

STUDIES ON VIRUS DISEASES OF *Capsicum Sp.*
IN TAMIL NADU.

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INTRODUCTION

The cultivated pepper (Capsicum spp.), or chilli, as it is commonly known in India, has been introduced into India by the Portuguese in the sixteenth century from its native country of Tropical America and West Indies. Chilli is one of the chief spices of India cultivated over an area of about 5,67,000 hectares with a production of about 40,800 metric tons, of which a major portion is consumed within the country. Some quantity is also exported to Ceylon mostly as dry pods and as an essential constituent of curry powder and hot pickles to several other countries.

Of the different species of Capsicum, C.frutescens L. and C.annuum L. are by far the most cultivated ones. In Tamil Nadu C.annuum is grown over large areas, though C.frutescens is cultivated to a limited extent in isolated pockets.

Many diseases caused by fungi and bacteria have been reported to cause severe losses to the cultivated chilli crop. Many viruses also infect and cause equally, if not more, severe losses under field conditions. Literature available upto 1960 on virus diseases of chilli had been reviewed by Ramakrishnan (1961). He had listed 17 viruses namely, cucumber mosaic virus, tobacco mosaic virus, tobacco etch virus, alfalfa mosaic virus, pepper vein banding mosaic virus, potato virus Y, cranberry-false blossom virus, tomato spotted

wilt virus, aster ringspot virus, Trinidad pepper mosaic virus, Indian chilli mosaic virus, Puerto Rican pepper mosaic virus, Trinidad pepper vein banding virus, Italian pepper mosaic virus and pepper yellow leaf virus, to occur on chilli under natural conditions. Potato virus X and 16 other viruses have been reported to cause infection on chilli on artificial inoculation. Subsequently, Ramakrishnan (1959) and Paulus et al. (1960) have reported the natural occurrence of potato virus X on chilli in California. Ozalp (1961) reported the occurrence of PVX from Aegean area. Rao et al. (1970) reported the natural occurrence of a ring spot strain of PVX on chilli from India.

Since 1960 many workers have reported the occurrence of viruses that were already recorded on chilli from different places. Potato virus Y has been reported from Israel (Nitzany and Tanne, 1962), Costa Rica (Gamez, 1962), Aegean area (Ozalp, 1961), Japan (Miyamoto et al., 1963, 1964), Southern California (Laird et al., 1964), Hungary (Horvath, 1967) and Argentina (Gracia et al., 1968). Cucumber mosaic virus has been reported by Nitzany and Tanne (1962) from Israel and Kapellar (1966) from Hungary. Stolbur virus has been reported from Yugoslavia by Aleksic et al. (1969). Kapellar (1966), Gracia et al. (1968) and Vlasov (1970) reported the occurrence of alfalfa mosaic virus from Hungary, Argentina and U.S.S.R. respectively. Tobacco etch virus has been reported by Ozalp (1961), Nitzany and Tanne (1962),

Miyamoto et al. (1963, 1964) and Vlasov (1970) from Aegian area, Israel, Japan and U.S.S.R. respectively. Miyamoto et al. (1964) have also reported another pepper virus strongly resembling Rotterdam virus B and tobacco rattle on chilli from Japan.

Paulus et al. (1963) reported a soil-borne virus of chilli from California transmitted by Trichodorus sp. The virus caused stunting of the plant, severe stem necrosis, irregular yellow green mottle, ring spots and enations. Necrotic spots were reported on fruits.

In India chilli mosaic disease had been reported from various parts by McRae (1924), Kulkarni (1924), Uppal (1929) and Vasudeva (1954). These reports did not give any detailed description of the symptoms or the identity of the virus or viruses involved. Jha and Raychaudhuri (1956) reported a virus occurring on chilli and described the symptoms, transmission and physical properties of the virus and designated it as Indian chilli mosaic virus. Mariani and Sastry (1958) reported that the virus could also be transmitted by Myzus persicae Sulz. and Aphis evonymii Fabricius. in addition to A.gossypii Glov. reported by Jha and Raychaudhuri (1956). Studies on the relationship of the virus to its vector A.gossypii was reported by Mariani and Sastry (1962).

Joshi and Bhargava (1962) recorded a vein banding mosaic virus disease of chilli (Capsicum frutescens L.)

from Uttar Pradesh. The virus was reported to be a serologically related variant of PVY.

Mishra (1963) reported a virus causing mosaic symptoms and necrosis on chillies (C. annuum L.) from Delhi. The virus induced systemic necrosis of the veins and stem and mosaic mottling. The virus had a thermal inactivation point of 60°-65°C, a dilution end point of 1:500 to 1:1000, and a longevity in vitro of 1-2 hr. at 32°-35°C. A. gossypii, A. craccivora Koch., and Myzus persicae were reported to be its vectors.

Kandaswamy et al. (1963) have reported the occurrence of tobacco mosaic virus in Tamil Nadu. Based on symptomatology and transmission, they considered that cucumber mosaic virus, alfalfa mosaic virus, pepper vein banding virus or chilli mosaic virus may also be present in chilli crop.

Anjaneyulu and Appa Rao (1967) recorded the natural occurrence of cucumber mosaic virus in chilli from Andhra Pradesh.

Nariani (1968) reported tomato leaf curl virus, a strain of Nicotiana virus 10 to infect chilli. Dhanraj and Seth (1968) reported a new strain of leaf curl virus infecting chilli from Delhi.

Jeyarajan and Ramakrishnan (1961, 1969) reported chilli mosaic disease caused by PVY from Tamil Nadu.

Detailed studies have, however, not been made to identify and ascertain different viruses occurring on chilli crop in Tamil Nadu. A systematic study has therefore been undertaken in great detail to investigate the different viruses that cause mosaic disease of chilli in Tamil Nadu and fill up the lacuna.

MATERIALS AND METHODS

A. Collection and maintenance of virus isolates

A survey of the chilli growing tracts in Tamil Nadu was undertaken and virus infected material brought from the different areas was used to isolate the virus cultures for further study. A collection of thirty virus isolates was made from Coimbatore, Tirunelveli, Tiruchirappalli, Salem, Chingleput, North Arcot, South Arcot and Ramnad districts.

The virus isolates were maintained on chilli plants (K1, an annuum type released by the Agricultural Research Station, Koilpatti) in an insect proof glass-house after transmission by sap inoculation or by protected transmission with the vector Aphis gossypii Glover. Sub cultures were made once in every month. Parathion was periodically sprayed on the plants in the glasshouse as an additional precaution against insects. Powdery mildew was prevented by dusting with sulphur whenever necessary. The plants were also sprayed with Trithion to control mites at times.

B. Transmission

(i) Sap inoculation: Young leaves showing characteristic symptoms of virus infection were homogenised with the addition of 3 ml. of 0.1M phosphate buffer for every gram of leaf material in a pestle and mortar. The homogenate was squeezed through muslin cloth and the extracted sap was used as standard inoculum. Inoculations were carried out by rubbing

the inoculum on the upper surface of the second pair of leaves dusted with carborundum powder, using a glass spatula. The leaf was supported from below by a small piece of clean cardboard during inoculation.

(ii) Aphid transmission: Virus free colonies of aphids were maintained by the multiplication of a single viviparous, wingless female on suitable host plants. The aphids tested for their efficiency in virus transmission and their maintenance hosts were as follows:-

1. Aphis gossypii Glov. on Capsicum annuum L.
2. Aphis craccivora Koch. on Vigna sinensis Endl.
Aphis craccivora Koch. on Glyricidia maculata H.E. & K.
3. Aphis nerii Boyer de Fonscolombe on Calotropis gigantea L.
4. Aphis evonymi Fabricius on Solanum nigrum L.
5. Myzus persicae Sulz. on Nicotiana tabacum L.
6. Toxoptera citricidus Kirk. on Citrus aurantifolia Swingle
7. Rhopalosiphum maidis (Fitch). on Sorghum vulgare Pers.
8. Pentalonia nigronervosa Coq. on Musa paradisiaca L.

Only adult apterous forms were used in the transmission studies. They were initially starved for two hours and then transferred to detached young leaves showing characteristic symptoms of infection kept in a petri dish and allowed to feed for 15 to 30 minutes. They were then transferred to healthy test plants and allowed to feed for 24 hours. The test plants were then sprayed with parathion to kill the

aphids and kept for observation inside the glasshouse. Except where otherwise mentioned in the text the above aphid transmission method was used.

C. Host range studies

The effect of the different virus isolates on different plant species was studied by sap inoculation to a number of plants belonging to 52 genera representing 15 families. Plants which did not show any visible symptoms of the virus infection after 5 weeks were indexed on healthy test plants to determine their role as symptomless virus carriers.

The species of plants used for the study of host range of the virus isolates are listed below:

SOLANACEAE (24)

1. Nicotiana tabacum L. C.V white burley
2. N. tabacum L. C.V xanthi
3. N. tabacum L. C.V Harrison special
4. N. glutinosa L.
5. N. rustica L.
6. N. glauca Graham
7. N. paniculata L.
8. N. companiculata L.
9. Datura stramonium L.
10. D. ferox L.
11. D. metel L.
12. Capsicum annuum L.

- 13. C.frutescens L. var. tabasco
- 14. C.frutescens L. var. buccatum
- 15. C.microcarpon DC
- 16. Solanum nigrum L.
- 17. S.melongena L.
- 18. Solanum demissum Lindl x S.tuberosum L. - Hybrid (A₆)
- 19. Lycopersicon esculentum Mill.
- 20. Nicandra physaloides (L) Pers.
- 21. Petunia hybrida Vilm
- 22. Physalis peruviana L.
- 23. P.floridana Rydb.
- 24. P.minima L.

LEGUMINOSAE (24)

- 25. Vigna sinensis Endl.
- 26. Phaseolus aureus L.
- 27. P.mungo L.
- 28. P.vulgaris L.
- 29. Pisum sativum L.
- 30. Cajanus cajan L. Millsp.
- 31. Cyamopsis psoraloides DC
- 32. Lathyrus odoratus L.
- 33. Dolichos lab lab L.
- 34. Glycine max Merr.
- 35. Glyricidia maculata Stued.
- 36. Medicago sativa L.
- 37. Trifolium pratense L. 'Kenland'

- 38. T. incarnatum L.
- 39. T. repens L.
- 40. Crotalaria juncea L.
- 41. Arachis hypogaea Wild.
- 42. Melilotus alba Desv.
- 43. Cicer arietinum L.
- 44. Clitoria ternata L.
- 45. Vicia faba L.

CHENOPODIACEAE (6)

- 46. Chenopodium amaranticolor Coste & Reyn.
- 47. C. album L.
- 48. C. murale L.
- 49. Beta vulgaris L.
- 50. Basella alba L.
- 51. B. rubra L.

COMPOSITAE (5)

- 52. Zinnia elegans Jacq.
- 53. Flavaria australasica Hk
- 54. Helianthus annuus L.
- 55. Lactuca sativa L.
- 56. Carthamus tinctorius L.

CRUCIFERAE (2)

- 57. Nasturtium officinale Br.
- 58. Brassica juncea L.

AMARANTHACEAE (3)

59. Gomphrena globosa L.
 60. Amaranthus viridis L.
 61. A.gangeticus L.

CUCURBITACEAE (8)

62. Cucumis sativus L.
 63. C.melo L.
 64. Cucurbita moschata Duch.
 65. C.pepo L.
 66. C.maxima Duch
 67. Citrullus vulgaris Schrad.
 68. Trichosanthes anguina L.
 69. Momordica charantia L.

MALVACEAE (1)

70. Althaea rosea L.

POLYGONACEAE (2)

71. Fagopyron esculentum Gaertn.
 72. Rumex nepalensis Spr.

GRAMINEAE (1)

73. Zea mays L.

CARYOPHYLLACEAE (1)

74. Dianthus barbatus Pall ex. ser.

AIZOACEAE (1)

75. Trianthema portulacastrum L.

LABIATAE (1)76. Ocimum sanctum L.COMMELINACEAE (1)77. Commelina jacobii C. Fisch.APOCYNACEAE (1)78. Vinca rosea L.D. Physical properties of the virus isolates1. Thermal inactivation point

Three ml portions of the virus inoculum extracted with 0.1 M phosphate buffer was distributed into thin walled glass tubes of uniform size. The tubes with the inoculum were subjected to temperatures ranging from 40° to 95°C for ten minutes in a thermostatically controlled water bath. After the ten minute exposure, the tubes were immersed in ice-cold water for 10 minutes. The heat treated sap was inoculated on chilli plants for each treatment in the case of virus isolates that did not have any local lesion hosts. In the case of virus isolates that produced local lesions, the local lesion host was used as the test plant. Untreated sap was used as a check.

2. Dilution end point

Inoculum was extracted by grinding the leaves in a sterile pestle and mortar without the addition of buffer. Serial dilutions of the expressed sap were made in 0.1 M phosphate buffer (pH 7.0) and each dilution was inoculated to

test plants. Plants inoculated with the undiluted sap served as the check.

3. Longevity in vitro

The standard inoculum was distributed into small glass vials in aliquots of 3 cc and one set of such vials was stored at the room temperature (26°-28°C) and another set at 5°C for 100 days. Samples of the stored inoculum were taken at periodical intervals and inoculated on to chilli test plants. Freshly extracted sap served as control.

E. Studies on virus-vector relationships

The relationship between the viruses and aphid vectors was studied in detail in the case of the virus isolates C₁₀, C₂₄ and C₃₀ and the vectors Aphis gossypii and A. craccivora. In the case of the virus isolates C₁₀ and C₂₄ detailed studies were made in respect of virus transmission with Aphis craccivora collected from the source plants cowpea and Glyricidia maculata. Later studies were made by maintaining A. craccivora on cowpea plants in insect cages. Young chilli plants at four leaf stage were used in all the studies on virus vector relationship.

(1) Effect of preliminary or preacquisition fasting on the efficiency of the aphid vectors to acquire the virus

Batches of 20 aphids were fasted for varying periods ranging from 15, 30, 45 and 60 minutes and 2, 4 and 6 hours.

They were then given an acquisition feeding period of 10 minutes before allowing them a test feed on healthy plants for 24 hours.

(ii) Determination of the acquisition threshold of the vectors

Batches of 20 aphids were starved for 2 hours. After preacquisition fasting the aphids were given an acquisition feeding period ranging from 5, 15, 30 and 60 seconds and 2, 5, 15, 30 and 60 minutes on diseased plants. The aphids were transferred to healthy test plants after the acquisition feed and allowed 24 hour test feeding.

(iii) Determination of the inoculation threshold of the vectors

Batches of 20 virus free aphids were starved for 2 hours and then fed on infected plants for 15 minutes. The aphids were transferred on healthy test plants after the acquisition feed. Different batches of viruliferous aphids were then allowed different inoculation or test feeding periods ranging from 15, 30 and 60 seconds and 2, 5, 15, 30 minutes and 1, 4 and 24 hours.

(iv) Determination of the number of viruliferous aphids per plant required for efficient transmission

Single aphids and 5, 10, 20 and 30 aphids in batches were fasted for 2 hours and allowed an acquisition feed for 15 minutes on infected plants. Then, they were transferred to healthy test plants for a test feeding of 24 hours.

(v) Study of the persistence of virus in the vectors during fasting

Virus free aphids were starved for 2 hours and then allowed to feed for 15 minutes on infected plants to acquire the virus. After the acquisition the aphids were starved for 15, 30, 45 and 60 minutes and 2, 4 and 24 hours. After the post acquisition fasting, the aphids were placed on healthy test plants to feed for 24 hours.

(vi) Persistence of the virus in the aphid vectors during feeding

Virus free aphids were fasted for 2 hours and then allowed an acquisition feed for 15 minutes on infected plants. They were then transferred individually to series of healthy test plants in succession. Viruliferous aphids were given a test feed of 5 minutes, 10 minutes and 15 minutes on each successive test plant in two series and allowed 24 hours feed on the last plant of each series.

F. Serological tests

Antisera were obtained from the following sources.

<u>Antiserum</u>	<u>Source</u>
1. Antiserum for tobacco mosaic virus	- Microbiological Associates Inc. Washington
2. Antiserum for alfalfa mosaic virus	- Microbiological Associates Inc. Washington

- | | | |
|---|---|---|
| 3. Antiserum for potato virus X | { | Mr. J.M. Todd, Scottish
Agricultural Department,
United Kingdom |
| 4. Antiserum for potato virus Y | } | |
| 5. Antiserum for cucumber mosaic
virus | - | Dr. Kimble, U.S.D.A. |

Ouchterlony agar double-diffusion tests were made as per the procedure described by Ball (1961). Tube precipitin tests were made as per the procedure described by Matthews (1957).

EXPERIMENTAL RESULTS

I. Virus diseases of chilli

A. (i) Survey and collection of virus isolates

A survey was undertaken in important chilli growing areas of Tamil Nadu to collect virus diseased chilli plants. The disease was found to be widespread with its incidence ranging from 4 to 75 per cent. Thirty virus isolates were collected from different areas. The percentage of disease incidence, the number of collections made, the details of localities from where they were collected together with the designation of the isolates are presented in the following Table.

Table 1: Collection of chilli virus isolates from different areas of Tamil Nadu.

District	Place of collection	Disease incidence	No. of collections	Isolate No.
Coimbatore	Agricultural College Campus	4.5-20%	2	C ₂₁ , C ₂₃
	Periyanaickenpalayam	30%	2	C ₁ , C ₂
	Pollachi	50-60%	4	C ₃ , C ₁₄ , C ₁₅ , C ₁₆
	Bhavanisagar	20-35%	6	C ₄ , C ₅ , C ₆ , C ₁₀ , C ₁₆ , C ₂₆
Salem	Salem	30-40%	1	C ₉
Dharmapuri	Krishnagiri	15-21%	1	C ₇

Chinglepet	Kancheepuram	14-26%	1	C ₂₀
North Arcot	Vellore	15-20%	1	C ₈
South Arcot	Cuddalore	10-15%	1	C ₁₂
Tiruchi- rapalli	Thuraiyur	4%	1	C ₁₉
	Ariyalur	6%	1	C ₂₅
	Tiruchirapalli	5-10%	1	C ₂₉
	Manikandan	9%	1	C ₂₈
Madurai	Oddanchatram	40-50%	2	C ₁₁ , C ₂₄
Tirunelveli	Koilpatti	5-40%	1	C ₁₈
	Sathur	35-60%	1	C ₂₂
	Sankarankoil	50-75%	2	C ₁₃ , C ₃₀
Hamnad	Aruppukottai	24-30%	1	C ₁₇

The distribution of the chilli viruses in Tamil Nadu is presented in the map. (Plate A).

(ii) Symptoms on the diseased plant

The symptoms exhibited by the diseased plants at the time of collection and the symptoms induced by the individual virus isolates were classified into six groups and presented below.

The groupings were based on detailed studies of transmission, host range, physical properties and serological tests discussed in later pages of this work.

The same groupings were adopted in presenting the results in all experiments.

Table 2 : Symptoms exhibited by the diseased plants in f

Collection number	Stunting of plants	Reduction in leaf size	Chlorosis	Vein clearing	Vein banding	Mosaic mottling	Crinkling	Blistering	Deformation	Twisting and overlapping	Fleeging	Other leaf symptoms
C1			+			+	+					
C2			+			+	+					
C3	+		+			+						
C4	+		+			+						
C5	+		+			+						
C6	+		+			+						
C7		++	+	+	+	+++	+		++	+		
C8		++	+	+	+	+++	+		++	+		
C9		++	+	+	+	+++	+		++	+		
C10		++	+	+	+	+++	+		++	+		
C11		++	+	+	+	+++	+		++	+		
C12		++	+	+	+	+++	+		++	+		
C13		++	+	+	+	+++	+		++	+		
C14		++	+	+	+	+++	+		++	+		
C15		++	+	+	+	+++	+		++	+		
C16		++	+	+	+	+++	+		++	+		
C17		++	+	+	+	+++	+		++	+		
C18			+		+	+	+	+	+			
C19		++	+	+	+	+++	+		++	+		
C20		++	+	+	+	+++	+		++	+		
C21			+		+	+	+	+	+			
C22			+		+	+	+	+	+			
C23		++	+	+	+	+++	+		++			
C24		++				+++		+	++			
C25		++				+++		+	++			
C26			+			+					+	HM
C27			+			+					+	HM
C28			+			+					+	HM
C29			+			+						(CR
C30					+	+						(OLP
												(CR
												(OLP

HM = Heiroglyphic markings; CR = Chlorotic rings; OLP = Oak leaf
 +, ++, +++ = intensity of symptoms

Group I: The collection C₁ and C₂ exhibited chlorosis of leaves, the leaves being much paler than normal. Mosaic mottling of light to moderate shades of green was observed. The plants were less bushy than the normal and the leaves were also slightly crinkled.

Group II: In the case of the collection C₃, C₄, C₅ and C₆, the leaves exhibited interveinal chlorosis and light and dark green mosaic pattern. The diseased plants were slightly stunted. The symptoms were not very distinct from the first group except for slight differences in the severity of mosaic pattern and slight stunting of the diseased plants.

Group III: Chlorosis, fine diffuse mottle and slight deformation of the leaves were the symptoms characteristic of the collection C₁₈, C₂₁, C₂₂. The leaves also showed crinkling, vein banding and slight blistering. The collection C₇, C₈, C₉, C₁₀, C₁₁, C₁₂, C₁₃, C₁₄, C₁₅, C₁₆, C₁₇, C₁₉, C₂₀ and C₂₃ exhibited clearing of veins of young top leaves, mosaic mottling of severe type with crinkling of leaves. The leaves were so severely deformed, that the lamina was very much reduced in size to varying degrees. They were elongated from base to tip and reduced in breadth. The reduction in lamina was very prominent in young and middle leaves. The older leaves exhibited chlorosis and dark green vein banding. Some leaves were also twisted, bent at the middle and folded towards the base. The fruits were severely crinkled and smaller in size. This group was distinctly different from

the previous two groups in exhibiting vein banding and deformation of the leaves with reduction in the size of the lamina to varying degrees. The collection C₁₈, C₂₁ and C₂₂, though exhibiting less severe leaf deformation and mosaic pattern than the others in the third group, had, however, vein banding symptoms.

Group IV: The collection C₂₄ and C₂₅ exhibited severe mosaic mottling symptoms on the lamina, slight blistering and severe deformation of leaves with reduction of lamina. This group differed from the groups I and II in the severity of mosaic pattern and leaf malformation with reduction in the lamina and from group III in not exhibiting vein banding symptoms.

Group V: The collection C₂₆, C₂₇ and C₂₈ exhibited chlorosis, moderate mosaic mottling symptoms and flagging of some older leaves. Heiroglyphic markings were noticed on a few older leaves. This group, though exhibiting symptoms that could not be distinguished clearly from the first two groups except for a few older leaves showing heiroglyphic markings and flagging, was reckoned as distinct, since its constituent isolates differed in other characters of transmission, physical properties and host range as well as serological reaction, discussed elsewhere.

Group VI: The collection C₂₉ and C₃₀ showed uniform pattern of mosaic mottling of leaves of larger patches of light and

Table 3 : Symptoms induced by various virus isolates on Capsicum annuum on artificial inoculation

Isolate number	leaves developing subsequently													fruits		plants									
	Inoculated leaves	Chlorotic spots and abscession	Chlorosis	Reduction in size	Chlorosis	Vein clearing	Vein banding	Mosaic	Puckering	Blistering	Crinkling	Wavy margin	Irregularity	Deformation	Twisting and folding	Flaking	Nerosis and hanging of leaves	Leaf tip elongation	Other leaf symptoms	Reduction in size	Crinkling	Paleness	Yellow markings	Stunting	Death
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)		
G1	+				+		+	+	+							+							+		+
G2	+				+		+	+	+							+							+		+
G3				+	+		+	+		+											+	YM	+		
G4				+	+		+	+		+											+	YM	+		
G5				+	+		+	+		+											+	YM	+		
G6				+	+		+	+		+											+	YM	+		
G7				+	+		+++	+		+			+								+				
G8				+	+		+++	+		+			+								+				
G9				+	+		+++	+		+			+								+				
G10				+	+		+++	+		+			+								+				
G11				+	+		+++	+		+			+								+				
G12				+	+		+++	+		+			+								+				

Table 3 (CONTD.)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)		
G13					+	+	+	+++			+	+	+		+									+	+	
G14					+	+	+	+++			+	+	+		+										+	+
G15					+	+	+	+++			+	+	+		+										+	+
G16					+	+	+	+++			+	+	+		+										+	+
G17					+	+	+	+++			+	+	+		+										+	+
G18				+	+	+	+	+		+																
G19					+	+	+	+++			+	+	+		+										+	+
G20					+	+	+	+++			+	+	+		+										+	+
G21				+	+	+	+	+		+																
G22					+	+	+	+		+																
G23					+	+	+	+++			+	+	+		+										+	+
G24								++			+	+	+		+											
G25								++			+	+	+		+											
G26			+	+				+			+	+	+													HM
G27			+	+				+			+	+	+													HM
G28			+	+				+			+	+	+													HM
G29								+																		(CR + (OLP
G30								+																		(CR + (OLF

HM = heiroglyphic markings; CR = Chlorotic rings; OLP = Oak leaf pattern YM = Yellow markings
 +, ++, +++ = intensity of symptoms

dark green areas. Older leaves were exhibiting often chlorotic rings, oak leaf patterns and vein banding. This group is distinct from group III in not showing leaf deformations or reduction in lamina and differs from group IV in exhibiting vein banding. The symptoms of this group is less distinct than groups I and II as well as group V that showed flagging of a few old leaves.

From the study of field symptoms it became evident that symptoms alone could not be used as criteria for distinguishing the viruses, except to broadly differentiate them into groups on the basis of extreme reduction in lamina with severe mosaic patterns and vein banding.

(iii) Symptoms on *Capsicum annuum* on artificial inoculation

On artificial inoculation, the plants of 'Sathur samba' a local cultivar of *C.annuum* exhibited the following symptoms.

Group I: The isolates C₁ and C₂ produced diffuse yellow chlorotic spots on inoculated leaves in about 4 to 6 days following inoculation. The inoculated leaves abscised in another two days. This acute stage was followed by chronic symptoms in newly formed leaves with clearing of the veins followed by light and dark green mosaic mottling, puckering and blistering. The infected plants were slightly stunted. In the case of a few of the severely infected plants necrotic patches appeared along the veins. The necrosis extended along

the petiole reaching the stem. The leaves dried and hung down. The necrosis of the stem proceeded downwards killing the young stem at first and ultimately the plant later.

Group II: The isolates C₃, C₄, C₅ and C₆ did not produce any symptoms on the inoculated leaves. Young leaves near the top of the plant exhibited vein clearing in about 10-12 days following inoculation. Chlorotic to pale green patches and mosaic mottling symptoms followed by crinkling of leaves appeared later. The infected plants were slightly stunted. Only few fruits were formed which were reduced in size and crinkled with whitish yellow markings when green.

Group III: The isolates C₁₈, C₂₁ and C₂₂ did not produce any symptoms on the inoculated leaves. After 14 to 15 days following inoculation emerging leaves exhibited mild chlorosis, fine diffuse mottle and slight reduction in the size of the lamina. The leaf margins were wavy. Vein banding was observed as a characteristic symptom. The lamina also exhibited slight blistering.

The isolates C₇, C₈, C₉, C₁₀, C₁₁, C₁₂, C₁₃, C₁₄, C₁₅, C₁₆, C₁₇, C₁₉, C₂₀ and C₂₃ also failed to produce any symptoms on the inoculated leaves. Twelve to sixteen days after inoculation young newly formed leaves showed symptoms of vein clearing and subsequently severe mosaic mottling of light and dark green areas. The leaves had wavy margin with crinkled lamina. The older leaves exhibited pale chlorotic

interveinal areas and dark green vein banding. The leaves were elongated from base to apex and reduced in breadth. The lamina was reduced to varying degrees in young leaves on both sides of the midrib. The lamina was also completely lacking with the midrib thin and drawn out as a filiform tendril. Some of the middle leaves were bent at the middle and rolled down below the basal half. Fruits produced on infected plants were small in size and crinkled.

Group IV: The isolates C₂₄ and C₂₅ did not exhibit any symptoms on the inoculated leaves. About 14 to 16 days after inoculation young newly formed leaves exhibited vein clearing. Mosaic mottling of light and dark green areas were observed subsequently. The leaves exhibited crinkling, blistering, twisting and waviness of the margin. The lamina was reduced to varying degrees on both sides of the midrib and in a few instances leading to filiform structure. Vein banding symptoms were, however, not produced by these isolates.

Group V: The isolates C₂₆, C₂₇ and C₂₈ showed mild chlorosis of the inoculated leaves about 8 days after inoculation. The emerging leaves exhibited clearing of the veins after 12 days from the time of inoculation. Subsequently, mild symptom of mosaic mottling was observed along with vein banding. The leaf margins were wavy. The lamina was either normal in size or slightly reduced. Older leaves exhibited hieroglyphic markings and flagging. In severely infected plants older leaves showed necrosis at the base that caused leaf fall.

Table 4 : Transmission tests with sap and aphid vectors of the chilli virus isolates

Iso- num- ber	Sap Inoculation		Aphis gossypii		Aphis cracivora		Myzus persicae		Aphis evonymi		Aphis nerii	
	% trans- mis- sion (in days)	Incu- bation period (in days)	% trans- mis- sion (in days)	Incu- bation period (in days)	% trans- mis- sion (in days)	Incu- bation period (in days)	% trans- mis- sion (in days)	Incu- bation period (in days)	% trans- mis- sion (in days)	Incu- bation period (in days)	% trans- mis- sion (in days)	Incu- bation period (in days)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
C ₁	90	4										
C ₂	100	4										
C ₃	80	10										
C ₄	80	11										
C ₅	70	12										
C ₆	70	10										
C ₇	70	12	50	14	60	16	50	16	20	18	--	--
C ₈	80	12	70	14	60	14	60	12	18	17	--	--
C ₉	60	14	60	14	50	16	60	12	22	15	--	--
C ₁₀	60	14	70	15	60	15	30	12	25	15	--	--
C ₁₁	50	13	70	16	60	15	50	14	19	16	--	--
C ₁₂	50	14	50	12	40	14	60	12	21	16	--	--

Not transmitted by any of the aphid species

Table 4 (Contd.)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
G ₁₄	80	12	60	14	40	15	50	13	24	17	--	--
G ₁₅	70	13	30	14	50	14	60	12	26	15	--	--
G ₁₆	80	12	70	12	70	13	80	11	22	14	--	--
G ₁₇	60	16	60	14	40	14	50	12	28	15	--	--
G ₁₈	80	12	70	12	70	14	50	12	18	17	--	--
G ₁₉	60	16	60	15	30	17	50	14	19	16	--	--
G ₂₀	40	16	60	16	40	16	50	14	21	16	--	--
G ₂₁	70	14	50	16	60	15	60	14	18	15	--	--
G ₂₂	50	15	60	13	60	13	40	11	24	17	--	--
G ₂₃	50	14	60	15	50	15	40	14	23	17	--	--
G ₂₄	60	11	60	14	50	14	40	12	20	12	18	20
G ₂₅	50	12	60	14	40	15	50	12	20	15	22	18
G ₂₆	60	11	50	13	--	--	50	12	--	--	--	--
G ₂₇	60	9	40	12	--	--	60	11	--	--	--	--
G ₂₈	50	10	40	12	--	--	50	11	--	--	--	--
G ₂₉	60	10	30	13	40	13	60	11	--	--	--	--
G ₃₀	60	12	40	16	40	16	60	12	--	--	--	--

-- = Not infected

Group VI: The virus isolates C₂₉ and C₃₀ caused clearing of the veins of newly formed expanding leaves in about 10 to 14 days after inoculation. The inoculated leaves did not exhibit any symptoms. Infected leaves exhibited uniform systemic mosaic mottling subsequently. The mosaic consisted of larger patches of light green or slightly pale yellow areas. The leaf tip was slightly elongated. Older leaves often showed chlorotic rings and oak leaf patterns. The fruits formed were slightly crinkled and pale in colour.

B. Transmission tests

Transmission of the virus isolates were tested both by sap inoculation and by the use of different species of aphids, namely Aphis gossypii, A. craccivora, A. evonymii, A. nerii, Myzus persicae, Toxoptera citricidus, Rhopalosiphum maidis and Pentalonia nigronervosa. The results are presented in the Table 4. The isolates C₁ and C₂ (group I) and the isolates C₃, C₄, C₅ and C₆ (group II) were only sap inoculable. The aphid species tested could not transmit these isolates. The virus isolates in group III namely C₇ to C₂₃ in serial order and the virus isolates in Group IV namely C₂₄ and C₂₅ were transmitted by four of the aphid species Aphis gossypii, Aphis craccivora, Aphis evonymii and Myzus persicae. Aphis craccivora failed to transmit the virus isolates C₂₆, C₂₇ and C₂₈ in Group V.

Aphis evonymii failed to transmit the virus isolates C₂₆, C₂₇ and C₂₈ in Group V and C₂₉ and C₃₀ in Group VI.

Aphis nerii was able to transmit the virus isolates C₂₄ and C₂₅ in Group IV alone successfully but failed to transmit any of the other virus isolates.

Toxoptera citricidus, Rhopalosiphum maidis and Pentalonia nigronervosa did not transmit any of the virus isolates tested.

C. Studies on host range and symptomatology of the virus isolates

The virus isolates were inoculated on different host plants listed earlier and their reactions were studied in detail. Symptoms induced by individual isolates within a group did not vary and are therefore presented here in groups. (Table 5, Plates 1-6)

Nicotiana tabacum C.V. White Burley

The virus isolates C₁ and C₂ did not produce any reaction on the inoculated leaves. Vein clearing was the first symptom observed 10-12 days after inoculation on newly emerging young leaves. Subsequently produced leaves showed mosaic mottling consisting of patches of light and dark green areas. Leaf margins were slightly distorted and the leaf-size was also slightly reduced.

The virus isolates of group II namely, C₃, C₄, C₅ and C₆ produced necrotic spots on inoculated leaves in 3-4 days. This was followed by mosaic mottling of new, expanding leaves in 12-14 days.

The isolates of group III namely C₇ to C₂₃ in seriatum, numbering 17, infected this plant systemically. Inoculated leaves failed to exhibit any symptoms. Vein clearing was observed on the new expanding leaf in 12 days followed by general chlorosis and mosaic mottling. Vein banding especially, near the finer veins was the marked symptom.

The virus isolates of group IV namely C₂₄ and C₂₅ did not cause any symptoms on the inoculated leaves. New expanding leaves showed mild clearing of the veins 12-14 days after inoculation. Mosaic mottling of light and dark green areas appeared later. The leaves were slightly reduced in size and the infected plants were slightly stunted.

The isolates of group V namely C₂₆, C₂₇ and C₂₈ infected this plant in about 8 days showing mild vein clearing of the young expanding leaves. No symptoms were observed on the inoculated leaves. Mild mosaic mottling appeared later. Leaves of the infected plants also showed slight vein banding. Older leaves exhibited heiroglyphic markings and necrotic areas bordering the veins.

The isolates of group VI namely C₂₉ and C₃₀ caused systemic vein clearing of the expanding leaves about 10 days after inoculation. Subsequently, the leaves developed mosaic mottling symptoms. Older leaves occasionally exhibited oak leaf patterns.

N. tabacum C.V. Harrison's Special

The isolates belonging to all groups did not show any symptom on the inoculated leaves. Young expanding leaves exhibited vein clearing in about 10-12 days except the isolates in group II. All the isolates caused mosaic mottling symptoms. Distortion of leaf margins were observed in plants inoculated with group I and group III isolates. Size of the leaves was reduced in case of group I and group IV isolates. Vein banding was observed in plants inoculated with group III and group V isolates. Inoculated plants were stunted in case of group IV isolates.

N. tabacum var. xanthi

The isolates of group I caused local necrotic lesions on the inoculated leaves in 2-4 days after inoculation. No systemic symptoms were produced. Isolates of other groups did not show any symptom on inoculated leaves. Isolates of group III and IV exhibited vein clearing followed by mosaic mottling, reduction in lamina size and slight stunting of the plants. Isolates of group II, group V and group VI caused mosaic mottling symptoms.

N. glutinosa

The isolates of group I caused local necrotic lesions on the inoculated leaves in 2-4 days as in the case of the host N. tabacum var. xanthi. On N. glutinosa however, the lesions enlarged and coalesced together and the infection spread to the petioles and to the stem causing a blackening

of the area and killing the apical part of the plant under summer conditions. Isolates of group V induced vein-banding symptoms. All the other isolates of different groups caused symptoms similar to those that were produced on N.tabacum var xanthi.

N.paniculata and N.companiculata

Both the species had reacted with systemic symptoms of mosaic mottling to all the virus isolates in all the groups in about 14-16 days after inoculation.

N.glauca

Plants of this species reacted with mosaic mottling symptoms of the emerging leaves, 15 days after inoculation with the isolates of group I and II. Isolates of group III induced mosaic mottling and severe vein banding. Isolates of group IV, V and VI did not produce any symptoms.

N.rustica

This species reacted with brown local lesions on the inoculated leaves with group I and group II virus isolates. The isolates of the groups IV and VI did not infect this plant. Isolates in group III reacted with severe mosaic and chlorosis. Isolates in group V reacted with local lesions followed by mild mosaic mottling.

Datura stramonium

Isolates of groups I and II caused local lesions of 4 mm size in 3-4 days on inoculated leaves. There was no

however, showed symptoms of vein necrosis, wilt and abscission of lower leaves which were not observed in the variety buccatum. Both the varieties reacted with mild mosaic mottling of newly formed leaves in 12 days when infected with the isolates of group VI.

C. microcarpon

The isolates of group I did not infect this species and cause any symptoms. The isolates of group II caused local brown lesions of 1 mm size in 5 days on the inoculated leaf. No systemic symptoms were produced. The isolates in group III, IV, V and VI caused mild mosaic systemically on newly produced leaves in 12 to 15 days after inoculation.

Solanum nigrum

The isolates of group I and III caused in this plant systemic symptoms of faint mosaic mottling in about 15 days. But the isolates of group IV caused systemic symptoms of severe mosaic. The isolates of group II, group V and group VI did not infect this species.

S. demissum x S. tuberosum Hybrid A6

Detached leaves of this hybrid plant on inoculation with group III isolates exhibited brown local lesions after inoculation in a moist chamber at 22°C and constant light after 4-5 days; none of the isolates of any other groups caused any symptom on this hybrid.

Lycopersicon esculentum

Plants of this species reacted with mosaic mottling symptoms systemically in 10-12 days with isolates of group I and group II. No reaction was observed with isolates belonging to the other four groups.

Petunia hybrida

Group I isolates caused severe systemic mosaic symptoms on newly formed leaves in 10 days. Isolates of group II also caused similar symptoms. Isolates of group III and group VI caused mild systemic mosaic symptoms and vein banding in 12 to 15 days. Isolates of group III also induced veinal necrosis. Isolates of group IV and V caused severe mosaic mottling and crinkling of leaves.

Nicandra physaloides

Group I isolates on inoculation caused severe mosaic, crinkling of leaves and stunting of the plant. Similar symptoms were produced by the virus isolates of group III and group IV. Isolates of group II, group V and group VI caused only mild mosaic symptoms.

Physalis peruviana

This species on inoculation reacted after 15 days with mild mosaic to the isolates from group I. Group II, III, IV and group VI isolates did not infect. Isolates from group V caused mild mosaic and vein banding of the young leaves.

P.floridana

Plants of this species reacted with mild mosaic to isolates from group III and group V in about 12 days. Vein banding was also induced by isolates of group V. This plant was not infected by the isolates in groups I, II, IV and VI.

Chenopodium amaranticolor and C.murale

Both the species reacted with local lesions on inoculated leaves in about 5 to 7 days with the isolates from group I. C.amaranticolor reacted with local lesions in about 5-7 days on inoculation with the isolates from group II while C.murale reacted with severe systemic mosaic mottling, smalling of leaves and stunting followed by local lesions. The isolates of the groups III, IV, V and VI had failed to produce any of the symptoms in these two species.

C.album

Group I isolates caused local brown lesions on inoculated leaves in a week. Isolates of other groups did not infect C.album.

Phaseolus vulgaris (local variety)

The isolates of group I, II, III, IV or V did not cause any symptoms. Group VI isolates caused small brown local lesions of 1 mm size in 4 days after inoculation on the inoculated first pair of leaves.

Medicago sativa

Group VI isolates infected and caused mild mottling symptoms, 14 days after inoculation on young leaves. The mottling disappeared in older leaves. Isolates of other groups had failed to infect these plants.

Trifolium repens

Fourteen days after inoculation, emerging leaves exhibited mild mosaic mottling with the isolates in group VI. The isolates of other groups did not infect this plant.

Vicia faba

Chocolate brown necrotic local lesions of 2-3 mm size developed on inoculated leaves with group VI isolates only. Necrotic streaks or patches had also developed on the stem and in a few cases the plants died. No symptoms were produced by isolates of other groups.

Zinnia elegans

Mild mosaic mottling developed systemically after 12 days on newly formed leaves with group I isolates. Isolates of group V and VI also caused mild mosaic, 10-12 days after inoculation. Isolates of group IV caused severe mosaic mottling and crinkling. There was no reaction to the isolates of group II and group III.

Gomphrena glabosa

The isolates from group I infected and caused chlorosis and mosaic mottling on newly formed leaves of

this plant after 12 days following inoculation. The isolates from group II caused local round, reddish necrotic lesions in 4-6 days on inoculated leaves. No systemic infection took place. Virus isolates from groups III, IV, V and VI did not infect this plant.

Cucumis sativus

Isolates from group IV produced clearing of the veins of new leaves of this plant in 10 days. Leaves produced later (second or third pair of true leaves) exhibited mild mosaic mottling symptoms. The isolates from other groups had not caused infection.

The symptoms observed on various host plants are summed up in Table 5.

All the isolates tested had failed to infect the following species.

Solanum melongena, Physalis minima, Vigna sinensis,
Phaseolus aureus, P.mungo, Pisum sativum, Cajanus cajan,
Cyamopsis psoraloides, Lathyrus odoratus, Dolichos lab lab,
Glycine max, Glyricidia maculata, Trifolium pratense,
T.incarnatum, Crotalaria juncea, Arachis hypogaea,
Melilotus alba, Cicer arietinum, Clitoria ternata,
Beta vulgaris, Basella alba, B.rubra, Flavaria australasica,
Helianthus annuus, Lactuca sativa, Carthamus tinctorius,
Nasturtium officinalis, Brassica juncea, Amaranthus viridis,

Table 5 : Symptoms on different host plants caused by the 6 groups of virus isolates

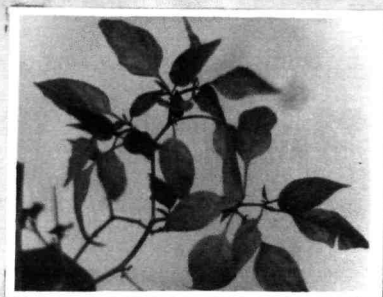
Host plant	Virus groups					
	Group I	Group II	Group III	Group IV	Group V	Group VI
<u>Nicotiana tabacum</u> var. <u>White Burley</u>	Sys.VC.MM RL.D	nLL Sys.MM	Sys.VC.C MM.VB.	Sys.VC. MM.RL.S.	Sys.VC.m. MM.VB. nec.areas along the vein.H.	Sys.VC.MM. O.L.P.
<u>N. tabacum</u> var. <u>Harrison's Special</u>	Sys.VC.MM. RL.D.	Sys.MM.	Sys.VC. MM.VB.D.	Sys.VC. MM.RL.S.	Sys.VC. MM.VB.	Sys.VC.MM.
<u>N. tabacum</u> var. <u>Ranhi</u>	nLL	Sys.MM.	Sys.VC.MM. RL.S.	Sys.VC. MM.RL.S.	Sys.MM.	Sys.MM.
<u>N. glutinosa</u>	nLL coalesced and killed apical parts	Sys.MM.	Sys.VC.MM. RL.S.	Sys.VC.MM. RL.S.	Sys.MM. VB.	Sys.MM.
<u>N. paniculata</u>	Sys.MM	Sys.MM	Sys.MM	Sys.MM	Sys.MM	Sys.MM
<u>N. glauca</u>	Sys.MM	Sys.MM	Sys.MM	Sys.MM	Sys.MM	Sys.MM
<u>N. rustica</u>	Sys.MM	Sys.MM	MM.Se.VB.	-	-	-
<u>Datura stramonium</u>	nLL	nLL	Se.M.C.	-	LL.MM.	-
<u>D. metel</u>	nLL	nLL	-	Sys.MM.C. F.S.	Sys.MM.D. F.S.	Sys.MM
<u>D. innoxia</u>	nLL	-	-	Sys.MM.C. F.S.	Sys.MM. F.S.	Sys.MM

Host plant	Virus groups					
	Group I	Group II	Group III	Group IV	Group V	Group VI
<u>Capsicum frutescens</u> var. <u>tabasco</u>	nLL.E.A.	nLL.E.A.	Sys.se.MM. cr.VB.	Sys.se.MM. cr.	Sys.VC.VN. C.mmm. W.A. of lower leaves	Sys.mmm.
<u>C. frutescens</u> var. <u>buccatum</u>	nLL.E.A.	nLL.E.A.	Sys.se.MM. cr.	Sys.se. MM, cr.	Sys.VC. C.mmm.	Sys.mmm
<u>C. microcarpon</u>	-	nLL	Sys.mmm	Sys.mmm	Sys.mmm	Sys.mmm
<u>Solanum nigrum</u>	Sys.faint MM	-	Sys.MM	Sys.se.MM.	-	-
<u>S. dasycarpum</u> x <u>S. tuberosum</u> Hybrid AGW	-	-	LL	-	-	-
<u>Lycopersicon</u> <u>esculentum</u>	Sys.MM	Sys.MM	-	-	-	-
<u>Nicotiana physaloides</u>	Sys.se.MM cr.S.	Sys.mmm	Sys.se. MM, cr.S.	Sys.se.MM cr. S.	Sys.mmm	Sys.mmm
<u>Physalis peruviana</u>	Sys.mmm	-	-	-	Sys.mmm.VB	-
<u>P. floridana</u>	-	-	Sys.mmm	-	Sys.mmm.VB	-
<u>Petunia hybrida</u>	Sys.se.MM	Sys.se.MM	Sys.mmm.VB. VN.	Sys.se.MM cr.	Sys.se.MM cr.	Sys.mmm. VB.

Host plant	Virus groups					
	Group I	Group II	Group III	Group IV	Group V	Group VI
<u>Chenopodium amarantifolium</u>	LL	LL	-	-	-	-
<u>C. murale</u>	LL	LL.Sys.se. MM.	-	-	-	-
<u>C. album</u>	LL	-	-	-	-	-
<u>Phaseolus vulgaris</u>	-	-	-	-	-	LL
<u>Medicago sativa</u>	-	-	-	-	-	Sys.mmm
<u>Trifolium repens</u>	-	-	-	-	-	Sys.mmm
<u>Vicia faba</u>	-	-	-	-	-	nLL, n. streaks and patches on stem death of the plant
<u>Zinnia elegans</u>	Sys.mmm	-	-	Sys.se. MM.or.	Sys.mmm	Sys.mmm
<u>Gomphrena globosa</u>	Sys.C.MM.	nLL	-	-	-	-
<u>Oreumis sativus</u>	-	-	-	Sys.VC.mmm	-	-

A - Abscission
C - Chlorosis
cr - Crinkling
D - Distortion
E - Epinasty
F - Filiformity
H - Heliroglyphic markings
LL - Local lesions
m - mild
MM - Mosaic mottling
n - Necrotic
OLP - Oak leaf pattern
RL - Reduction in size of the leaf
S - Stunting
se - Severe
Sys - Systemic
VC - Vein clearing
VN - Vein necrosis
VB - Vein banding
W - Wilt

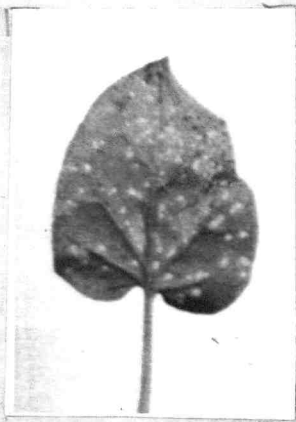
PLATE 1



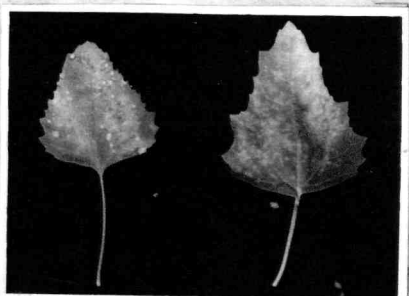
A



D

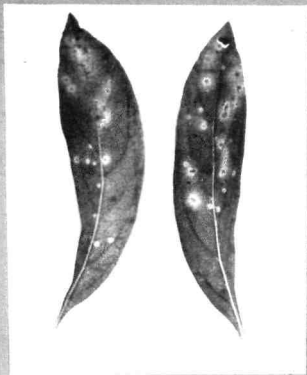


B



C

PLATE 2



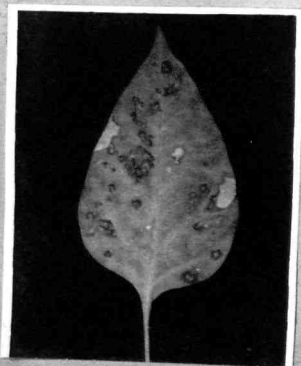
B



C



A



E



D

Plate 2 - Potato Virus X

- A. Symptom on chilli plant
- B. Local lesions on Gomphrena globosa
- C. Symptom on Nicotiana glutinosa
- D. Local lesions on Chenopodium amaranticolor
- E. Local lesions on Capsicum frutescens var. tabasco.

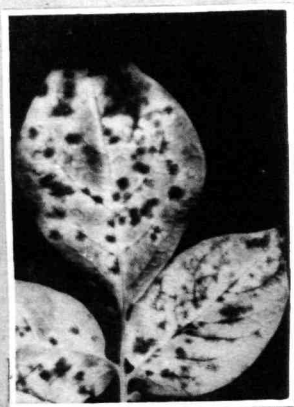
PLATE 3



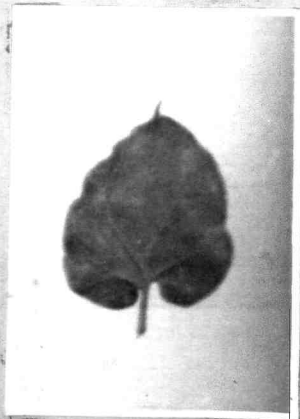
A



B



D



C

Plate 3 - Potato Virus Y

- A. Symptom on chilli plant
- B. Symptom on chilli leaves
- C. Symptom on Nicotiana glutinosa
- D. Local lesions on Potato hybrid 'A6'

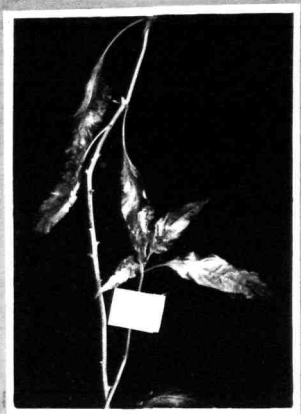
Plate 4 - Capsicum mosaic Virus

- A. Symptom on chilli plant
- B. Symptom on chilli leaves
- C. Symptom on Datura stramonium
- D. Symptom on Nicotiana glutinosa

PLATE 5



A



B



C



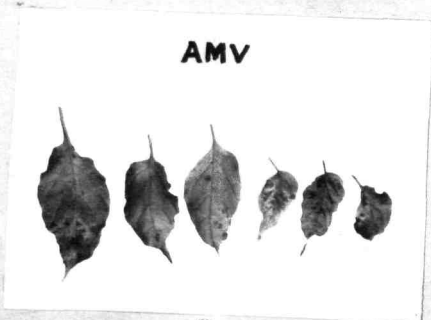
D

Plate 5 - Tobacco etch Virus

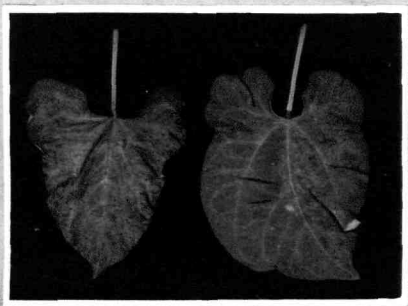
- A. Symptom on chilli plant
- B. Symptom of leaf abscission of chilli
- C. Symptom of etch on cilli
- D. Symptom of etch tobacco (White Burley)



A



B



C

Plate 6 - Alfalfa mosaic Virus

- A. Symptom on chilli plant
- B. Symptom on chilli leaves
- C. Local lesions of Phaseolus vulgaris

Table 6 : Thermal inactivation point of the chilli virus isolates

Ther- mal S. tem- No. para- ture	Virus isolates																																	
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'	2'
	Number of plants infected out of 30																																	
1. Un- trea- ted																																		
sep	47	48	25	26	27	26	24	25	26	29	25	27	26	29	24	27	27	28	28	26	27	27	24	25	22	24	24	25	24	25	24	25	24	
2. 40°C	34	32	19	18	17	21	19	20	21	22	22	18	23	25	21	19	17	23	24	18	16	22	17	16	14	14	15	18	16					
3. 50°C	29	30	13	14	13	14	14	15	14	11	14	9	17	18	15	14	3	19	18	14	11	16	11	12	7	8	7	13	11					
4. 55°C	-	7	8	6	7	3	3	4	3	4	4	2	6	7	5	7	-	8	7	-	5	-	-	3	2	2	7	6						
5. 60°C	21	19	4	3	3	4	0	0	0	0	0	0	2	3	2	2	3	4	3	5	4	2	3	3	0	0	0	2	2					
6. 65°C	-	2	1	2	1	-	-	-	-	-	-	-	0	0	0	0	1	0	0	2	1	0	0	-	-	-	-	0	0					
7. 70°C	14	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
8. 75°C	8	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
9. 80°C	5	6	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
10. 85°C	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
11. 90°C	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
12. 95°C	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						

- Not tested 0 Not infected
 1' Local lesions on H. tabsonii var. xanthi
 2' Local lesions on C. microcarpon
 Isolates C₇ to C₃₀ were tested on chilli plants

D. Studies on Physical Properties of the virus isolates.

Thermal inactivation point

The thermal inactivation point of the virus isolates was determined as outlined under 'Materials and Methods'. The results are furnished in Table 6.

Isolates of group I were found infective after exposure to 85°C for 10 minutes, but they were inactivated at 90°C exposure for the same period.

The isolates of group II were inactivated at 70°C but remained active at 65°C.

The isolates in Group III had varying thermal inactivation point as detailed below.

The isolates C₇, C₈, C₉, C₁₀, C₁₁, C₁₂ and C₁₃ were infective after exposure to 55°C and became innocuous at 60°C.

The isolates C₁₄, C₁₅, C₁₆, C₁₇, C₁₉, C₂₀ and C₂₃ had their thermal inactivation point between 60°C and 65°C. The isolates C₁₈, C₂₁ and C₂₂ had their thermal inactivation point between 65° and 70°C.

The isolates in group IV had a thermal inactivation point between 60° and 65°C.

The isolates in group V were able to withstand 55°C but not 60°C.

Table 7 : Dilution end point of chilli virus isolates

S. Dilu- No. tions	Isolate numbers																																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30								
	Number of plants infected out of 30																																					
1. Undiluted	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C		
Sap	52	49	27	26	28	27	23	26	28	26	28	26	28	27	29	27	28	26	26	28	27	26	24	24	24	24	24	26	26	23	26	-	-	-	-	-		
2. 1:10	41	43	21	20	19	11	12	20	19	18	20	19	21	21	17	19	16	17	16	20	18	21	21	19	14	13	14	16	17	-	-	-	-	-	-	-	-	
3 1:100	23	22	17	16	18	16	4	5	14	13	12	13	12	15	11	11	9	11	10	12	11	18	15	15	4	5	4	13	13	-	-	-	-	-	-	-	-	
4 1:500	-	-	-	-	-	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
5 1:1000	11	13	11	9	11	8	0	0	6	5	4	5	4	6	7	4	4	4	3	4	7	5	11	7	8	0	0	0	7	6	-	-	-	-	-	-	-	
6 1:2000	-	-	-	-	-	-	-	-	1	1	1	2	1	2	2	1	2	1	1	2	2	1	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7 1:5000	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8 1:10000	4	4	5	3	4	3	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	0	0	0	0	0	0	0	0	0	0	0	
9 1:20000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10 1:100000	2	1	1	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- not tested 0 not infected

1' Local lesions on Nicotiana tabacum var. xanthi

2' Local lesions on Capsicum microcarpon

Isolates C₇ to C₃₀ were tested on chilli plants

The isolates in group VI had their thermal inactivation point between 60° and 65°C.

Dilution end point

The results are presented in Table 7. Isolates in groups I and II had a dilution end point above 1:100,000. The isolates in group III had a varied dilution end point as detailed below.

The isolates C₇ and C₈ were infective at a dilution of 1:100 and became innocuous at 1:500. The isolates C₉ to C₂₂ in seriatum were infective at a dilution of 1:2000 but not at a dilution of 1:5000. The isolate C₂₃ was infective at a dilution of 1:10,000 but not at 1:20,000.

The isolates in Group IV namely C₂₄ and C₂₅ were infective at a dilution of 1:10,000 and not above.

The virus isolates C₂₆, C₂₇ and C₂₈ in group V were infective at a dilution of 1:100 but not at 1:500.

The isolates in Group VI namely C₂₉ and C₃₀ were infective at a dilution of 1:2000 but not at 1:5000.

Longevity in vitro

The results are presented in Tables 8 and 9. The isolates of groups I and II were infective for over 30 days but less than 100 days when stored in vitro at 26-28°C.

Table 9 : Longevity in vitro of the virus isolates at 5°C

S. No.	Age of the sep	Virus isolates																														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
		Number of plants infected out of 10																														
1	4 hours	48	52	29	28	28	32	6	7	7	6	7	6	7	6	5	7	5	7	8	7	6	7	6	5	7	8	6	8	7		
2	8 hours	47	50	24	26	26	28	4	2	2	4	4	5	4	4	6	5	6	4	6	5	5	6	5	5	6	4	4	7	3	4	5
3	24 hours	41	49	21	21	20	21	2	1	2	4	2	2	4	5	2	4	2	6	4	5	1	2	5	4	2	5	1	3	2		
4	48 hours	37	39	16	19	20	20	1	1	2	2	1	2	3	2	2	2	1	3	3	3	1	1	4	3	1	3	1	1	1		
5	3 days	35	37	14	16	19	18	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1		
6	4 days	32	33	11	12	13	14	-	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	2	-	-	-	-	-		
7	5 days	27	27	10	9	10	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
8	6 days	21	24	10	10	8	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
9	7 days	15	17	4	5	3	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
10	14 days	13	13	4	4	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
11	30 days	11	10	3	2	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
12	100 days	8	8	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		

- = not tested ; 0 = not infected

1' Local lesions on *N. tabacum* var. *Zanthei*

2' Local lesions on *C. microcarpon*

Isolates C₇ to C₃₀ were tested on chilli plants

Table 8 : Longevity in vitro (at room temperature - (26-28°C) of the virus isolates

S. No. of the sap	Age	Virus isolates																															
		C ₁	C ₂	C ₃	C ₄	C ₅	C ₆	C ₇	C ₈	C ₉	C ₁₀	C ₁₁	C ₁₂	C ₁₃	C ₁₄	C ₁₅	C ₁₆	C ₁₇	C ₁₈	C ₁₉	C ₂₀	C ₂₁	C ₂₂	C ₂₃	C ₂₄	C ₂₅	C ₂₆	C ₂₇	C ₂₈	C ₂₉	C ₃₀		
		1'	1'	2'	2'	2'	2'																										
		Number of plants infected out of 10																															
1	Fresh	49	56	30	28	27	30	7	6	8	8	7	7	6	8	7	7	6	7	8	7	8	7	8	7	8	8	8	8	7	8	7	
2	4 hours	48	52	28	27	27	30	6	5	6	7	5	5	6	6	4	7	5	7	7	6	5	7	6	5	7	6	4	5	4	5	5	
3	8 hours	46	50	24	26	24	25	3	2	4	5	4	3	3	5	6	4	5	3	4	6	6	3	3	5	5	2	2	1	3	4		
4	24 hours	35	47	20	19	18	18	1	1	1	1	2	2	3	4	3	2	1	2	4	4	1	1	5	5	1	1	1	2	2			
5	48 hours	22	25	14	16	17	16	1	1	1	1	1	2	2	1	1	1	1	1	2	1	1	1	1	3	2	0	0	1	1			
6	3 days	19	21	11	13	14	13	0	0	0	0	0	0	0	1	1	1	1	0	1	1	0	0	0	0	1	1	0	0	0			
7	4 days	17	18	9	10	11	9	0	0	0	0	0	0	1	1	1	1	0	1	1	0	1	0	0	1	1	0	0	0	0			
8	5 days	17	17	9	9	11	6	0	-	-	-	-	0	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-			
9	6 days	14	13	8	9	8	4	0	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-			
10	7 days	10	9	2	5	4	3	0	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-			
11	14 days	7	8	2	2	2	1	0	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-			
12	30 days	5	5	1	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
13	100 days	0	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			

0 = Not infected; - = Not tested
 1' Local lesions on N. tabacum var. xanthi
 2' Local lesions on C. microcarpon
 Isolates C₇ to C₃₀ were tested on chilli plants

The isolates of group III had a longevity in vitro of 2 days at room temperature except C₁₄, C₁₅, C₁₆, C₁₇, C₁₉ and C₂₀ which had a 4 days longevity.

The isolates of group IV had a longevity in vitro of 4 days. The isolates of group V had a longevity in vitro of only 24 hours. The isolates of group VI withstood 2 days storage in vitro at room temperature (26°-28°C).

The isolates of group I had a storage life in vitro of about 100 days when stored at 5°C, while those of group II had a longevity in vitro between 30 and 100 days at 5°C.

The isolates C₇ to C₂₃ of group III varied considerably in their period of longevity in vitro when stored at 5°C. The isolates C₇, C₈, C₁₈, C₂₁, C₂₂ and C₂₃ had a longevity of 3 days. The isolates namely C₉, C₁₀, C₁₁, C₁₂ and C₁₃ had a longevity of 4 days, while C₁₉ and C₂₀ withstood storage for 5 days. The isolate C₁₄, C₁₅, C₁₆ and C₁₇ withstood 6 days storage.

The isolates of group IV had a longevity of six days.

The isolates of group V had a storage life of 3 days in vitro at 5°C.

The isolates of group VI had 4 days longevity in vitro at 5°C.

E. Studies on the Vector-Virus relationships of chilli virus isolates representing three groups

Detailed studies on the Vector-Virus relationships were carried out with three chilli virus isolates, each one representing the groups III, IV and VI respectively. These virus isolates were determined based on host range, symptomatology, physical properties and serological reactions as potato virus Y, capsicum mosaic virus and alfalfa mosaic virus respectively.

Both the aphid species namely Aphis gossypii Glov. and Aphis craccivora Koch. were used as vectors. A.gossypii collected from cotton plants and A.craccivora collected from Glyricidia maculata were employed for the studies on the effect of preacquisition fasting of the aphid vectors on the efficiency of virus transmission. Subsequent experiments were conducted with Aphis gossypii maintained on Capsicum annuum and A.craccivora maintained on Vigna sinensis in insect cages.

1. Effect of preacquisition fasting of the aphid vectors on the efficiency of transmission

The virus isolates C₁₀, C₂₄ and C₃₀ representing potato virus Y (Group III), capsicum mosaic virus (Group IV) and alfalfa mosaic virus (Group VI) respectively were studied in detail in relation to the vectors A.gossypii and A.craccivora.

The results are presented in Table 10^{Fig-1}. The data indicated that the vector A.gossypii was able to transmit

Fig.1. Effect of pre-acquisition fasting of the two aphid vectors on transmission of three chilli virus isolates

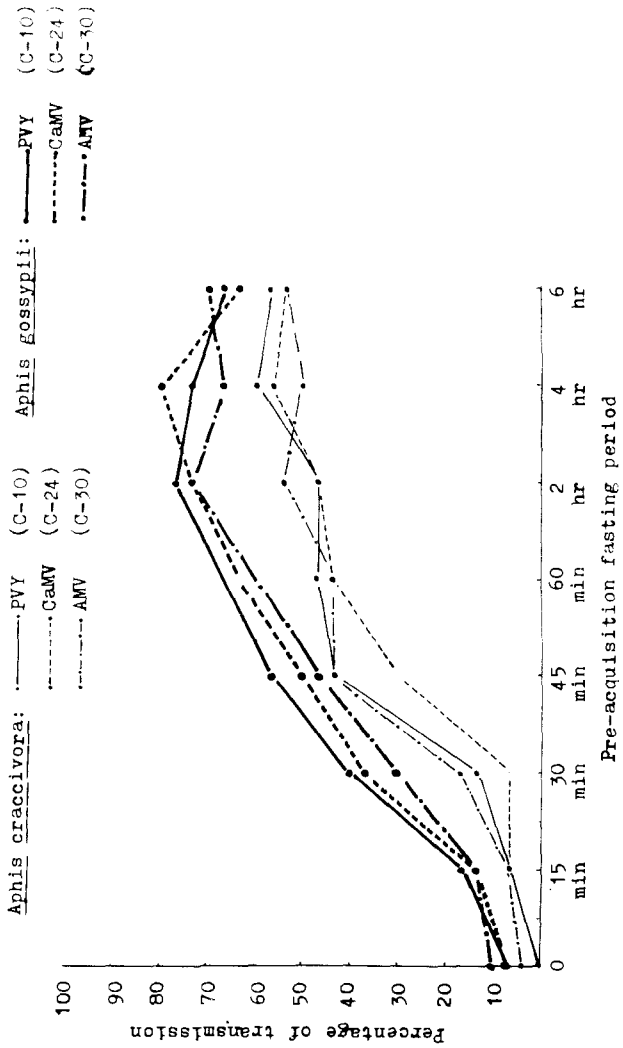


Table 10: Pre-acquisition fasting of the two aphid vectors on transmission of three chilli virus isolates

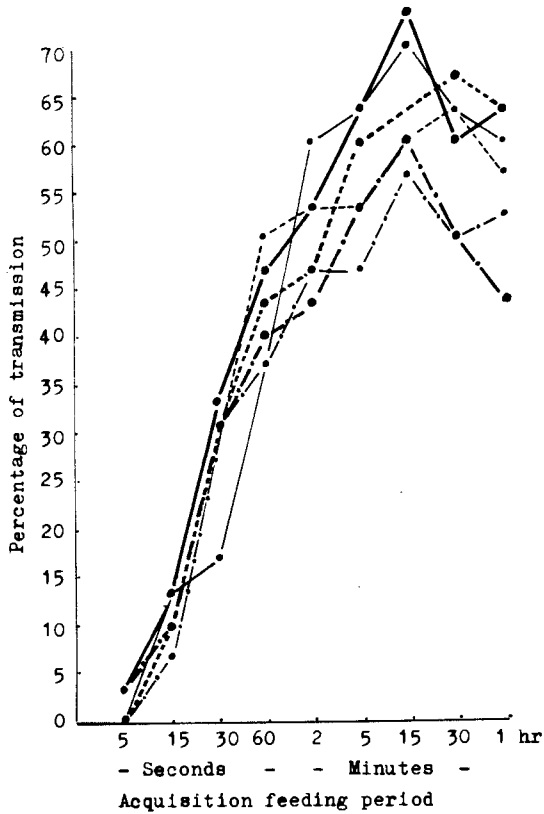
S. No.	Fasting period	<u>Aphis gossypii</u>				<u>Aphis craccivora</u>			
		PVY isolate (C10)	CaMV isolate (C24)	AMV isolate (C30)	PVY isolate (C10)	CaMV isolate (C24)	AMV isolate (C30)		
		No. of plants in- fected out of 30	% of trans- mission	No. of plants in- fected out of 30	% of trans- mission	No. of plants in- fected out of 30	% of trans- mission	No. of plants in- fected out of 30	% of trans- mission
1.	No fasting	2	6.7	2	6.7	3	10.0	0	0
2.	15 minutes	5	16.7	4	13.3	4	13.3	2	6.7
3.	30 minutes	12	40.0	11	36.7	9	30.0	4	13.3
4.	45 minutes	17	56.7	15	50.0	14	46.7	13	43.3
5.	60 minutes	20	66.7	19	63.3	18	60.0	14	46.7
6.	2 hours	23	76.6	22	73.3	22	73.3	14	46.7
7.	4 hours	22	73.3	24	80.0	20	66.7	18	60.0
8.	6 hours	20	66.7	19	63.3	21	70.0	17	56.7
	Total	121		116		108		82	
Total number of plants infected									
		<u>A. gossypii</u> 345		<u>A. craccivora</u> 236					
		720		720					
Percentage of infection = 47.9								= 32.8	

all the three viruses even without preacquisition fasting. However, the order of transmission was found to be as low as 6.7, 6.7 and 10.0 per cent for the three viruses respectively. The efficiency of transmission increased with the increase in preacquisition fasting period of the aphids. Fasting for a period of two hours was found to be optimum to secure transmission percentages as high as 76.7 and 73.3 for potato virus Y and alfalfa mosaic viruses respectively. In the case of the capsicum mosaic virus maximum percentage of infection (80.0%) was obtained with four hours of preacquisition fasting.

In the case of the vector A. craccivora, a preliminary preacquisition fasting was found to be essential for successful transmission of the viruses, though the alfalfa mosaic virus was found to be transmitted to a small extent of 3.3 per cent without the preliminary fasting of the vector. The efficiency of transmission increased with the period of preacquisition fasting, the optimum period being two hours in the case of the alfalfa mosaic virus, and, four hours for potato virus Y and capsicum mosaic virus. Fasting the vectors beyond the optimum period was found to reduce the percentage of transmission. From the total number of successful transmissions of all the three viruses it was observed that A. gossypii accounts for 47.9 per cent transmission. Only 32.8 per cent transmission was obtained with the vector, A. craccivora. A. gossypii was found to be a more efficient

Fig.2. Effect of acquisition feeding on transmission of chilli virus isolates

Aphis craccivora: ——— PVY (C-10)
 - - - - CaMV (C-24)
 ····· AMV (C-30)



Aphis gossypii: ——— PVY (C-10)
 - - - - CaMV (C-24)
 ····· AMV (C-30)

Fig.2. Effect of acquisition feeding on transmission of chilli virus isolates

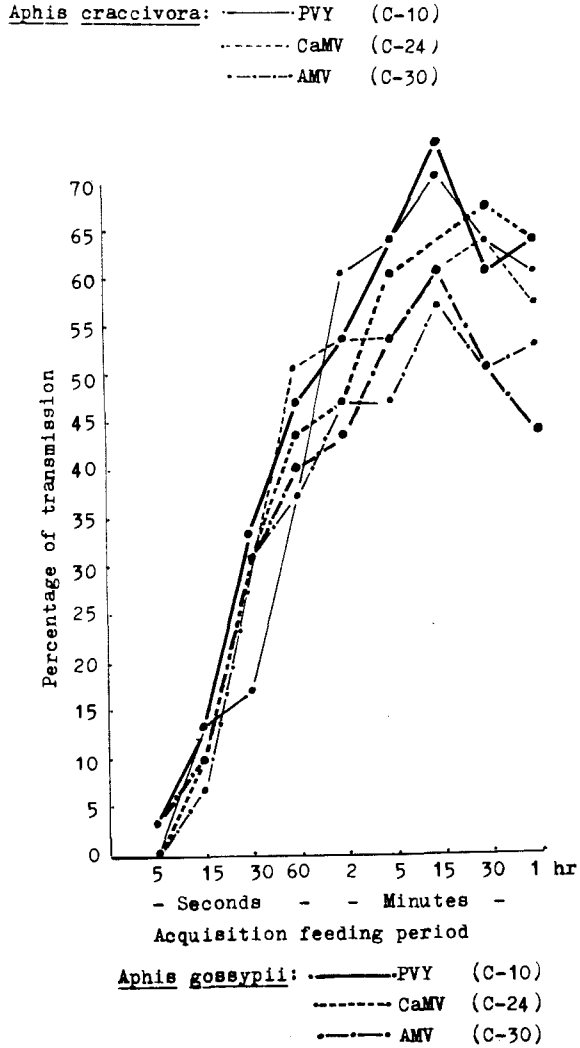


Table 11 : Effect of acquisition feeding on transmission of chilli virus isolates

Acquisition No. feeding period	Aphis gossypii						Aphis craccivora						
	PVY isolate (C10)		CaMV isolate (C24)		AMV isolate (C30)		PVY isolate (C10)		CaMV isolate (C24)		AMV isolate (C30)		
	No. of plants in- fected mis- out of sion 30	% of trans- in- fection	No. of plants in- fected mis- out of sion 30	% of trans- in- fection	No. of plants in- fected mis- out of sion 30	% of trans- in- fection	No. of plants in- fected mis- out of sion 30	% of trans- in- fection	No. of plants in- fected mis- out of sion 30	% of trans- in- fection	No. of plants in- fected mis- out of sion 30	% of trans- in- fection	
1. 5 seconds	1	3.3	0	0	1	3.3	0	0	0	0	0	0	
2. 15 seconds	4	13.3	3	10.0	3	10.0	4	13.3	4	13.3	2	6.7	
3. 30 seconds	10	33.3	9	30.0	10	33.3	5	16.7	9	30.0	9	30.0	
4. 60 seconds	14	46.7	13	43.3	12	40.0	11	36.7	15	50.0	11	36.7	
5. 2 Minutes	16	53.3	14	46.7	13	43.3	18	60.0	16	53.3	14	46.7	
6. 5 Minutes	19	63.3	18	60.0	16	53.3	19	63.3	16	53.3	14	46.7	
7. 15 Minutes	22	73.3	19	63.3	18	60.0	21	70.0	18	60.0	17	56.7	
8. 30 Minutes	18	60.0	20	66.7	15	50.0	19	63.3	19	63.3	15	50.0	
9. 1 Hour	19	63.3	19	63.3	13	43.3	18	60.0	17	56.7	16	53.3	
Total	123		115		101		115		114		98		
Total number of plants infected													: 327/810
Percentage of infection													: 40.2
													: 41.9
													: 339/810

vector than A. craccivora in the transmission of the three viruses.

2. Acquisition threshold of the two aphid vectors in respect of the three viruses

The results are presented in Table 11^{Fig.2.}. The vectors A. gossypii and A. craccivora varied in their ability to pick up the virus isolates and become viruliferous with varying periods of acquisition feed. A. gossypii was able to acquire potato virus Y and alfalfa mosaic virus within five seconds, but required fifteen seconds to pick up capsicum mosaic virus. The transmission percentages were 73.3 and 60.0 respectively for potato virus Y and alfalfa mosaic virus, when the vector was fed for a maximum period of fifteen minutes. The maximum percentage of 66.7 transmission was recorded with 30 minutes acquisition feed in the case of the capsicum mosaic virus.

A. craccivora was not able to transmit any of the three virus isolates with five seconds of acquisition feeding period. Fifteen seconds were found to be the minimum acquisition feed required for transmission. Maximum percentage of transmission was obtained with fifteen minutes acquisition feed in case of potato virus Y and alfalfa mosaic virus while 30 minutes acquisition feed was required for the capsicum mosaic virus. The results indicated that the vector A. gossypii was able to transmit the viruses to 41.9 per cent of the test plants while A. craccivora was able to transmit the viruses to 40.2 per cent of the test plants.

Fig. 3. Effect of inoculation feeding (Test feeding) period

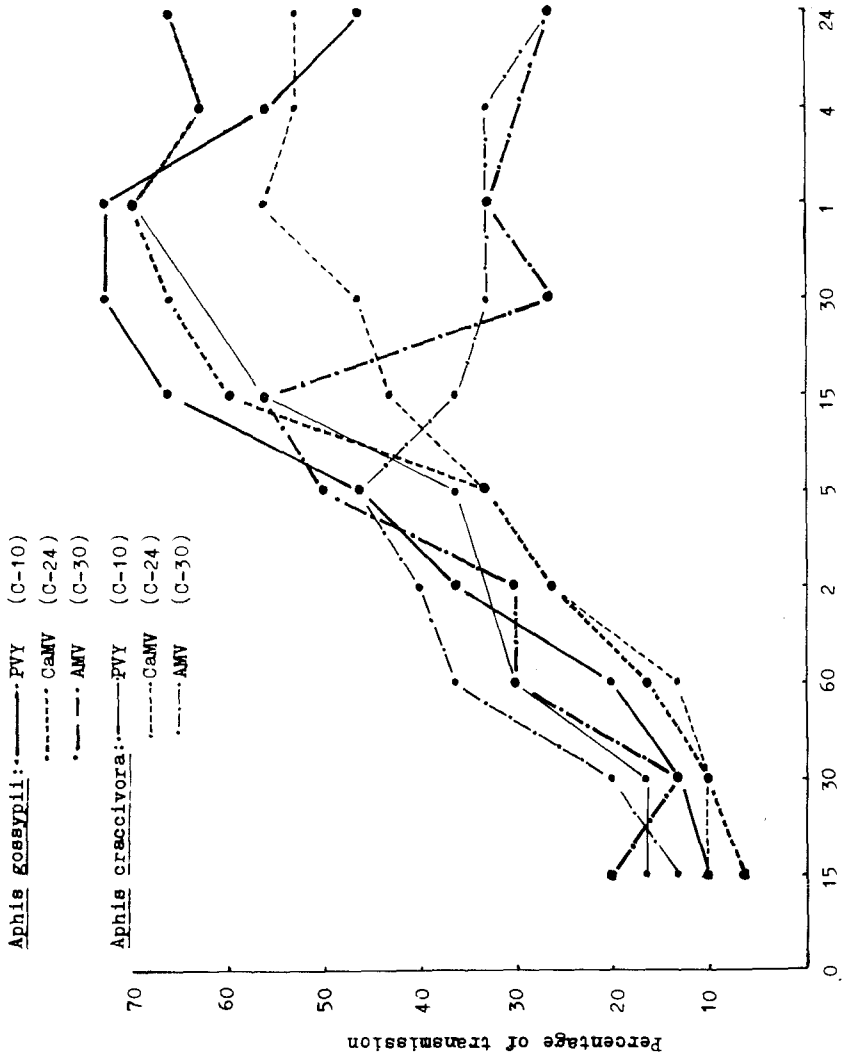


Table 12: Effect of inoculation feeding period on transmission of chilli virus isolates

Inoculation No. feeding period	<u>Aphis gossypii</u>						<u>Aphis craccivora</u>						
	FVY isolate (C10)	CaMV isolate (C24)	AMV isolate (C30)	No. of plants in- trans- fected out of 30	% of trans- mission	No. of plants in- trans- fected out of 30	FVY isolate (C10)	CaMV isolate (C24)	AMV isolate (C30)	No. of plants in- trans- fected out of 30	% of trans- mission	No. of plants in- trans- fected out of 30	
1. 15 seconds	3	10.0	2	6.7	6	20.0	5	16.7	3	10.0	4	13.3	
2. 30 seconds	4	13.3	3	10.0	4	13.3	5	16.7	3	10.0	6	20.0	
3. 60 seconds	6	20.0	5	16.7	9	30.0	9	30.0	4	13.3	11	36.7	
4. 2 Minutes	11	36.7	8	26.7	9	30.0	10	33.3	8	26.7	12	40.0	
5. 5 Minutes	14	46.7	10	33.3	15	50.0	11	36.7	10	33.3	14	46.7	
6. 15 Minutes	20	66.7	18	60.0	17	56.7	17	56.7	13	43.3	11	36.7	
7. 30 Minutes	22	73.3	20	66.7	8	26.7	19	63.3	14	46.7	10	33.3	
8. 1 Hour	19	63.3	21	70.0	10	33.3	21	70.0	17	56.7	10	33.3	
9. 4 Hours	17	56.7	19	63.3	9	30.0	19	63.3	16	53.3	10	33.3	
10. 24 Hours	14	46.7	20	66.7	8	26.7	20	66.7	16	53.3	8	26.7	
Total	130		126		95		136		104		96		
Total number of plants infected													: 336/900
Percentage of infection													: 37.3

3. Inoculation threshold of the two aphid vectors in respect of the three viruses

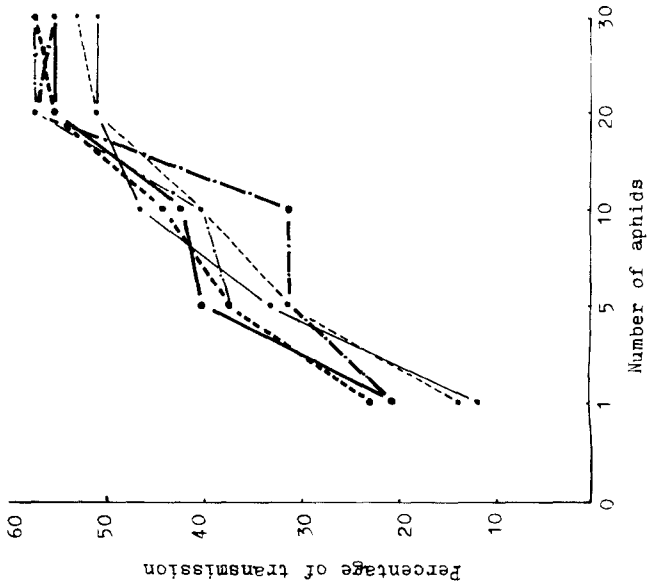
The results are presented in Table 12. ^{Fig 3.} Both the species of aphids were found to transmit the three viruses even within 15 seconds of test feeding. However, the percentage of transmission was found to be poor with the short period of feeding. A.gossypii transmitted the potato virus Y with a maximum percentage of 73.3 in 30 minutes test feed. The capsicum mosaic virus was transmitted to a maximum of 70 per cent in one hour. The alfalfa mosaic virus was transmitted in 15 minutes test feed to a maximum of 56.7 per cent.

The vector A.craocivora transmitted the potato virus Y isolate in 30 minutes test feed to 63.3 per cent of the test plants and to 70 per cent of the plants in one hour test feed. The capsicum mosaic virus was transmitted to 56.7 and 53.3 per cent of the test plants in one and four hours test feeding periods respectively. Alfalfa mosaic virus was transmitted to a maximum of 46.7 per cent in 5 minutes test feed. Increase in the inoculation feeding period reduced the efficiency of transmission.

When the total efficiency of transmission was considered the vector A.gossypii transmitted the virus isolates to 39 per cent of the test plants and A.craocivora transmitted the virus isolates to 37.3 per cent of the test plants.

Fig.4. Number of aphids and percentage of transmission

Aphis gossypii: — PVY (C-10) Aphis craccivora: — PVY (C-10)
 - - - CaMV (C-24) - - - CaMV (C-24)
 - · - AMV (C-30) - · - AMV (C-30)



Handwritten text, likely bleed-through from the reverse side of the page. The text is extremely faint and illegible due to the quality of the scan. It appears to be a list or a series of entries, possibly names and dates, but the characters are too light to transcribe accurately. Some faint words like "1860" and "1861" are visible, suggesting a chronological list.

Table 13: Minimum number of Aphids required for optimum transmission

		<u>Aphis gossypii</u>						<u>Aphis craccivora</u>					
		FVY isolate (C10)		CaMV isolate (C24)		AMV isolate (C30)		FVY isolate (C10)		CaMV isolate (C24)		AMV isolate (C30)	
No. of S. aphids	No. per plant	No. of plants in-trans- fected out of sion	% of in-trans- fected out of sion	No. of plants in-trans- fected out of sion	% of in-trans- fected out of sion	No. of plants in-trans- fected out of sion	% of in-trans- fected out of sion	No. of plants in-trans- fected out of sion	% of in-trans- fected out of sion	No. of plants in-trans- fected out of sion	% of in-trans- fected out of sion	No. of plants in-trans- fected out of sion	% of in-trans- fected out of sion
1.	1	9	20.0	10	22.2	9	20.0	5	11.1	6	13.3	10	22.2
2.	5	18	40.0	17	37.8	14	31.1	15	33.3	14	31.1	17	37.8
3.	10	19	42.2	20	44.4	14	31.1	21	46.6	18	40.0	18	40.0
4.	20	25	55.6	25	55.6	26	57.8	23	51.1	23	51.1	26	57.8
5.	30	25	55.6	26	57.8	25	55.6	23	51.1	24	53.3	26	57.8
	Total	96		98		88		87		85		97	
Total number of plants infected out of						:282/675						:269/675	
Percentage of infection						: 41.8						: 39.9	

Fig. 5. Persistence of the virus in vectors during fasting

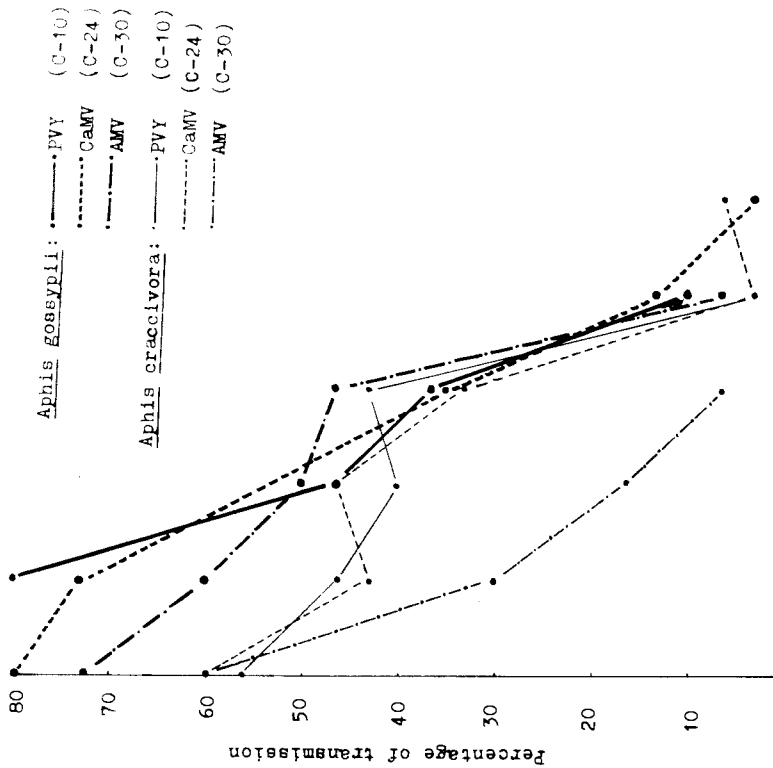


Table 14: Persistence of the virus in the vectors during fasting

Period of No. post acquisition fasting	<u>Aphis gossypii</u>				<u>Aphis craccivora</u>				
	PVY isolate (C10)	CaMV isolate (C24)	AMV isolate (C30)	AMV isolate (C30)	PVY isolate (C10)	CaMV isolate (C24)	AMV isolate (C30)	AMV isolate (C30)	
	No. of plants affected out of 30	No. of plants affected out of 30	% of trans- mission out of 30	% of trans- mission out of 30	No. of plants affected out of 30	No. of plants affected out of 30	% of trans- mission out of 30	% of trans- mission out of 30	
1. No fasting	24	24	80.0	22	73.3	17	56.7	18	60.0
2. 15 Minutes	24	22	73.3	18	60.0	14	46.7	13	43.3
3. 30 Minutes	14	16	53.3	15	50.0	12	40.0	14	46.7
4. 45 Minutes	11	9	30.0	14	46.7	13	43.3	10	33.3
5. 60 Minutes	3	4	13.3	2	6.7	3	3.3	3	3.3
6. 2 Hours	0	1	3.3	0	0	0	0	2	6.7
7. 4 Hours	0	0	0	0	0	0	0	0	0
8. 24 Hours	0	0	0	0	0	0	0	0	0
Total	77	76	71	59	60	34			

4. Minimum number of aphids required per plant for optimum virus transmission

The results are presented in Table 13^{Fig. 4.}. All the viruses were successfully transmitted by single adult aphids of A.gossypii as well as A.craacivora. Maximum transmission was obtained with 20 aphids in case of potato virus Y and alfalfa mosaic virus. With the capsicum mosaic virus maximum transmission was obtained with 30 aphids.

A.gossypii gave 41.8 per cent total successful transmissions and A.craacivora was able to transmit the viruses to 39.9 per cent of the test plants.

5. Persistence of the viruses in aphid vectors during fasting

The data is presented in Table 14^{Fig. 5.}. The viruliferous aphids in the case of A.gossypii were able to retain potato virus Y and alfalfa mosaic virus upto one hour and capsicum mosaic virus upto 2 hours. In the case of A.craacivora the viruliferous aphids were able to retain the potato virus Y for one hour and capsicum mosaic virus for two hours, but lost the alfalfa mosaic virus in 45 minutes.

6. Persistence of the viruses in aphid vectors during feeding

The results are presented in Table 15. The potato virus Y, capsicum mosaic virus and alfalfa mosaic virus were transmitted up to the third successive test plant by A.gossypii when the interval of transfer was five minutes. As the interval of transfer was increased to ten minutes rarely a third plant was infected. At 15 minutes interval

of transfer only the first plant in the series was infected. A. craccivora also exhibited similar results. The persistence of the viruses studied during continuous feeding was 15 minutes and was more than 60 minutes during fasting.

7. Relative efficiency of the vectors in transmission

An overall picture of transmission efficiency of the two vectors A. gossypii and A. craccivora from the first four of the experiments on vector virus relationship is presented in Table 16.

Table 16 : Relative vector efficiency in the experiments on vector-virus relationship

Experiment	Total number of plants infected/ Total number of plants inoculated, percentage infection				Diffe- rence
	<u>Aphis</u> <u>gossypii</u>		<u>Aphis</u> <u>craccivora</u>		
a) Preacquisition starvation	$\frac{345}{720}$	47.9 ¹	$\frac{236}{720}$	32.8 ²	15.1
b) Acquisition feeding	$\frac{339}{810}$	41.9*	$\frac{327}{810}$	40.2 ⁺	1.7
c) Inoculation feeding	$\frac{351}{900}$	39.0*	$\frac{336}{900}$	37.3 ⁺	1.7
d) Number of aphids	$\frac{282}{675}$	41.8*	$\frac{269}{675}$	39.9 ⁺	1.9

- 1) Aphids collected from cotton plants in the field
- 2) Aphids collected from Glyricidia maculata in the field
- * Aphids maintained on Capsicum annuum in cages
- + Aphids maintained on Vigna sinensis in cages

The above results indicated that A.gossypii was comparatively more efficient than A.craccivora as a vector in giving maximum virus transmission.

Another important observation made from the above results was that in the first experiment to study the effect of preacquisition starvation the difference in the efficacy between A.gossypii and A.craccivora was in the order of 15.1 per cent while in the case of the subsequent experiments to study the effect of acquisition feeding period, inoculation feeding period and the number of aphids, the differences in the efficacy of transmission were only 1.7, 1.7 and 1.9 per cent respectively.

In order to examine the possibility that the plant sap of Glyricidia maculata might contain some inhibitory principle which could have been responsible for the difference in transmission ability, additional experiments were carried out and the results are presented below.

8. Detection of virus inhibitor in the plant sap of Glyricidia maculata

Young leaves of Glyricidia maculata and Vigna sinensis were crushed in a pestle and mortar separately and sap extracted. The inoculum of potato virus Y (chilli virus isolate, C₁₀) was prepared by extraction of infective sap from the young severely infected leaves. Equal volumes of the inoculum and sap extracted from the two plants were mixed in separate batches in one set. Equal volumes of the

inoculum and 1:10, 1:20, 1:40, 1:80 and 1:100 dilutions of the plant leaf extracts were mixed and each set was inoculated to 20 young healthy chilli plants. The data is presented below in Table 17.

Table 17: Effect of inhibitor in plant sap at different dilutions on virus transmission

Dilution of plant extract	<u>Gliricidia maculata</u>		<u>Vigna sinensis</u>		Water (Control)
	Plants infected out of 20	% reduction over control	Plants infected out of 20	% reduction over control	
Undiluted	10	44.44	16	11.11	18
1:10	13	13.33	15	-	15
1:20	14	12.50	15	6.25	16
1:40	15	6.25	16	-	16
1:80	15	-	16	+6.67	15
1:100	16	-	16	-	16

The data indicated that there was inhibition of infection when Gliricidia maculata plant extract was mixed with the inoculum of potato virus Y up to 44.44 per cent over control. There was a gradual decrease in the effect of inhibition when the inoculum was mixed with equal quantity of the two fold dilution of the Gliricidia maculata plant sap.

9. Effect of preacquisition feeding on *Glyricidia maculata* and *Vigna sinensis* by the aphid vectors on the transmission of potato virus Y (isolate C₁₀ of chilli mosaic virus)

The effect of preacquisition feeding of *A. craccivora* and *A. gossypii* on *Glyricidia maculata* was tested as compared to similar feeding on *Vigna sinensis*. Healthy colonies of *A. craccivora* and *A. gossypii* were collected and starved for two hours. The vectors were fed on young leaves of *Glyricidia maculata* and *Vigna sinensis* for varying periods of 5, 15, 30, 60 seconds, 5, 30 and 60 minutes in separate batches. After the preacquisition feeding on these leaves, the aphids were allowed an acquisition feed of 15 minutes on young infected chilli leaf showing severe mosaic symptoms in batches. The viruliferous aphids were transferred to young healthy chilli plants and allowed an inoculation feeding of 4 hours after which the test plants were sprayed with parathion. The plants were kept for observation inside the glasshouse. The results are presented in Table 18.

Table 18: Effect of preacquisition feeding on Glyricidia maculata and Vigna sinensis on the transmission of the chilli virus isolate C₁₀ (PVY)

S. No.	Period of pre-acquisition feeding	<u>Aphis craccivora</u>		<u>Aphis gossypii</u>	
		<u>G. maculata</u> % reduction over control	<u>V. sinensis</u> % reduction over control	<u>G. maculata</u> % reduction over control	<u>V. sinensis</u> % reduction over control
	Control		56.67		60.0
1	5 seconds	23.53	11.76	22.22	11.11
2	15 seconds	29.41	17.64	38.89	22.22
3	30 seconds	52.94	41.18	55.56	38.89
4	One minute	64.71	47.06	72.22	55.56
5	5 minutes	94.12	82.35	94.44	88.89
6	30 minutes	100.00	100.00	100.00	100.00
7	One hour	100.00	100.00	100.00	100.00

The observations presented in the table indicated the general inference that the increase in preacquisition feed on Glyricidia maculata as well as Vigna sinensis reduced the percentage of successful transmissions. However, there was also another important observation that the aphids fed on Glyricidia maculata showed less number of successful transmissions than those fed on Vigna sinensis. The percentage difference in transmission was low at lower periods of preacquisition feed and it increased gradually up to 5 minutes of preacquisition feed. As the preacquisition feeding period was increased, the transmission was reduced to nil.

10. Effect of post acquisition feeding on *Glyricidia maculata* and *Vigna sinensis* by the aphid vectors on the transmission of the potato virus Y (isolate C₁₀ of chilli mosaic virus)

The effect of post acquisition feeding of *A. craccivora* and *A. gossypii* on *Glyricidia maculata* was compared with that of feeding on *Vigna sinensis*. Healthy colonies of both the species of aphids were collected, starved for two hours and allowed to feed on young infected chilli leaves for an acquisition feed of 15 minutes in batches. The viruliferous aphids were transferred to leaves of *Glyricidia maculata* and *Vigna sinensis* in batches for post acquisition feeding periods of 5, 15, 30, 60 seconds, 5, 30 and 60 minutes. They were then picked up carefully and allowed an inoculation feeding period of 4 hours on young chilli test plants. The test plants were sprayed after this period with parathion and kept for observation in an insect proof glasshouse. The results are presented in Table 19.

Table 19: Effect of post acquisition feeding on Glyricidia maculata and Vigna sinensis on the transmission of chilli virus isolate C₁₀ (PVY)

S. No.	Period of pre-acquisition period	<u>Aphis craccivora</u>			<u>Aphis gossypii</u>		
		Con-trol	G. <u>maculata</u>	V. <u>sinensis</u>	Con-trol	G. <u>maculata</u>	V. <u>sinensis</u>
	Control	53.33	-	-	56.67	-	-
1	5 seconds	-	12.50	6.25	-	15.00	5.88
2	15 seconds	-	50.00	12.25	-	31.00	16.00
3	30 seconds	-	87.50	29.25	-	75.00	30.00
4	One minute	-	87.50	40.00	-	81.00	41.00
5	5 minutes	-	100.00	56.25	-	100.00	58.00
6	30 minutes	-	100.00	100.00	-	100.00	100.00
7	One hour	-	100.00	100.00	-	100.00	100.00

The results gave a general indication that the increase in post acquisition feeding time on Glyricidia maculata as well as Vigna sinensis reduced the number of successful virus transmissions. Viruliferous aphids fed on Glyricidia maculata always gave less number of virus transmissions than those fed on Vigna sinensis. The difference in transmission between aphids fed on the two plant species increased as the period of post acquisition feeding time increased. At five minutes period of post acquisition feed no large scale differences could be observed. Both the aphid species failed to transmit

Fig.6. Effect of Pre-acquisition fasting

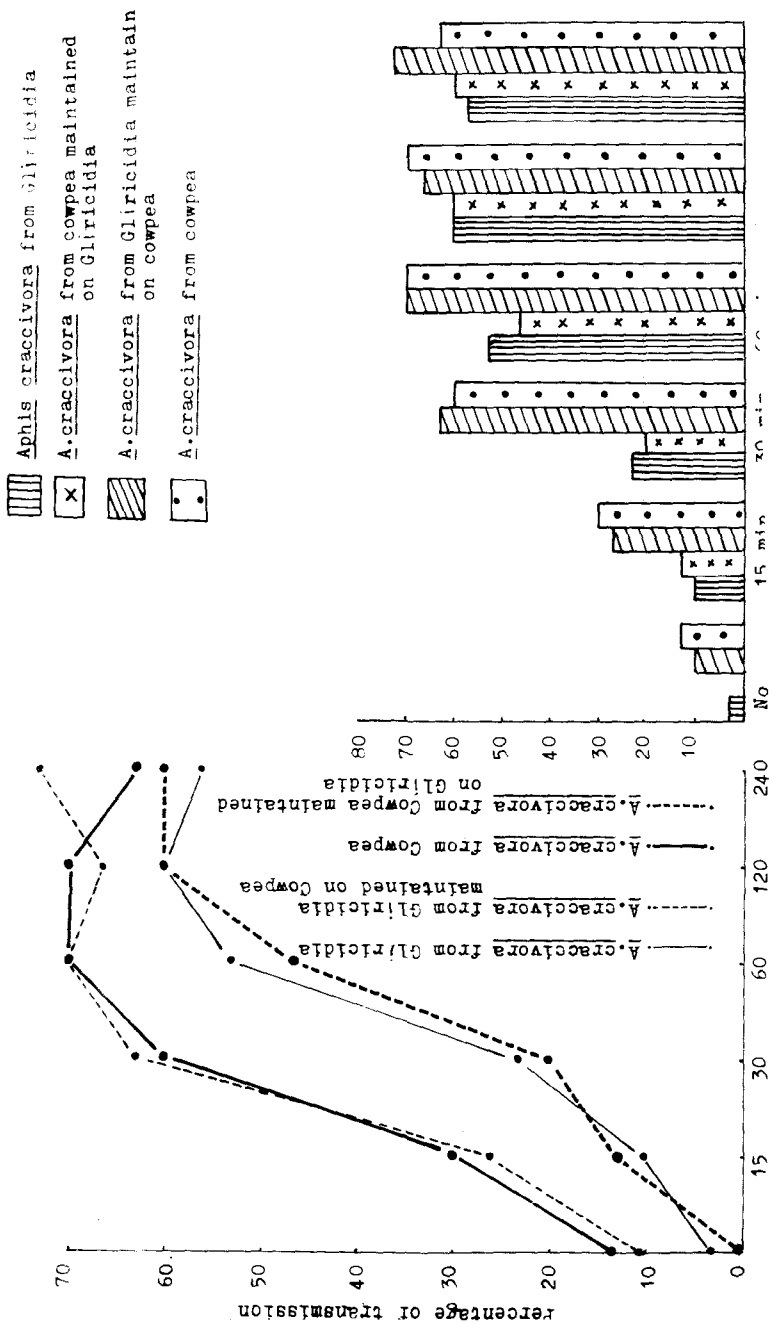


Table 20: Effect of preacquisition fasting of Aphis craccivora from different host plants on transmission of chilli virus isolate C₁₀ (FVY).

S. No.	Period of preacquisition fasting	<u>Aphis craccivora</u> from <u>Glyricidia</u> maintained on cowpea		<u>Aphis craccivora</u> from cowpea maintained on <u>Glyricidia</u>					
		No. of plants infected out of 30	% of trans-mission	No. of plants infected out of 30	% of trans-mission				
1.	No fasting	1	3.3	3	10.0	4	13.3	0	0
2.	15 Minutes	3	10.0	8	26.7	9	30.0	4	13.3
3.	30 Minutes	7	23.3	19	63.3	18	60.0	6	20.0
4.	60 Minutes	16	53.3	21	70.0	21	70.0	14	46.7
5.	120 Minutes	18	60.0	20	66.7	21	70.0	18	60.0
6.	240 Minutes	17	56.7	22	73.3	19	63.3	18	60.0
	Total	62		93		92		60	



the virus after 30 minutes of, post acquisition feed on glyricidia and cowpea.

11. Effect of preacquisition fasting of Aphis craccivora from two different sources of collection maintained on two different host plant species

A. craccivora collected from Glyricidia maculata and Vigna sinensis were maintained on same host plant species or alternately on the other host plant. Batches of aphids were collected and fasted for periods of 15, 30, 60, 120 and 240 minutes. They were then fed on young severely infected chilli leaves for 15 minutes of acquisition feed. The viruliferous aphids were then released on healthy young chilli plants for 4 hours inoculation feed. The test plants were then sprayed with Parathion and kept for observation in the insect proof glasshouse.

The results are presented in Table 20^{Fig. 6.}. In all the cases the percentage of transmission increased with the increase in the preacquisition fasting period. A. craccivora collected from V. sinensis as well as from G. maculata and maintained on V. sinensis always gave more percentage of successful virus transmissions than the aphids collected from G. maculata or those collected from V. sinensis but maintained on G. maculata.

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PLATE 7

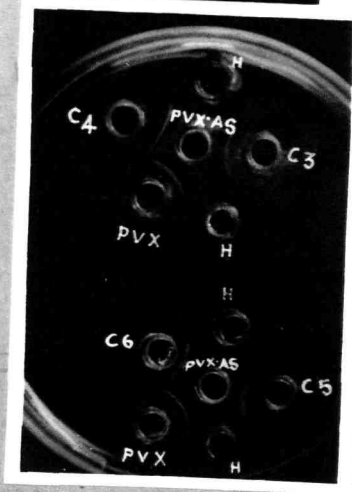
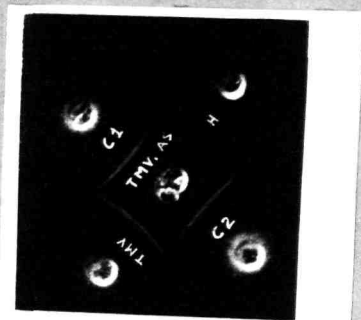


Plate 78 Ouchterlony agar double diffusion test

Top - Isolates C₁ and C₂ with TMV antiserum
Bottom - Isolates C₃, C₄, C₅ and C₆ with
PVX antiserum

F. Serological tests

1(a). The group I isolates both C₁ and C₂ reacted with the antiserum for tobacco mosaic virus received from Microbiological Associates, Inc. U.S.A., positively.

The results are furnished in Table 21.

Table 21: Tube precipitin test : Reaction of chilli virus isolates of group I

Sap tested	Antiserum for TMV from U.S.A.						
	0	Dilution of sap					
		1:2	1:4	1:8	1:16	1:32	1:64
Healthy	-	-	-	-	-	-	-
C ₁ isolate	+++	+++	+++	+++	++	+	-
C ₂ isolate	+++	+++	+++	+++	++	+	-
TMV (culture)	+++	+++	+++	+++	+++	++	-

+)
 ++) Intensity of reaction
 +++)

(b). Ouchterlony agar double diffusion test

Both the isolates of group I gave positive precipitin lines similar to tobacco mosaic virus in the gel diffusion test. Plate 7.

2(a). The group II isolates C₃, C₄, C₅ and C₆ reacted with the antiserum for PVX and gave positive precipitin test. The results are furnished below in Table 22.

PLATE 8

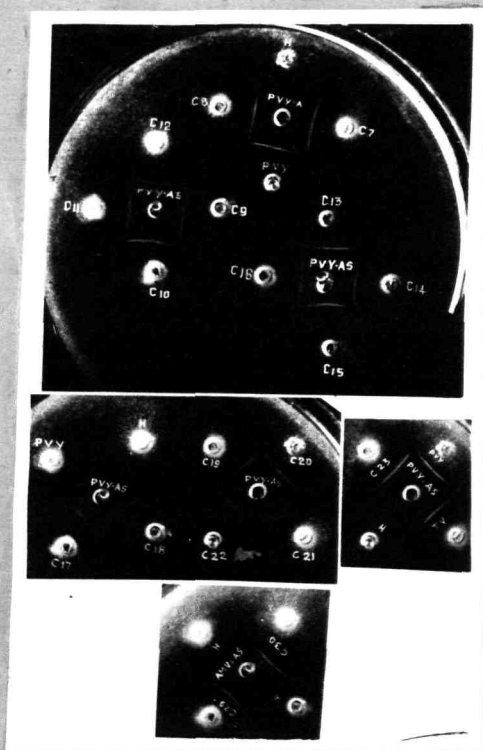


Plate 2 - Ouchterlony agar double diffusion test

- Top Group III isolates C₇ to C₁₆ with PVY
 antiserum
- Middle left - Group III isolates C₁₇ to C₂₂
 with PVY antiserum
- Middle right - Group III isolates C₂₃ and
 Trianthema mosaic virus isolate (TV)
 with PVY antiserum
- Bottom - Group VI isolates C₂₉ and C₃₀ with
 AMV antiserum

Table 22: Tube precipitin test : Reaction of chilli virus isolates of group II with antiserum for PVX

Sap tested	0	Dilution of sap					
		1:2	1:4	1:8	1:16	1:32	1:64
C ₃	+++	+++	+++	++	+	-	-
C ₄	+++	+++	++	++	+	-	-
C ₅	+++	+++	++	+	+	-	-
C ₆	+++	+++	++	+	+	-	-
Healthy sap	-	-	-	-	-	-	-
PVX from potato	+++	+++	+++	++	+	-	-

+) Intensity of reaction
 ++)
 +++)

(b). Ouchterlony agar double diffusion test

All the four isolates exhibited precipitin lines with the PVX antiserum similar to PVX from potato. *Plate 7.*

3. Ouchterlony gel double diffusion test

Group III isolates totalling seventeen (C₇ to C₂₃) gave positive precipitin lines with the antiserum for PVY. Healthy sap did not react with the antiserum. *Plate 8.*

4. Group IV isolates did not give any positive reaction with the antisera for potato virus Y, cucumber mosaic virus and alfalfa mosaic virus in Ouchterlony gel double diffusion tests and tube precipitin test.

5. Group V isolates also did not react with the antisera for PVY, CMV and AMV.

6. Group VI isolates C₂₉ and C₃₀ reacted positively with the antiserum for alfalfa mosaic virus in Ouchterlony gel double diffusion serological test. Healthy sap did not react with the antiserum. Plate 8.

G. Reaction of chilli varieties and Capsicum species to three chilli viruses

Varieties of chilli (Capsicum annuum L. and C.frutescens L. and C.sinensis, C.microcarpon, C.pendulum, C.peruvianum and C.columbianum numbering 158 in all were screened for their reaction to three chilli viruses namely potato virus Y (PVY - isolate C₁₀), capsicum mosaic virus (CaMV - isolate C₂₄) and alfalfa mosaic virus (AMV - isolate C₃₀). The results are presented below based on the type of reaction of the varieties.

1. Varieties not infected by any of the viruses
Capsicum annuum c.v. '685'
2. Varieties that were not infected by potato virus Y but reacted with mild symptoms to Capsicum mosaic virus and alfalfa mosaic virus
C.annuum c.v. 'P11', cuaremeno and C.pendulum c.v.'SA90'
3. Varieties that reacted with mild symptoms for all the three viruses
C.annuum c.v., B15A, B24A₂, B32A, B39A, C2A, C15A,

C20B, C81, G₂, G₃, Hy17-1-1, N.P.41, 692, 693, 703,
728, 757, 760, 765, 857, 962, 971, 1187, 1190,
I33d-1-1-4 and C.columbianum 1766.

4. Varieties that exhibited mild symptoms for potato virus Y and capsicum mosaic virus but severe symptoms with alfalfa mosaic virus
C.annuum c.v. 'CA766 and C52A'
5. Varieties not infected by potato virus Y, showing severe symptoms with capsicum mosaic virus and mild symptoms to alfalfa mosaic virus
C.annuum c.v. 'P34'
6. Varieties that reacted with mild symptoms for potato virus Y and alfalfa mosaic virus and severe symptoms with capsicum mosaic virus
C.annuum c.v., A123, A125, A154, A47A, B86A, C1B, C22B,
1264, AC4939 Lincoln Bell, AC4943 hungarian Hot wax,
C.peruvianum 1749, C.microcarpon, C.sinensis 1491, 1554
1555 and 1556
7. Varieties that exhibited mild symptoms to potato virus Y but severe symptoms to capsicum mosaic virus and alfalfa mosaic virus
C.annuum c.v. B29A, 562 and 618-14.
8. Varieties that exhibited severe symptoms to potato virus Y and mild symptoms to capsicum mosaic virus and alfalfa mosaic virus
C.annuum c.v. C56B, I.C.2474, 759, N.P.34, AC4938 Pimento
A.C.4942 Anaheim and C.peruvianum 221

PLATE 9



A



B



C



D

II. Identification of a virus isolate from Trianthema portulacastrum L.

During the collection of chilli virus infected plants at the Agricultural College campus fields, a few plants of Trianthema portulacastrum L. growing as a gardenland weed was found to show symptoms of mosaic mottling. The infected plants were collected and studied in detail to examine the role played by this weed as an alternate host for any one of the viruses affecting chilli crop.

The infected Trianthema portulacastrum plants were normal in growth but exhibited only slight chlorosis of the leaves especially at the middle and mosaic mottling of the leaves.

(1) Transmission

The causal virus was transmitted by mechanical inoculation to chilli plants, with difficulty. Sap extracted with 0.5 per cent sodium sulphite gave only 10 per cent infection on chilli. The virus was also transmitted from chilli to Trianthema portulacastrum by transmission with the aid of the aphid vector Aphis brassivora.

The virus was transmitted by Aphis gossypii and also by Myzus persicae from chilli to chilli. The virus culture was maintained on chilli for further study. The results of the transmission experiments are presented below in Table 25.

Table 23 : Transmission tests with Trianthema portulacastrum virus

Inoculation method	Percentage of transmission	Inoculation period
Mechanical transmission	30	14 days
<u>Aphis craccivora</u>	43	16 days
<u>Aphis gossypii</u>	56	16 days
<u>Myzus persicae</u>	60	14 days

(ii) Studies on symptomatology

The infected Capsicum annuum plants exhibited the following symptoms. Fourteen days after inoculation newly formed leaves showed clearing of the veins. Severe mosaic mottling of light and dark green areas developed later. The lamina was crinkled and reduced in size. Older leaves exhibited vein banding. Leaves were malformed and were reduced to varying degrees. The lamina was completely reduced in a few young leaves leaving a thin midrib.

Trianthema portulacastrum exhibited mosaic pattern and chlorosis on artificial inoculation similar to the symptoms on naturally infected plants.

(iii) Host range and symptoms on various host plants

The Trianthema virus isolate induced the following symptoms on different host plants tested. Plate 9.

Nicotiana tabacum var. White Burley:

Infected plants exhibited chlorosis of the leaves

mild mosaic mottling and vein banding 14 days after inoculation.

N.tabacum var. xanthi:

Clearing of the veins was the first symptom observed 10 days after inoculation. The leaves were slightly reduced in size and showed mosaic mottling.

N.glutinosa:

Newly emerging leaves exhibited chlorosis and mosaic mottling. The leaves were slightly deformed with reduction in size of the lamina.

N.glauca:

Newly emerging leaves exhibited mild mosaic mottling symptoms and vein banding.

N.rustica:

The virus induced chlorosis and severe mosaic mottling. The infected plants were slightly stunted.

Capsicum frutescens var. tabasco and var. buccatum:

The infected plants exhibited severe mosaic mottling and crinkling of the leaves. The leaves also showed vein banding.

Solanum demissum x S.tuberosum Hybrid 'A6'

Detached leaves developed brown local lesions 5 days after incubation in a moist chamber at 22°C.

Nicandra physaloides

Infected plants were very much reduced in size and stunted. Leaves were severely crinkled and showed severe mosaic pattern.

Physalis floridana

Leaves exhibited chlorosis and mild mosaic mottling.

Petunia hybrida

Leaves were crinkled and slightly reduced in size. Mild mosaic mottling and vein banding were exhibited by the leaves.

The following plants were not infected by the virus isolate.

Datura stramonium, D.metel, D.ferox, Lycopersicon esculentum, Physalis peruviana, Chenopodium amaranticolor, C.murale, C.album, Phaseolus vulgaris, Medicago sativa, Trifolium repens, Vicia faba, Zinnia elegans, Gomphrena globosa, Cucumis sativus, Solanum melongena, Vigna sinensis, Phaseolus aureus, P.mungo, Pisum sativum, Cajanus cajan, Cyamopsis psoraloides, Lathyrus odoratus, Dolichos lab lab, Glycine max, Glyricidia maculata, Crotalaria juncea, Melilotus alba, Cicer arietinum, Clitoria ternata, Beta vulgaris, Flavaria australasica, Carthamus tinctorius, Brassica juncea, Cucurbita pepo, Fagopyron esculentum, Commelina jacobii and Vinca rosea.

(iv) Studies on the physical properties of the virus isolate

Physical properties namely, the thermal inactivation point, the dilution end point and the longevity in vitro of the virus isolate were studied with chilli as the test plant. The results are presented in Table 24.

Table 24 : Physical properties of the Trienthema virus isolate

Thermal inactivation point		Dilution end point		Longevity <u>in vitro</u>	
Temperature	Number of plants infected	Dilution	Number of plants infected	Age of the sap	Number of plants infected
Control (untreated)	25/30	Control (undiluted)	23/30	Control (fresh sap)	8/10
50°C	18/30	1:10	18/30	6 hr	5/10
55°C	8/30	1:100	15/30	8 hr	4/10
60°C	3/30	1:1000	6/30	12 hr	3/10
65°C	0/30	1:10000	2/30	24 hr	1/10
70°C	0/30	1:10,000	0/30	48 hr	1/10
75°C	0/30	1:100,000	0/30	72 hr 96 hr	0/10 0/10

Numerator - Number of plants infected

Denominator - Number of plants inoculated

(a) Thermal inactivation point: The isolate withstood exposure to 60°C but was inactivated at 65°C for 10 minutes

(b) Dilution end point: The isolate was infective at a dilution of 1:10,000 but not at 1:20,000.

(c) Longevity in vitro: The virus had a longevity in vitro of 48 hours at 26°-28°C.

(v) Serological tests

Ouchterlony gel double diffusion test

Trianthema virus isolate gave positive precipitin line with the antiserum for potato virus Y. Healthy sap did not react with the antiserum. Plate 8.

DISCUSSION

1. Identification of chilli mosaic virus isolates

Thirty chilli mosaic virus isolates were studied. These isolates could be differentiated into six major groups based on symptomatology, mode of transmission, specific reactions on certain differential hosts, physical properties and serological relationships.

Six of the virus isolates serially numbered from C₁ to C₆ were not transmitted by any of the aphid vectors. Though all these six isolates had a dilution end point above 1 in 100,000 they could be classified into two groups based on the thermal inactivation point. The isolates C₁ and C₂ had a thermal inactivation point between 85 and 90°C, while the isolates C₃, C₄, C₅ and C₆ had a thermal inactivation point between 65 and 70°C. Differences also existed between the two groups referred as group I and II respectively in the succeeding paragraphs in their reaction on different host plants and serological relationships.

Nineteen of the virus isolates C₇ to C₂₅ were transmitted by sap inoculation and by the aphid vectors namely, Aphis gossypii, A. craccivora, A. evonymii and Myzus persicae. The isolates C₂₄ and C₂₅ were transmitted by A. nerii also. There were differences among them regarding specific host reactions, physical properties and serological relationships. Three isolates C₂₆, C₂₇ and C₂₈ were

transmitted by sap as well as by the aphid vectors namely, A.gossypii and Myzus persicae but not by A.craccivora, A.nerii and A.evonymii.

Based on the differences further 24 virus isolates are discussed below as group III (C₇ to C₂₃ seventeen isolates), group IV (isolates C₂₄ and C₂₅), group V (isolates C₂₆, C₂₇ and C₂₈) and group VI (isolates C₂₉ and C₃₀).

The isolates in group I (C₁ and C₂) were characterized by chlorosis of the leaf, mosaic mottling and crinkling of the leaves. The infected plants were less bushy than normal. Both the isolates had an acute and chronic phase of the disease when inoculated to chilli plants. During the acute phase, diffuse yellow chlorotic spots appeared on the inoculated leaves which later abscised. The chronic phase showed mosaic mottling of the leaves and the plants were stunted. Necrotic patches appeared on the petiole and stem, killing the plants in severe cases. These isolates caused characteristic local necrotic lesions on inoculated leaves of Nicotiana tabacum var xanthi, N.glutinosa, N.rustica, Datura stramonium, D.metel, D.ferox, Capsicum frutescens var. tabasco and C.frutescens var. buccatum. C.microcarpon was not infected. Local lesions were also produced on Chenopodium amaranticolor, C.aurale and C.album. Twentyone plant species belonging to Leguminosae and eight species belonging to Cucurbitaceae were not infected. Both the isolates were infective at a dilution of 1:100,000.

The longevity in vitro was over 30 days at room temperature (26°-28°C) and over 100 days at 5°C.

Ramakrishnan (1961) had reviewed the literature on virus diseases of pepper wherein 17 different viruses were reported to occur naturally on pepper. Among these the only virus reported to be sap transmissible and had no vectors was tobacco mosaic virus, which is an important virus infecting chilli next only to cucumber mosaic virus. Kovachevsky (1942) had described the symptoms caused by this virus in detail. The disease was characterized by an acute and a chronic phase. During the acute phase growth ceased in 6-7 days. Leaves turned yellow along the veins, became necrotic and fell off. Stems also became necrotic and destroyed the plants. In the chronic phase the plants grew slowly and the leaves appeared bleached with a faint mosaic pattern. Holmes (1937) reported four types of symptoms namely (a) systemic chlorosis (b) local lesions on leaves and abscission (c) delayed necrosis with a systemic disease and (d) systemic necrosis and stem streaking resulting in the death of the plant.

Nakata and Takimoto (1940) described a ring strain causing large, bright yellow mottling on the leaves. It had a dilution end point of 1:1,000,000 and a thermal inactivation point of 90°C. Marmor tabaci Holmes var. Siccans described by Doolittle and Beachler (1942) passed on to pepper causing symptoms similar to those caused by TMV. The virus had a dilution end point exceeding 1:1,000,000 and a thermal

inactivation point between 90 and 95°C. Tomato atypical mosaic virus (Miller and Thornberry, 1958) had a dilution end point of 10^{-8} similar to TMV but was inactivated at 72°C. This virus also infected pepper plants. Two strains of TMV on pepper in South America described by Zabala and Della Coste (1947) were inactivated at 80°C. Seedborne strain of TMV described by McKinney (1952) had a dilution end point of 88 to 89°C. The TMV strain similar to latent strain of McKinney described by Greenleaf ^{et al.} (1964) had a thermal inactivation point between 85 and 90°C and caused severe mottle of Capsicum frutescens var. tabasco. Tomato streak virus, a strain of TMV is distinct in producing local lesions on tobacco plants. The Rib grass strain of TMV had a thermal inactivation point of 93°C.

The virus isolates of Group I (C_1 and C_2), now studied, were similar to TMV in several respects. Both the isolates gave positive serological reaction with TMV antiserum in tube precipitin test as well as Ouchterlony agar double diffusion test and as such were identified as strains of tobacco mosaic virus but they might be considered as distinct and different from other strains of TMV reported on chilli so far, since they differed sharply both in their physical properties and in their reaction on tobacco or pepper in all respects.

The isolates in Group II namely, C_3 , C_4 , C_5 and C_6 were characterized by chlorotic pale green patches, mosaic

mottling of leaves, stunting of the plant and reduced fruit set. The fruits were reduced in size and crinkled with whitish yellow markings when green. These isolates caused necrotic local lesions on Nicotiana rustica, Datura stramonium and D.metel. PVX and its strains were reported to cause mild mottling to extensive necrosis and ringspots on D.stramonium. All the four isolates studied had induced local lesions on Chenopodium amaranticolor and systemic mosaic mottling with crinkling, reduction of leaf size and stunting in C.murale, Gomphrena globosa had reacted with typical round reddish necrotic local lesions. Capsicum microcarpon reacted with necrotic local lesions. C.frutescens var. tabasco and C.frutescens var. buccatum reacted with symptoms similar to those caused by TMV.

According to literature, potato virus X and its strains are the sap inoculable viruses having no vector that infect chilli other than TMV. Ramakrishnan (1959) and Paulus et al. (1960) reported the natural occurrence of PVX on chilli. According to Ramakrishnan (1959), "California Wonder" chilli plants infected with PVX were stunted with mosaic pattern and dark green vein banding on the leaves. The leaves were narrowed and reduced in size. Schultz et al. (1937), Roland (1950) and Chester (1936) have observed severe systemic necrosis on chilli by a latent potato strain of PVX. David and Storer (1941) described primary dark necrotic rings and systemic bleaching, puckering with occasional necrosis and death of apical portions of the plants.

The chilli virus isolates in group II differ in their symptomatology described above in some respects. The isolates studied, however, gave positive serological precipitin reactions with the antiserum for PVX both in tube precipitin test and Ouchterlony agar double diffusion test. These isolates were identified as PVX. As the reaction on Datura stramonium and the symptoms produced differed from the ones reported for other strains, these isolates turned out to be of a distinct strain.

Seventeen isolates C₇ to C₂₃ in group III were characterized by their causing a general reduction in size of the leaves of the chilli plant, severe mosaic mottling, crinkling, blistering, leaf malformation and distortion, and twisting or folding of leaf. Three of them C₁₈, C₂₁ and C₂₂ however exhibited slightly moderate type of symptoms compared to the other 14 isolates. Vein banding was exhibited by all the isolates. Fruits were crinkled and reduced in size. Nicotiana tabacum reacted with vein banding symptoms with these isolates. The three species of Datura tested were not infected by any of these isolates. Potato hybrid A₆ reacted with local lesions only when inoculated with these isolates, while the leguminous plants tested were not susceptible. All the isolates tested reacted positively with PVY antiserum in Ouchterlony agar double diffusion test with precipitin lines. Based on these specific characteristics these seventeen isolates were therefore, identified as strains of PVY.

Roque and Adsuar (1941) described a clearing of the veins, systemic mottling and vein banding in chilli by the Puerto Rican pepper mosaic virus. The leaves became wrinkled and the plant was stunted. Fruits were small in size, mottled and badly distorted. Simons (1956) described a pepper vein banding mosaic virus exhibiting chlorosis of leaves, vein banding and mosaic mottling with leaf distortion on chilli from Florida. Both the viruses were found to have affinity to potato virus Y. Trinidad pepper vein banding virus (Lale, 1954, 1956) had very much similar physical properties and produced symptoms comparable to that of the Florida virus. A potato virus Y strain on chilli described by Jayarajan and Ramakrishnan (1961) had also produced similar symptoms, besides exhibiting filiformity of leaves.

The isolates studied in the present investigations produced similar or comparable symptoms to those described above. They, however, differed from those described by Kovachevsky (1942), and David and Stormer (1941) for potato virus Y on chilli in not producing flecking on leaves. The physical properties of the virus isolates varied widely. The isolates C₇ and C₈ had a thermal inactivation point between 55 and 60°C, a dilution end point between 1:100 and 1:500 and a longevity in vitro of 2 days at room temperature (26°-28°C) closely resembling the Puerto Rican pepper mosaic virus, and the PVY strain on chilli described by Jayarajan and Ramakrishnan (1961). The isolates C₉, C₁₀, C₁₁, C₁₂ and C₁₃ also had a thermal inactivation point between 55 and 60°C

a dilution end point between 1:2000 and 1:5000 and a longevity in vitro of 48 hours. These isolates did not resemble any of the strains of PVY reported on ohilli. The isolates C₁₄, C₁₅, C₁₆, C₁₇, C₁₉ and C₂₀ had a thermal inactivation point between 60 and 65°C, a dilution end point between 1:2000 and 1:5000, a longevity in vitro of 4 days. These isolates resembled closely the Trinidad chilli (pepper) mosaic virus described by Ferguson (1951). The isolates C₁₈, C₂₁ and C₂₂ exhibited milder symptoms than all the 14 other isolates tested in this group. These three isolates differed from others in having a high thermal inactivation point between 65 and 70°C although they closely resembled the Ferguson's Trinidad chilli mosaic virus in other characteristics. The isolate C₂₃ had a thermal inactivation point between 60 and 65°C, a dilution end point between 1:10,000 and 1:20,000 and a longevity in vitro of 48 hours. This isolate resembled pepper vein banding mosaic virus described by Simons (1958) from Florida and the vein banding mosaic virus described by Joshi and Bhargava (1962).

The isolates in group IV namely C₂₄ and C₂₅ were characterised by severe mosaic mottling, twisting and waviness of the leaf margin, orinkling, blistering and severe deformation of the leaf leading to filiformity. These isolates differed from the potato virus Y strains described in the previous group in not exhibiting vein banding. They were transmitted by Aphis gossypii, A. craccivora, A. evonymii, A. nerii and Myzus persicae. Datura stramonium and D. metel

were infected with systemic mosaic mottling, chlorosis, filiformity of leaves and stunting of the plant. Potato hybrid A₆, Physalis peruviana and P.floridana were not infected. Zinnia elegans and Cucumis sativus were infected with mosaic mottling. These isolates have not reacted with the antiserum for potato virus Y, alfalfa mosaic virus or cucumber mosaic virus. Both the isolates differed from Indian chilli mosaic virus (Jha and Raychaudhuri, 1956) in having a higher thermal inactivation point of 60°-65°C, a lower dilution end point of 1:10,000 to 1:20,000 and longevity in vitro of 4 days. A mosaic virus causing necrosis on chillies described by Mishra (1963) was also different from these two isolates in symptomatology, host range and physicoel properties. Since these two isolates differed from the viruses reported on chilli, they were recognised as new viruses and designated as Capsicum mosaic virus.

The isolates in group V namely, C₂₆, C₂₇ and C₂₈ were characterized by chlorosis of the leaves mosaic mottling, vein banding and flagging of the leaves. The leaf lamina was slightly reduced with wavy margins. Older leaves exhibited necrosis at the base followed by leaf fall. Infected tobacco plants showed necrotic areas bordering the veins similar to etching. Tabasco chilli seedlings showed symptoms of wilt on inoculation. Datura stramonium reacted with severe mottling, distortion of leaves and filiformity. Physalis peruviana and P.floridana reacted with chlorotic mottle, dwarfing of the plant and epinasty. These isolates had a

thermal inactivation point between 55 and 60°C, a dilution end point between 1:100 and 1:500 and a longevity in vitro of 24 hours. Serologically they were not related to potato virus Y, alfalfa mosaic virus or cucumber mosaic virus. They did not resemble any of the other viruses reported on chilli except tobacco etch virus in causing etch like symptoms on tobacco, necrosis and defoliation of chilli leaves (described by Laird et al., 1964), wilting in C.frutescens var. tabasco (described by Greenleaf, 1953) and physical properties very similar to etch virus. These isolates did not infect the C.annuum c.v. P 11. Cook and Anderson (1959) reported this variety to be tolerant to five strains of TEV and resistant to two other strains.

The two virus isolates C₂₉ and C₃₀ of group VI exhibit mosaic mottling and vein banding of infected leaves with chlorotic rings and oak leaf pattern. Though no malformation was observed, the leaf blades were slightly elongated. Fruits formed on the infected plants were pale and wrinkled. Infected tobacco plants exhibited oak leaf patterns. The isolates infected Medicago sativa and Trifolium repens causing mosaic mottling of leaves. Local lesions were induced on Phaseolus vulgaris and Vicia faba. Streaks developed on the stem of V.faba killing the plants subsequently. The thermal inactivation point of the isolates was between 60 and 55°C, the dilution end point was between 1:2000 and 1:5000 and the longevity in vitro was 48 hours. Both the isolates reacted

positively with the antiserum for alfalfa mosaic virus in Ouchterlony agar double diffusion test. They were therefore, identified as strains of alfalfa mosaic virus.

The symptoms described, however, differed from those described by Quantz (1956) and Kovachevsky (1942) in not exhibiting bright yellow green streaks and flecks. Both the isolates differed from the potato calico strain and tuber necrosis strain in the thermal inactivation point and in not causing local necrotic lesions on inoculated leaves. Though the isolates C₂₉ and C₃₀ were similar in their thermal inactivation point to the Idaho alfalfa mosaic virus strain (Zaunmeyer, 1963~~4~~) and vein necrosis strain (Zaunmeyer and Patino, 1960), yet they could be differentiated on the basis of host reaction in that they did not infect Vigna sinensis as the latter two strains did. Datura stramonium was reported to be resistant to the Idaho isolate, while Gomphrena globosa was affected with local lesions to vein necrosis strain. Alfalfa yellow spot strain of Zaunmeyer (1962~~3~~) differed in infecting V. sinensis systemically. Tobacco strain of AMV described by Silber and Heggstad (1965) differed in physical properties and reaction with V. sinensis. The isolates studied now resembled the pepper strain (Berkley, 1947) in symptomatology and thermal inactivation point closely, but differed in not infecting cucumber. These isolates were identified as ^{AMV} ~~MAV~~ strain closely resembling the pepper strain Marmor medicaginis Holmes var. capsici, Berkeley.

2. Reaction of chilli varieties to three viruses occurring on chilli

From the above results and discussion it was evident that tobacco mosaic virus, potato virus X, potato virus Y, tobacco etch virus, capsicum mosaic virus and alfalfa mosaic virus occurred on chillies in Tamil Nadu. Among these, the tobacco mosaic virus and potato virus X were confined to certain pockets in Coimbatore district. Tobacco etch virus was collected from parts of Coimbatore and Tiruchirappalli districts. Alfalfa mosaic virus was collected from Tiruchirappalli and Tirunelveli districts. These viruses were limited in their distribution and the symptoms caused on chilli plants were less severe than those caused by potato virus Y and capsicum mosaic virus. These viruses were not considered to cause severe crop losses.

The studies on the reaction of chilli varieties and Capsicum species to the widely distributed potato virus Y capsicum mosaic virus and alfalfa mosaic virus indicated that the c.v. Capsicum annuum '685' was not infected by any of the three viruses. A strain of Capsicum annuum 'P11' and a Mexican hot pepper 'Cuaresmeno' were not infected by potato virus Y and reacted with mild symptoms to capsicum mosaic virus and alfalfa mosaic virus. Ramakrishnan et al. (1966) had studied the reaction of chilli (Capsicum spp.) cultivars to tobacco mosaic virus and found that the strain C.annuum 'P11' to be resistant to it. Cook and Anderson (1959) also

reported that the strain of Capsicum annuum 'J11' had shown multiple virus resistance to tobacco mosaic virus, tobacco etch virus and potato virus Y. Roque and Adsuar (1941) found the Mexican hot pepper 'Cuaresmeno' to be resistant to the Puerto Rican pepper mosaic virus. Riollano et al. (1948) had bred a strain of pepper, resistant to the disease by crossing 'California wonder' with 'Cuaresmeno'. The Capsicum annuum var. 'P34' was not infected by potato virus Y though it reacted with mild symptoms to alfalfa mosaic virus and with severe disease to capsicum mosaic virus. These varieties mentioned above could, therefore, be utilized to develop tolerance or resistance to the three viruses studied in the present instance.

Capsicum pendulum Wild., c.v. 'SA 90' was not infected by potato virus Y and reacted with only mild symptoms to capsicum mosaic virus and alfalfa mosaic virus. But C. pendulum had been reported by Lippert et al. (1966) to be incompatible with C. annuum for hybridization and might be of no utility in breeding for tolerance or resistance to virus diseases.

Twenty six of the varieties tested reacted only with mild symptoms to all the three viruses. Two cultivars "CA766" and 'G52A' of C. annuum reacted with mild symptoms to PVY and CaMV. Hence these cultivars also could be used in breeding for tolerance to the viruses.

Rest of the 125 varieties exhibited severe symptoms to either PVY or CaMV or all the three viruses leading to economic losses.

3. Sources of inoculum for the potato viruses X and Y occurring on chilli

Among the six viruses reported tobacco mosaic virus, tobacco etch virus, alfalfa mosaic virus and capsicum mosaic virus may have alternate hosts in the weeds or other cultivated crops which could be the source of inoculum for the spread of these viruses to chilli fields. Potato is a temperate crop grown only in Nilgiris district (altitude 1800 to 2600 above m.s.l.) about 80 Km away from Coimbatore district where PVX on chilli is prevalent. Potato virus Y is found widespread in other districts also. As such it is not clear as to how the potato viruses PVX and PVY are found in the plains away from the main source of these viruses. Probably the potato tubers transported for consumption from the Nilgiris carry PVX and finds its way into the chilli fields as a contaminant. However, Aphid migration was considered unlikely in the present case and there are no reports that such migrations occur here. Johnson (1957) had however, reported on the dispersal^{er} by upper winds of Aphis craccivora in South wales. According to Caster^y (1962) "this would appear to be a situation favourable for the transmission of persistent viruses, but not of the non-persistent." Hence the nature of spread of potato virus Y

on a large scale on the chilli crop in the plains remained obscure. Probably the virus had other solanaceous hosts like Solanum wendlandii Hook. and S.nigrum through which they found their way to the plains.

Sakimura (1953) considered Solanum nodiflorum, Desv. as a major source of inoculum under natural condition for the spread of potato virus Y into chilli and tomato on the island of Oahu (Hawaii). Simons (1956, 1957) reported that S.elaeagnifolium Cav. and S.gracile Sendt. harboured vein banding virus of chilli, a strain of potato virus Y. Solanum aculeatissimum Jacq. was found to harbour viruses causing damage to the chilli crop in central Florida (Anon, 1957). Anderson and Corbett (1957) and Anderson (1959) reported severe damage to chilli plants in the proximity of weed hosts and they isolated a strain of potato virus Y from Solanum nigrum L., S.gracile, and Physalis angulata, L. Simons (1959) reported that the chilli virus (PVY) overwinters on S.gracile.

In the present study, Trianthema portulacastrum a common weed found in most of chilli fields was also found to show symptoms of mosaic. The virus isolated from infected plants of this species was identified as PVY. The virus was transmitted to chilli by Aphis graccivora and A.gossypii. This weed could as well have served as a source of inoculum for the spread of PVY to chilli crop.

4. Relationship of chilli viruses to their aphid vectors

From the results obtained in the study of Vector-virus relationships of potato virus Y, capsicum mosaic virus and alfalfa mosaic virus the following conclusions could be drawn.

Increase in the period of preacquisition fasting was found to increase the efficiency of the vector. Preacquisition fasting periods exceeding the optimum had reduced the vector efficiency. Efficiency of the vector increased with the increase in the acquisition feeding period upto an optimum and declined on further feeding. The vector efficiency had increased with the increase in inoculation feeding upto an optimum and tended to decline with further increase in inoculation feeding. Increase in the number of viruliferous inoculating aphids increased the percentage of transmission upto an optimum. These results were in agreement with those of many workers (Watson 1936, 1938; Watson and Roberts, 1939, 1940; Sylvester, 1954; Day and Irzykiewicz, 1954; Ehargava, 1951; Bradley, 1952). Persistence of the virus in the vectors was longer during fasting than feeding. This had corroborated earlier observations on cucumber mosaic virus (Joolittle and Walker, 1928; Ehargava, 1951), potato virus Y (Smith, 1931; Watson and Roberts, 1940), pea mosaic virus (Osborn, 1937), Henbane mosaic virus (Watson, 1938, Watson and Roberts, 1940), and lettuce mosaic virus (Kassanis, 1947). The results obtained had shown that the three viruses potato virus Y, capsicum mosaic virus and alfalfa mosaic virus on chilli studied now were transmitted in a non-persistent manner.

Many workers studied the mechanism of transmission of non-persistent viruses Hoggan (1931, 1933, 1934), Watson (1936, 1938), Watson and Nixon (1953), Sylvester (1949, 1950, 1952, 1954, 1961) and Day and Irzykiewicz (1954). They put forward many theories regarding the mechanism of transmission of non-persistent viruses by aphid vectors. Carter (1962), Roehow (1963) and Smith (1965) had recently reviewed the mechanism of transmission of plant viruses by aphid vectors. The virus was carried as a contaminant on the stylets of aphids according to the "mechanical transmission hypothesis" of Hoggan (1933). Watson and her co-workers suggested the "Inactivator-behaviour-theory" explaining the loss of infectivity to be due to the activity of a substance produced by the aphids during feeding and was not due to the mechanical cleansing of the stylets during successive probings. Day and Irzykiewicz (1954) came up with the "mechanical - inactivator - behaviour" hypothesis. This hypothesis postulated the contamination of the stylets by the virus during feeding and selective inactivation of the virus on the stylets by fastigial components of the saliva. van der Want (1954) laid emphasis on surface differences of the stylets of various aphid species in his "mechanical - surface - adherence" hypothesis and the specificity was explained to be due to the differential absorption to and elution of the virus, from the stylets. van Hoof (1957) showed with the help of low power electron-micrographs, surface differences like pockets and ridges, on stylets of different aphids, which

might serve for the adherence of the virus. According to the "mechanical-inactivator-compatibility" hypothesis proposed by Sylvester (1954), compatibility of the combination of the virus, saliva, and host cell inoculated decided the specificity in infection. The advance of stylets was associated with the secretion of saliva that formed a sheath in some penetrations as reported by Sylvester (1961). However, the function of the salivary sheath had not been detailed. Adams and McAllan (1956) had shown the enzymatic activity of the saliva. However, information on the role played by such an enzyme in penetration of plant cells by the stylet of the aphid had not been studied. Information on the effect of salivary secretions on the mechanism of transmission had also been lacking. Inhibitory effect of the saliva of Periplaneta americana L. and Nezara viridula L. on tobacco mosaic virus was reported by Day and Irzykiewicz (1954). Nagarajan (1967) showed all aphids to ^{can} certain salivary inactivator, and the quantity varied from species to species. He correlated the efficiency of transmission to be inversely proportional to the quantity of the inhibitor secreted.

Much emphasis had been placed by many workers on the inhibitor theory. This theory emphasised that the viruses were inactivated on the aphid stylets by the salivary fluids. The viruses themselves differ in their reaction to these inhibitors. Different species of aphids vary in their capability of production of the inhibitor.

Simons and Moss (1963) had shown that stylet treatment with an inhibitor from Aeonium arboreum Webb and Benth. or with urea significantly reduced PVY infectivity. Treatment of wild stylets with the juice of A. arboreum reduced their ability to acquire virus to about half the level for aphids treated with water. Their work is believed to be the first of its kind to show that an inhibitor is involved in a natural mechanism of resistance to aphid transmission of virus in plants and offers the first evidence that such inhibitory substance can reduce aphid transmission.

Jansen and Staples (1970) reported the effect of soybeans as source or test plants on vector efficiency and retention of infectivity of the bean leaf-beetle and the spotted cucumber beetle. The vector efficiency of Ceratoma trifurcata (Forst.) and Diabrotica undecimpunctata Howardi (Barber) were significantly reduced in the transmission of the severe strain of cowpea mosaic virus on soybean test plants. The retention of infectivity of the bean leaf-beetle was reduced when soybean was used as the virus source plant. The possibility of the soybean plants containing an inhibitor which interfered with the infection process or which inhibited the virus in the beetles had been suggested by these authors.

In the present instance the vectors Aphis craccivora collected from Gliricidia maculata were less efficient than those collected from Vigna sinensis. Extracts of leaves of G. maculata inhibited the virus in mechanical transmission.

With the increase in the dilution of the sap of G. maculata the inhibitory effect tended to reduce. With the increase in pre-acquisition feeding on G. maculata the percentage of transmission was reduced much more compared to feeding on V. sinensis. Similarly post acquisition feeding also reduced efficiency of transmission to a much higher rate in case of G. maculata. The effect of the inhibitory substance was reduced with the increase in preacquisition fasting period. A. oraccivora from G. maculata maintained on cowpea and A. oraccivora collected from cowpea were more efficient giving a much higher percentage of virus transmission than the aphids collected from cowpea maintained on Gliricidia or aphids collected direct from Gliricidia.

From the results obtained in the study of vector-virus relationships of the potato virus Y, the author is also of the same opinion that the virus transmission ability of the aphid vectors depends on the source plant of the vector. The inhibitory substance present in the source plant has, therefore, a bearing on the efficiency of the aphid vector in virus transmission.

SUMMARY

1. A survey of chilli crops in different parts of Tamil Nadu indicated the prevalence of mosaic disease. The disease incidence varied from 4 to 75 per cent in different localities.

2. Thirty virus isolates were collected from chilli crop in different parts of the State and studied in detail. The identity of these virus isolates was established by studying their symptomatology, transmission, physical properties, host range and serological behaviour.

3. Two of the virus isolates were identified as tobacco mosaic virus. Mosaic disease caused by TMV was found in a confined pocket in Coimbatore district. Four virus isolates were identified as potato virus X. The virus was confined to two localities namely, Pollachi and Bhavanisagar in Coimbatore district. Seventeen virus isolates were identified as potato virus Y. This virus was found to be widespread in 10 districts. Two isolates collected from Coimbatore district and one isolate collected from Tiruchirappalli district were identified as tobacco etch virus. Two isolates identified as alfalfa mosaic virus occurred in one locality in each of Tiruchirappalli and Tirunelveli districts. All these viruses were also reported to occur on chilli in different parts of the world. Two isolates now collected and studied in detail, however, differed from all the known viruses that were reported on chilli. These

were identified for the first time and designated as capsicum mosaic virus (CaMV).

4. The virus-vector relationships were investigated in the case of potato virus Y, capsicum mosaic virus and alfalfa mosaic virus and their vectors Aphis gossypii and A. craccivora. The viruses were found to be transmitted in a non-persistent manner.

5. The vector Aphis gossypii was able to transmit all the three viruses mentioned above without any preacquisition fasting, but with low efficiency. Preacquisition fasting increased the efficiency of transmission upto an optimum period of 2 hours for PVY and CaMV and 4 hours for AMV.

Preacquisition fasting was found essential for the transmission of PVY and CaMV by Aphis craccivora. A. gossypii became viruliferous in 5 seconds of acquisition feeding in the case of PVY and AMV. Fifteen seconds was required to acquire CaMV. A. craccivora required 15 seconds to acquire all these three viruses.

The percentage of transmission increased with the increase in the acquisition feeding period upto an optimum and tended to reduce with further increase in the acquisition feeding period. Both Aphis gossypii and A. craccivora were found to transmit the three viruses PVY, CaMV and AMV in an inoculation feeding period of 15 seconds. Increase in the inoculation feeding period also increased the percentage of

transmission upto an optimum period and then reduced with further increase in inoculation feeding period. Individual aphid was able to transmit the three viruses PVY, CaMV and AMV. Maximum percentage of transmission was obtained with 20 aphids in the case of PVY and AMV while 30 aphids were required for the maximum percentage of transmission of CaMV.

6. Aphis craccivora maintained on Vigna sinensis gave higher percentage of transmission than those collected from Gliricidia maculata. G. maculata plants had an inhibitor which reduced the transmission of PVY. The effect of the inhibitor tended to minimize with the dilution of the sap. With increase in the preacquisition feeding of the vector on G. maculata, the percentage of virus transmission was decreased. Post acquisition feeding on G. maculata also decreased the percentage of transmission. A. craccivora collected from cowpea as well as from G. maculata and maintained on cowpea were more efficient giving a much higher percentage of virus transmission than the aphids collected from cowpea and maintained on Gliricidia or the aphids collected direct from Gliricidia.

7. Trianthema portulacastrum a common weed found in most of the gardenlands was found infected by a mosaic virus. The virus was identified as PVY. The virus from this plant was transmitted to chilli plants with the vectors A. gossypii and A. craccivora. This weed could as well serve as a source of inoculum for the spread of PVY to chilli crop.

8. Available species varieties and cultivars of Capsicum were screened for their reaction to PVY, CaMV and AMV. C.annuum c.v.685 was not infected by any of the three viruses mentioned above. A strain of C.annuum 'P11' and 'Cuaremeno' were found to be immune to PVY and reacted with mild symptoms to CaMV and AMV. C.annuum c.v.'P34' was also not infected by PVY. Twenty six of the other varieties tested also reacted only with mild symptoms to these three viruses. Two cultivars CA766 and C52A of C.annuum reacted with mild symptoms to PVY and CaMV only. These cultivars could be of much use in breeding for tolerance or resistance to the viruses.

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