the virus for 12 to 15 days after acquisition feeding. The virus thus persists in its vector, but not for the full span of the whitefly. The virus was not transmitted to progeny of the vector. The following plants were susceptible the chilli leaf curl virus; *Petunia hybrida, Lycopersicum esculentum* cv. Fukuju and cv. Ponterosa, *Physalis floridana, P. peruviana, Ageratum conyzoides*, and *Datura stramonium*. *N. tabacum* cv. Samsun and cv. Bright Yellow produced slight leaf curvature and outward curling, but no enation symptoms.

There are two kinds of viruses causing leaf curl of tobacco: one which produces enations on leaves and the other which produces no enation. Tobacco leaf curl with enation (TLCV-1) was transmissible to tomato, *L. esculentum* cv. Ponterosa, and tobacco, *N. tabacum* cv. Bright Yellow and cv. Samsun, by whitefly and by grafting, but not to chilli, *Capsicum annuum* cv. M.I.H. Tobacco leaf curl without enation (TLCV-2) was transmissible to tomato, tobacco, and chilli by whitefly and by grafting.

There is observed no enation in leaf curl of tomato in Sri Lanka. This tomato leaf curl without enation (ToLCV) was transmissible to tomato, tobacco, and chilli by whitefly and by grafting. No enation was produced on tobacco.

Chilli leaf curl was not transmissible to tobacco by grafting, but it could be transmitted to tobacco by whitefly under this experimental conditions. No enation was produced on tobacco. It is thus clear that the virus of tobacco leaf curl with enation on tobacco (TLCV-1) is distinct from that of tobacco leaf curl without enation on tobacco (TLCV-2), and the latter (TLCV-2) may be the same virus as tomato leaf curl virus and chilli leaf curl virus.

5. Insecticidal control trials were made in Jaffna (dry zone) and in Peradeniya (wet zone) during Maha 1970/1971 and Yala 1971. Sumithion spray and Ekatin-TD granules applied to soil were highly effective against mosaic diseases in Peradeniya, while Ekatin-TD granules gave better control over PSP-204 granules and Sumithion and Cyanox sprays in Jaffna. The use of these insecticides markedly increased the yield of chilli. Ekatin-TD granules and PSP-204 granules were more effective than other insecticides against leaf curl. It could be that these systemic insecticides rather controlled mites and thrips, thereby reducing feeding injury by these insects which causes symptoms of leaf curl almost indistinguishable from those caused by virus. In flelds where Ekatin-TD granules were used, leaf curl symptoms did not appear for 60 days after transplanting in Maha season, but a low percentage of a mosaic disease was observed. The causal virus of this mosaic disease was identified as tobacco mosaic virus.

6. Presence of tobacco mosaic virus in cigarettes and beedi tobacco of Sri Lanka was tested. A common strain of tobacco mosaic virus was detected from 80 to 100% of cigarettes, but only a low percentage from beedi tobacco. No virus was detected from

beedi wrappers.

7. Presence of tobacco mosaic virus in chilli and tomato seeds was tested. The virus was found at a low percentage in chilli seeds from Thinnavelly Farm in Jaffna, but not in tomato seeds from the Division of Botany, Central Agricultural Research Institute, Peradenya.

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XI. Explanation of photography

1. Mosaic symptom on chilli plants (Right: infected with PVY (No. 4 virus); Left: infected with TMV (No. 1–A virus)).
2. Mosaic symptom on infected chilli plant with CMV.
3. Mosaic symptom on original chilli plant with PVY (No. 4 virus).
4. Chlorotic mottle symptom on *N. glutinosa* with PVY (No. 4 virus).
5. Local lesion symptom on inoculated leaf of potato (Arka) with PVY (No. 4 virus).
6. Cross protection test: Right, only inoculated with PVY-J; Left, inoculated with PVY-J as a first virus and then inoculated with PVY (No. 4 virus) as a second virus.
7. Virus particles of TMV (No. 1–A virus) from chilli.
8. Virus particles of PVY (No. 1–B virus).
9. Virus particles of PVY (No. 4 virus) from chilli in Jaffna.
10.–12. Sections of chilli leaves systemically infected with PVY (No. 4 virus) and a general view of PVY-induced cylindrical inclusions and pinwheel.
13. Pinwheel, showing central axis.
14. Field trials: transplanting time of chilli plants in Jaffna.
15. Field trials in Jaffna.
16. Leaf curl symptom on original chilli plant.
17. Leaf curl symptom on fruirts of original chilli plant.
18. First symptom of chilli leaf curl on inoculated chilli plant by whitefly.
19. First symptom of leaf curl on *Capsicum frutescens* cv. California Wounder (Right) and Healthy plant (Left).
20. Leaf curl symptom on *Petunia hybrida.*
21. Leaf curl symptom on *Physalis* sp. (Local variety of Sri Lanka).
22. Leaf curl symptom on *Datura stramonium.*
23. Leaf curl symptom on *Nicotiana tabacum* cv. Samsun.
24. Leaf curl symptom on *Nicotiana rustica.*
25. Leaf curl symptom on *Ageratum conyzoid*es.
26. Chilli leaf curl symptom on Samsun tobacco inoculated with whitefly (Left) and tobacco leaf curl symptom on Samsun tobacco inoculated with grafting. (Right).
27. Tobacco leaf curl symptom on tobacco and first symptom of enation on lower surface.

Plate 1

Plate 2

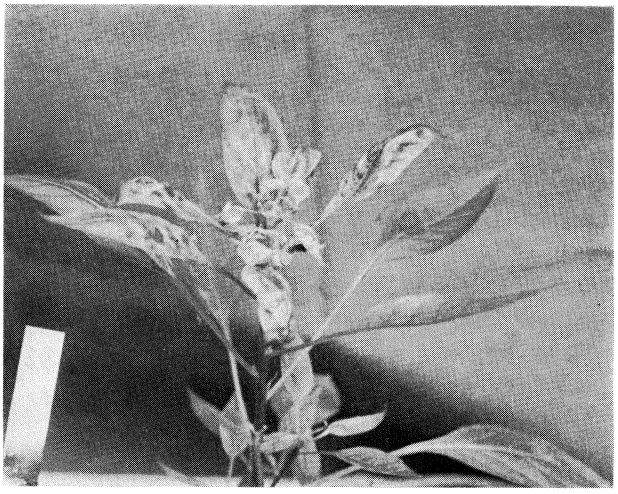


Plate 3

Plate 4

Plate 5

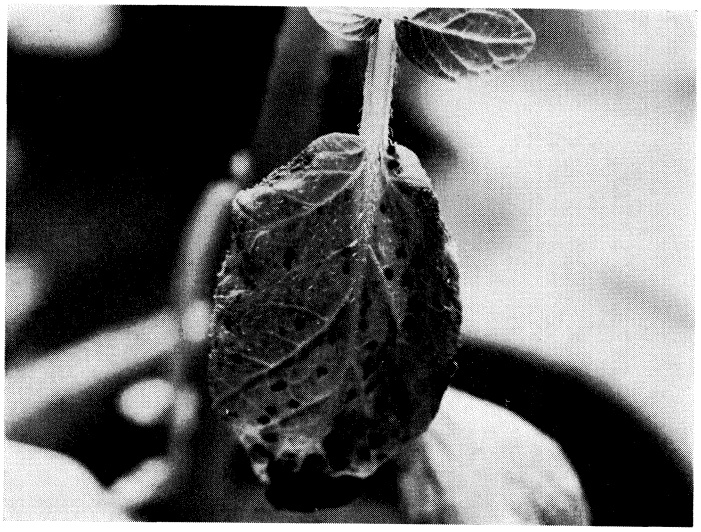
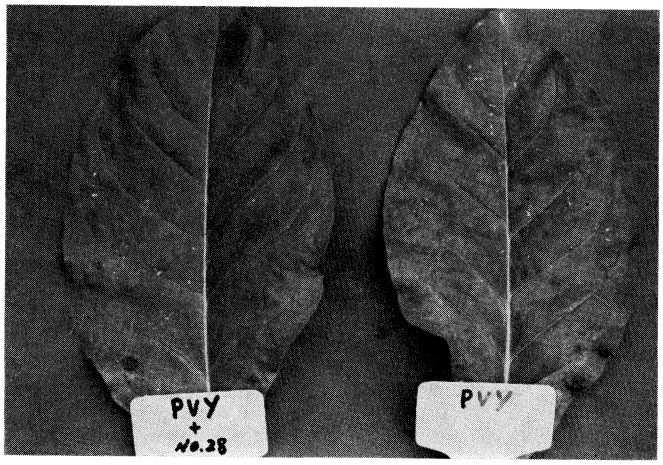


Plate 6



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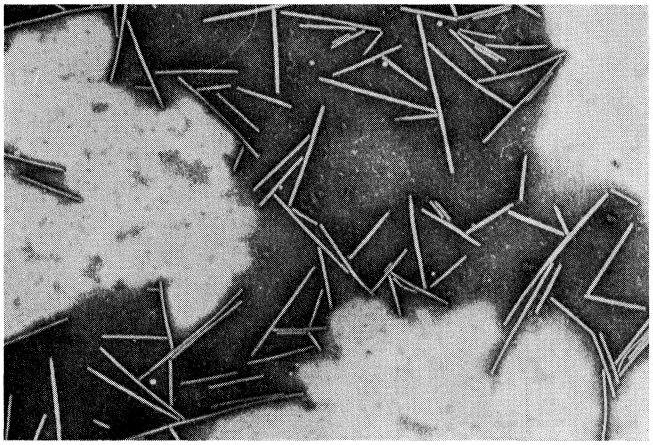

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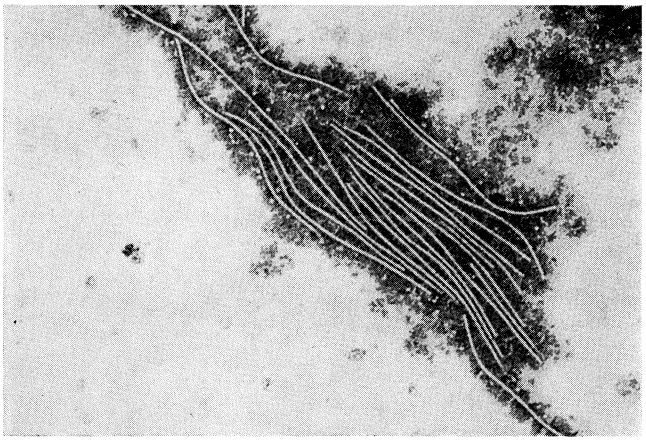

Plate 9

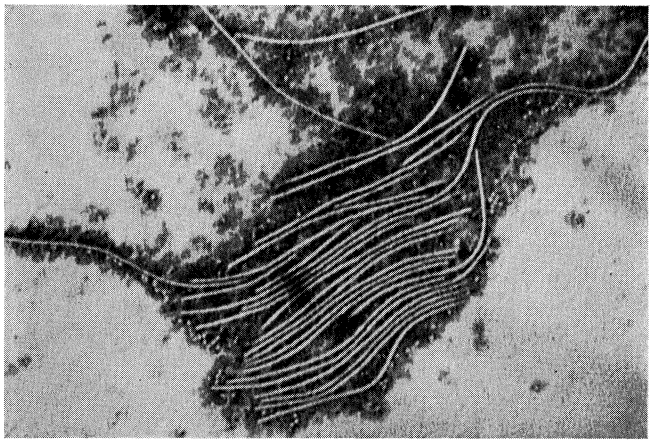

Plate 10

Plate 11

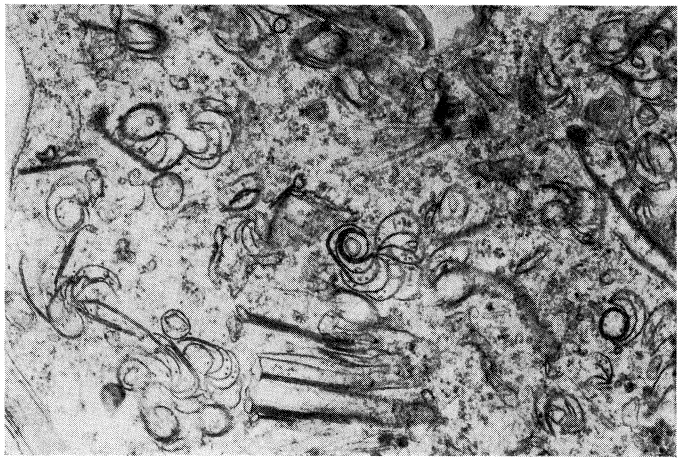

Plate 12

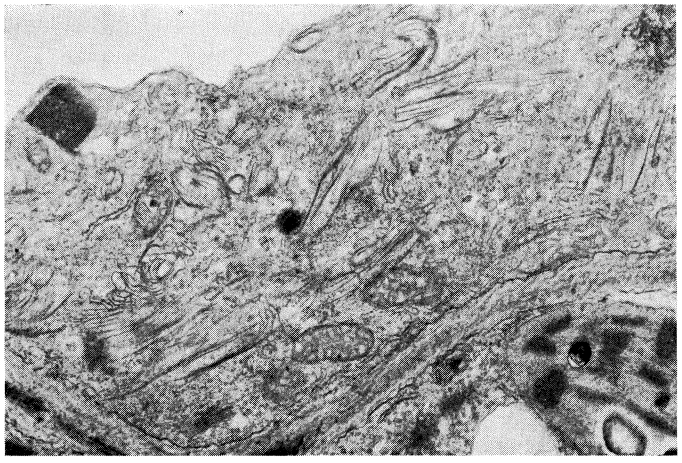

Plate 13

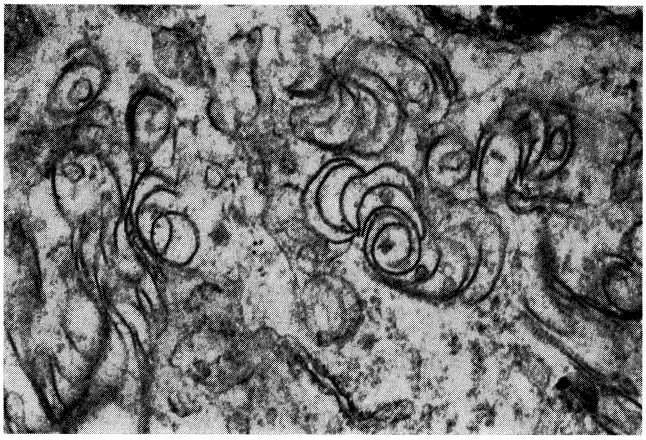

Plate 14

Plate 15

Plate 16

Plate 17

Plate 18

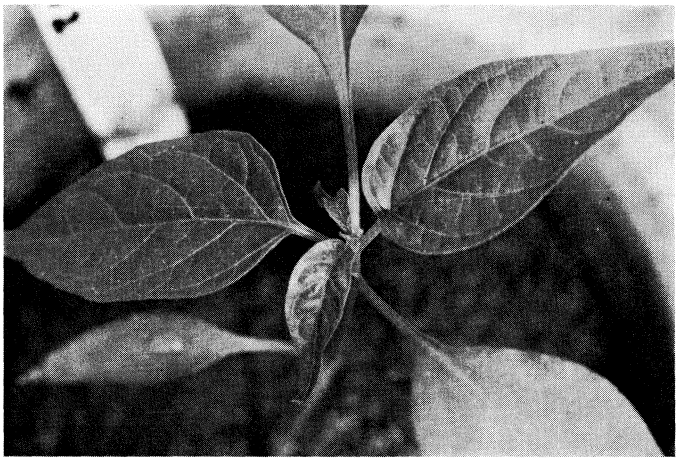

Plate 19

Plate 20

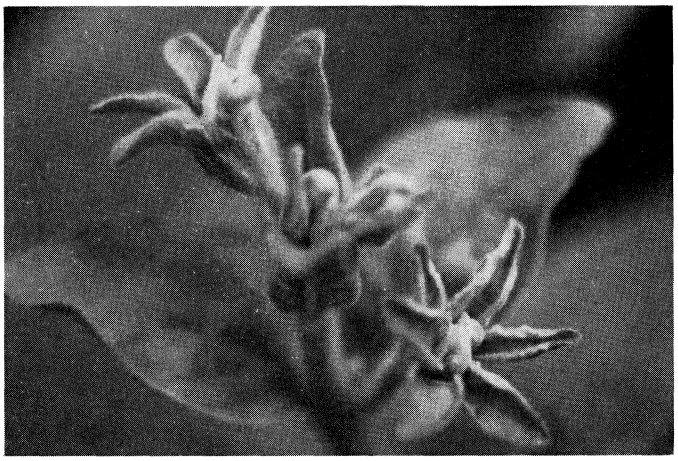

Plate 21

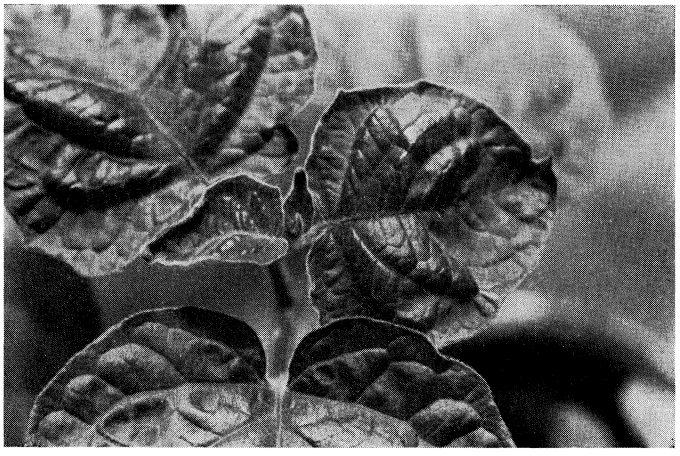

Plate 22

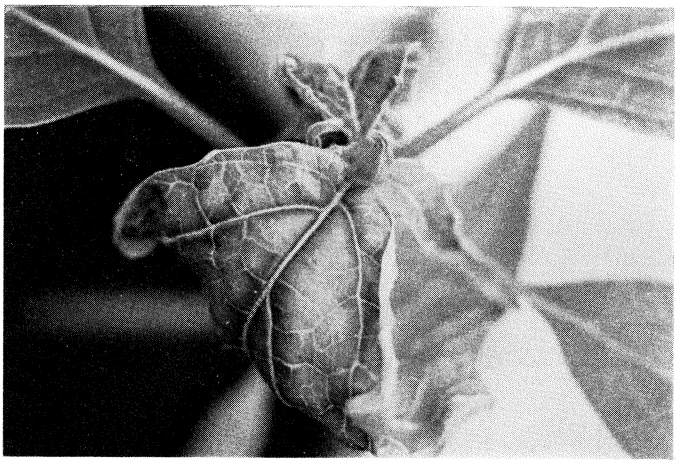

Plate 23

Plate 24

Plate 25

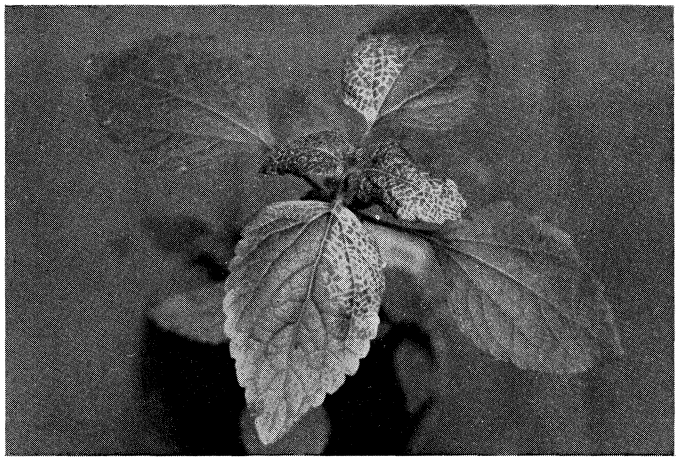

Plate 26

Plate 27