STUDIES ON CUCURBIT VIRUSES IN MADRAS STATE

IV. Some Aspects of the Relationships of Melon Mosaic Virus Strain to Its Three Aphid Vectors

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ABSTRACT

Melon Mosaic Virus (MMV) was non-persistent in its three aphid vectors. The pre-acquisition fasting threshold, acquisition threshold and the inoculation feeding threshold were 5 minutes, 10 seconds and 60 seconds respectively for *Myzus persicae*, 15 minutes, 30 seconds and 3 minutes respectively for *Aphis gossypii*, 60 minutes, 60 seconds and 5 minutes respectively for *A. nerii*. The optimum number of viruliferous aphids per plant for maximum transmission was 30 in all the vectors. Increasing the number of aphids above this optimum decreased the percentage transmission of the virus. In the case of *Myzus persicae*, the reduction in the percentage transmission was conspicuous when 240 aphids per plant were used whereas in *A. nerii* the decrease was noticed even when 100 aphids per plant were used. The percentage transmission by fasted aphids was more than by the non-fasted ones. Persistance of the virus during fasting was for 90, 45 and 30 minutes respectively in *M. persicae*, *A. gossypii* and *A. nerii* while during feeding it was 30, 15 and 10 minutes respectively. The aphid vectors were ranked in the following descending order of transmission efficiency: *Myzus persicae*, *Aphis gossypii* and *A. nerii*. The length of the pre-acquisition fasting period varied inversely as the efficiency. More efficient the vector, shorter was the fasting period. The acquisition threshold and incoulation feeding threshold also varied inversely as the efficiency of the vector. The fall in the efficiency of transmission when the number of aphid vector was increased above the optimum was considered to be due to a salivary inhibitor. Apterous forms of the aphid vectors were more efficient transmitters of the viruses than the alate forms.

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It was concluded that all aphids have a salivary inactivator, the quantity secreted varied from species to species, the efficiency of transmission being inversely correlated with the quantity of inhibitor secreted.

Introduction

A strain of Melon Mosaic Virus occurs commonly on pumpkin (*Cucurbita moschata* Duch.) in Tamil Nadu. The virus was sap transmissible and also through three aphids, *Myzus persicae*, *Aphis gossypii* and *Aphis nerii*. The present study was taken up to find out the relationship of the virus to the above vectors.

Materials and Methods

The study on the virus-vector relationships was carried out using an isolate of Melon Mosaic Virus. Virus-free colonies of aphid vectors, *Myzus persicae*, *Aphis gossypii* and *A. nerii* obtained by the multiplication of a single viviparous, wingless female were maintained on young healthy host plants in large insect-proof cages. Methods to determine the relationships were the same as described in detail by Nariani and Sastry (1962). Details are given under the respective experiments.

Results

Preliminary investigations revealed that *Myzus persicae*, *Aphis gossypii* and *A. nerii* were the chief aphid vectors of transmission of the virus, the percentage transmission being 70, 70 and 40 respectively while the following aphids, *Aphis craccivora*, *A. maydis*, *A. malvae*, *Toxoptera citricidus*, *Brevecorye brassicae*, *Aluacophora intermedia* and *A. foveicollis*, failed to trnasmit the virus strain.

(*a*) *Effect of preliminary fasting on the efficiency of the aphid vectors to acquire the virus.*—It was evident from the results that in all the three vectors a preliminary fasting was found necessary to effect successful transmission. However, the minimum fasting period required varied between the three vectors. In the case of *Myzus persicae*, a pre-acquisition fasting of 5 minutes was found sufficient for transmission of the virus while in the case of *Aphis gossypii* and *A. nerii*, the minimum pre-acquisition fasting was 15 minutes and 60 minutes respectively. However, in all the three vectors the percentage of transmission was comparatively low at the minimum pre-acquisition fasting. It was further observed that the optimum period of pre-acquisition fasting was 90 minutes for the three vectors, the percentage transmission of the viruses being 87·50, 62·50 and 37·50 respectively

Fasting of the vectors longer than the optimum was found to reduce the percentage of transmission in all the cases.

(*b*) *To determine the acquisition threshold of the vectors.*—The results indicated that the minimum time required by the vectors to pick up the virus from the infected plant (acquisition threshold) varied. While in the case of *M. persicae* the acquisition threshold was 10 seconds, it was 30 and 60 seconds respectively in *A. gossypii* and *A. nerii*. However, it was observed that the optimum acquisition feeding period was 5 minutes for all the 3 aphid vectors. The percentage transmission at 5 minutes acquisition feeding was 75·00, 62·50, and 50·00 respectively for *M. persicae*, *A. gossypii* and *A. nerii*. Longer acquisition feeding did not further improve the efficiency of transmission.

(*c*) *To determine the inoculation threshold of the vectors.*—The results indicated that the inoculation threshold of *M. persicae* was as short as 60 seconds while that of *A. gossypii* and *A. nerii* was 3 minutes and 5 minutes respectively. However, the percentage transmission was found to be poor with the above periods of feeding. The optimum period of inoculation feeding was found to be 60 minutes for all the three aphid vectors. Increasing the inoculation feeding period above 60 minutes reduced the efficiency of transmission.

(*d*) *To determine the number of viruliferous aphids per plant required for efficient transmission.*—The results of the experiment are presented in Table I.

It may be seen that in the case of *M. persicae*, a single viruliferous aphid per plant would result in successful transmission while in the case of *A. gossypii* and *A. nerii*, the minimum number of viruliferous aphids required per plant was 3 and 5 respectivey. However, it was observed that the optimum number of viruliferous aphids per plant required for maximum transmission was 15 in the case of *A. gossypii* and *A. nerii* and 20 in the case of *M. persicae*. It was also observed that colonization of the plant with more than the optimum number of aphids resulted in reduced percentage of transmission.

An important observations made from the above experiment was with regard to the number of viruliferous aphids per plant and the percentage transmission. The results showed that increasing the number of aphids per plant above the optimum resulted in reduced transmission. This observation was made for all the three aphid vectors, *viz.*, *M. persicae*, *A. gossypii* and *A. nerii* tested. In order to find out the reasons for such reduction in

the percentage transmission of the virus when large number of aphids per plant was used, further experiments were conducted using *M. persicae* and *A. nerii* as vectors and *Cucurbita pepo* var. *early white bush* as test plant.

TABLE I

Minimum number of viruliferous aphids per plant for successful virus transmission

Pre-acquisition fasting : 90 minutes
Acquisition feeding : 5 minutes,

Sl. No.	No. of aphids per plant	*M. persicae*		*A. gossypii*		*A. nerii*	
		Infected/ Inoculaed	% of transmission	Infected/ Inoculated	% of transmission	Infected/ Inoculated	% of transmission
1.	One	1/8	12·5	0/8	..	0/6	..
2.	Three	3/8	37·5	2/8	25·0	0/8	..
3.	Five	3/8	37·5	3/8	37·5	2/8	25·0
4.	Ten	5/10	50·0	3/8	37·5	3/8	37·5
5.	Fifteen	5/10	50·0	5/8	62·5	4/10	40·0
6.	Twenty	5/8	62·5	6/10	60·0	4/10	40·0
7.	Above twenty	3/8	37·5	5/10	50·0	2/8	25·0

1. *To find out the relation of number of viruliferous aphids per plant to percentage transmission of the virus isolate.* Healthy colonies of *Myzus persicae* and *Aphis nerii* were collected and starved for 90 minutes. After the preliminary fasting, the vectors were fed for five minutes on young infected pumpkin leaf showing severe mosaic symptom. After the acquisition feeding, the viruliferous aphids were transferred to young healthy *C. pepo* plants at the rate of 10, 20, 30, 40, 60, 80, 100, 120, 240 and 360 aphids per plant and allowed an inoculation feeding of 12 hours after which the plants were sprayed with parathion and kept inside an insect-proof glass house for observation. The results are presented in Table II.

The results presented in Table II further confirmed the earlier observation that increasing the number of viruliferous aphids per plant would decrease the percentage of transmission. In the present experiment it was found that the optimum number of viruliferous aphids per plant for maximum percentage of transmission was 30 to 40 in both *M. persicae* and *A. nerii*.

TABLE II

Relation of number of viruliferous aphids per plant to percentage of transmission

Treatment	No. of aphids per plant	No. of plants used	*Myzus persicae*			*Aphis nerii*		
			No. of plants infected	% of transmission	Incubation period in days	No. of plants infected	% of transmission	Incubation period in days
1	10	15	8	53·33	8	4	26·66	9
2	20	15	9	60·00	8	6	40·00	9
3	30	15	11	73·33	8	7	46·66	10
4	40	15	11	73·33	9	7	46·66	11
5	60	15	10	66·66	9	5	33·33	11
6	80	15	9	60·00	11	3	20·00	13
7	100	15	9	60·00	11	3	20·00	14
8	120	15	7	46·66	12	2	13·33	14
9	240	15	5	33·33	14	2	13·33	14
10	360	15	6	40·00	14	2	13·33	15

Increasing the number of aphids above this optimum level resulted in a decrease in the percentage transmission. In both *M. persicae* and *A. nerii* the decrease started from treatment 5 onwards, and this was found to be gradual. The percentage transmission of the virus was decreased to 33·33 from 73·33 in the case of *M. persicae* when 240 aphids per plant were used and from 46·66 to 13·33 in the case of *A. nerii* when 120 aphids per plant were used. Further it was observed that larger the number of aphids per plant, longer was the

incubation period. When the optimum number of aphids (30) per plant was used, the incubation period was 8 days in the case of *M. persicae* and 10 days in the case of *A. nerii*. When the number of aphids per plant was increased to 240 per plant, the incubation period with *M. persicae* was 14 days and the percentage transmission was 33·33; in the case of *A. nerii* the incubation period was 14 days and the percentage of transmission was 13·33 only.

2. *Detection of the salivary inhibitor.*—This experiment was carried out to detect the salivary inhibitor, if any, of the aphid vectors, *Myzus persicae* and *A. nerii*, using healthy pumpkin leaves. The details of the experiment are given below:

Healthy pumpkin leaves were harvested from young, vigorously growing plants and leaf pieces (approx. 3·5 sq. cm,) weighing one gram were cut from each healthy leaf. Healthy colonies of the aphid vectors, *M. persicae* and *A. nerii* were selected and were transferred to the healthy leaf pieces, at the rate of 100, 120 and 240 aphids per leaf piece. The aphids were used without any preliminary fasting. After allowing the aphids to feed on the healthy leaf pieces for 30 minutes, they were carefully removed. The leaf pieces thus fed with non-viruliferous aphids were macerated in one ml. distilled water and mixed with one ml. of virus infective sap (extracted from 1 g. of infected leaf in 3 ml. of distilled water) and immediately inoculated on the test plants. Two ml. of the mixture obtained by mixing 1 ml. of sap obtained from 1 g. of healthy leaf and 1 ml. of virus-infective sap, was used to inoculate five test plants (*C. pepo*). The following were the treatments:

Treatment 1: Control–1 g. of virus-infected leaf macerated in 2 ml. distilled water.

2: Sap extracted from healthy leaf not fed with aphid vectors and mixed with equal quantity of virus-infected sap.

3: Sap extracted from healthy leaf bits fed with 100, 120 and 240 non-viruliferous aphids and mixed with equal quantity of virus-infective sap.

The results are presented in Table III.

The results presented in Table III indicate that in treatment 1 where virus-infective sap alone was used, the percentage of infection was 80·00. Further, symptom development was observed in 8 days. In treatment 2, when healthy sap was mixed with virus sap, the percentage of transmission

was 66·66 and the symptom development was observed in 9 days. In treatment 3, when *Myzus persicae* was the vector, the percentage of transmission was 66·66, 66·66 and 53·33 respectively with 100, 120 and 240 aphids per leaf. Further, it was observed that the incubation period was 10 days when 100 and 120 aphids were used and 12 days when 240 aphids were used. In the case of *Aphis nerii*, the percentage of infection with 100, 120 and 240 aphids per leaf, was 66·66, 46·66 and 33·33 respectively and the incubation period was 10, 10 and 14 days respectively.

TABLE III

Detection of the salivary inhibitor in vivo

Treatments		No. of aphids per leaf					
		Myzus persicae			*Aphis nerii*		
		100	120	240	100	120	240
1	12/15	..	..	..	..	..	..
2	10/15	..	..	..	..	..	..
3	..	10/15	10/15	8/15	10/15	7/15	5/15

Numerator = No. of plants infected.
Denominator = No. of plants inoculated.

3. *Effect of pre-acquisition fasting on salivary inhibitors.*—In the above experiments it was observed that *Myzus persicae* and *Aphis nerii* when used in large numbers (240) and also without any pre-acquisition fasting probably produced an inhibitor which resulted in reduced transmission. In the present studies, comparison was made between aphids fasted prior to acquisition and aphids not fasted prior to acquisition.

One set each of healthy colonies of the aphid vectors (*Myzus persicae* and *Aphis nerii*) was selected and fed for 60 minutes on healthy pumpkin leaf at the rate of 240 aphids per 3 sq. cm. leaf piece. After the feeding, the aphids were carefully removed and the leaf piece macerated in 1 ml. of distilled water. The extract was immediately mixed with 1 ml. of viruliferous sap (extracted by grinding 3 sq. cm leaf bit of virus-infected leaf in 1 ml. of distilled water) and test plants (*C. pepo*) were inoculated. The resultant mixture of 2 ml. of the sap was used to inoculate 5 test plants.

In another set, the aphid vectors were fasted for two hours in empty petri plates after which they were fed on healthy pumpkin leaf pieces for one hour at the rate of 240 aphids per 3 sq.cm, leaf bit. After one hour of feeding, the aphids were removed and the leaf bit was macerated in 1 ml. of distilled water, mixed with 1 ml. of virus sap as in the above case and then resultant mixture was inoculated on five test plants. The following were the four treatments used:

Treatment 1 : Control–1 g. of infected leaf macerated in 2 ml. of distilled water (standard extract).

" 2 : Healthy pumpkin leaf of 3 sq. cm. macerated in 1 ml. distilled water and mixed with 1 ml. of the standard extract of the virus sap.

" 3 : Healthy pumpkin leaf piece of 3 sq. cm. fed for one hour with aphid vectors without preliminary fasting. The extract of one ml. of this sap was mixed with 1 ml. of virus sap.

" 4 : Healthy leaf piece of 3 sq. cm. fed for one hour with aphid vectors after a preliminary fasting of 2 hours. The extract of 1 ml. of this sap was mixed with 1 ml. of the virus sap.

The results presented in Table IV seems to indicate that when aphids were fasted and fed on leaves, less of the virus inhibitors are excreted into the leaf. In the case of *Myzus persicae*, the percentage infection was 60·00 when non-fasted aphids were used as against 73·33 when fasted aphids were used. As regards *Aphis nerii*, the percentage of infection was 41·70 in the case of non-fasted aphids.

TABLE IV

Effect of pre-acquisition fasting on salivary inhibitor

Treatments	*M. persicae*			*A. nerii*		
	No. of plants inoculated	No. of plants infected	Percentage of infection	No. of plants inoculated	No. of plants infected	Percentage of infection
1	15	12	80·00	12	9	75·00
2	15	11	73·33	12	9	75·00
3	15	9	60·00	12	5	41·70
4	15	11	73·33	12	8	66·66

4. *Detection of salivary inhibitor in the aphids.—Cucurbita pepo* (var. early white bush) plants of uniform age were selected. Plants having fully expanded first leaf were used for all the treatments. Healthy colonies of the aphid vectors (*Myzus persicae* and *Aphis nerii*) were made to feed directly on the first true leaf for one hour at the rate of 240 aphids per leaf. After one hour the aphids were removed and the leaf was immediately inoculated with virus sap. In another set, the first leaf on which the non-viruliferous aphids (240 Nos.) were fed for one hour, was subjected to the feeding of viruliferous aphids at the rate of 30 per leaf. The following were the treatments:

Treatment 1 : Plants inoculated with virus sap only.

" 2 : Plants inoculated through viruliferous aphid vectors only. In this case, the aphid vectors were starved for two hours and then given an acquisiion feeding of five minutes after which they were transferred to the first true leaf of the test plants at the rate of 30 per plant.

" 3 : Plants pre-treated with non-fasted aphids and then sap inoculated. In this case, the test plants at the first true leaf stage were subjected to the feeding of non-fasted aphids for one hour at the rate of 240 per leaf. Leaves thus treated were immediately inoculated with virus sap.

" 4 : Plants pre-treated with non-fasted aphids vectors and then inoculated through viruliferous aphids. In this case as in treatment 3, test plants were subjected to the feeding of non-fasted aphid vectors at the rate of 240 aphids per leaf for one hour after which the leaves were subjected to the feeding of viruliferous aphids at the rate of 30 per plant.

No significant differences in percentage infection were noticed between Treatments 1–3. However, in treatment 4, conspicuous reduction in the percentage transmission by both *M. persicae* and *A. nerii* was noticed. Further, it was confirmed that *A. nerii* was a less efficient vector compared to *M. persicae.* The reduction in the percentage of infection in Treatment 4 might be due to the presence of inhibitor injected by the non-fasted aphids into the leaf. However, this reduction was not noticeable in Treatment 3 where also the leaves were subjected to feeding of the non-fasted aphids but inoculated with sap.

The results are presented in Table V below:

TABLE V

Effect of salivary inhibitor on the transmission of the virus

Treatments		*M. persicae*			*A. nerii*		
		No. of plants inoculated	No. of plants infected	Percentage of infection	No. of plants inoculated	No. of plants infected	Percentage of infection
1	9/10	..	..	..	..	..	..
2	..	10	9	90·00	10	4	40·00
3	..	10	8	80·00	10	4	40·00
4	..	10	7	70·00	10	2	20·00

(*e*) *Persistence of the virus in the vectors during fasting.*—The results showed that the persistence of the virus during fasting was 90 minutes after acquisition in *Myzus persicae*; 45 minutes after acquisition in *Aphis gossypii* and 30 minutes in *A. nerii*. It was also observed that the rate of loss of the virus was gradual in the case of *Myzus persicae* while it was rapid in the other two aphid vectors.

(*f*) *Persistence of the virus in the vectors during feeding.*—The result indicated that the persistence of the virus during feeding was much longer in *M. persicae* compared to *A. gossypii* and *A. nerii*. When the viruliferous aphid (*M. persicae*) was transferred to a series of healthy test plants at an interval of 5 minutes, transmission was obtained till the fifth plant of the series. When the interval of transfer was 10 minutes between the plants, transmission was secured till the third plant of the series. This clearly indicated that the persistence of the virus in *M. persicae* during continuous feeding was 25 to 30 minutes as against 90 minutes during fasting. In the case of *A. gossypii* when three viruliferous aphids per plant (which was found to be minimum for transmission) were used, the persistence of the virus during feeding was 10 to 15 minutes as against 45 minutes during fasting. As regards *A. nerii*, the persistence of the virus during feeding was only 10 minutes as against 30 minutes during fasting.

(g) *Comparative efficiency of alate and apterous forms of aphids.*—The results indicated that both the alate and apterous forms of the aphids transmitted the virus isolate. However, the apterous forms were found to be more efficient vectors.

DISCUSSION

Reviewing the present results obtained in the study of aphid-virus relationships of the Melon Mosaic Virus (MMV), we are inclined to support the salivary inhibitor hypothesis to account for the specificity of aphid vectors to their viruses.

Sylvester (1961) reported that with efficient vectors, the effect of preliminary fasting will occur rapidly, *i.e.*, within 5 minutes of the time that the insects are removed from a feeding site. In an inefficient vector, on the other hand, the effects of preliminary fasting has been shown to accumulate over a period of hours. The present studies showed that a preliminary fasting of 5, 15 and 60 minutes are required for *M. persicae*, *A. gossypii* and *A. nerii* respectively to become viruliferous. This observation is in agreement with that of Sylvester (1961).

It has been observed that the length of the preliminary fasting period required varied inversely with the efficiency of transmission. In the most efficient vector, *M. persicae*, the length of preliminary fasting for the vector to become viruliferous was as short as 5 minutes while in the case of the least efficient vector, *A. nerii*, 60 minutes were required. When no preliminary fasting was given, none of the vectors acquired the virus. These observations could most conveniently be explained by postulating: (1) in non-fasted aphids the salivary inhibitor is present in sufficiently high quantities to inactivate the virus taken up during feeding. (2) during fasting the secretion of the inhibitor is reduced or even completely stopped, and (3) in the less efficient vector more of the inhibitor is secreted during feeding and, therefore, the fasting period has to be correspondingly prolonged to reduce the flow of the inhibitor.

It has been reported by many workers (Watson, 1936, 1938; Watson and Roberts, 1939, 1940; Sylvester, 1954; Day and Irzykiewicz, 1954) that preliminary fasting of the aphid vectors reduces the activity of the postulated salivary inhibitor. Sylvester (1961) reported that the concentration of labile virus inactivators in the saliva or alimentary canal is perhaps decreased by fasting. Earlier, Sylvester (1954) reported that the quantity and rate of production of inhibitor varies with the insect species and the

inhibitor is common to all aphids and acts on all viruses in a similar manner. Experiments conducted with fasted and non-fasted *M. persicae* and *A. nerii* indicated that when non-fasted vectors were fed on healthy leaf pieces and the leaf macerate mixed with the inoculum the percentage transmission was 60·00 and 41·70 respectively for *M. persicae* and *A. nerii*; when fasted aphids in a similar experiment were used the percentage was 73·33 and 66·66 respectively. It is presumed that the aphids while feeding injected saliva (and inhibitor) into the leaves and fasting reduced the quantity of inhibitor injected.

The acquisition threshold varied inversely as the efficiency of transmission. The most efficient vector *M. persicae* acquired the virus in 10 seconds while *A. nerii*, the least efficient one, acquired the virus in 60 seconds. Increasing the acquisition feeding period above the optimum decreased the percentage transmission. It has been reported by Watson and Roberts (1938), Day and Irzykiewicz (1954), Sylvester (1954), Bradley (1954, 1959), Nariani and Sastry (1962) that longer the aphid fed on the diseased plant, the less efficient it became as a vector. The fall in the percentage of infections with longer acquisition feeding periods has been explained by Watson and Roberts (1938) and Day and Irzykiewicz (1954) on the basis of production of inhibitors in insects during feeding. The differences in acquisition threshold could also be explained by postulating differences in quantity of inhibitor secreted–the least efficient vector secreting larger quantities than the most efficient and, therefore, larger quantities of the virus are required to neutralize the inhibitor leaving a residue of transmissible virus.

Persistence of the virus in the vector was longer during fasting than feeding. This corroborates earlier studies on cucumber mosaic virus (Doolittle and Walker, 1928; Bhargava, 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). Day and Irzykeiwics (1954) observed that the duration of persistence of infectivity of aphids has a bearing on the inactivator hypothesis. During fasting, the viruses would have less opportunity of coming in contact with a salivary inhibitor. During feeding some virus is wiped off the stylets but the very short survival time of viruses during feeding indicates that they are subjected to an additional inhibiting action. The results of the present study on persistence of the viruses in their aphid vectors seems to lend support to the inactivator hypothesis. Further, it was observed that in the efficient vector, *M. persicae*, the persistence of the virus during fasting

as well as feeding was always longer than in the inefficient vector, *A. nerii*. This seems to indicate that there are inherent differences between aphid species in the quantity or quality of inhibitor secreted.

Another interesting observation made in the present study was that when a larger number of viruliferous aphids above an optimum was used, there was a decrease in the transmission percentage. Additional experiments conducted with *M. persicae* and *A. nerii* and MMV showed that when 240 aphids per plant were used (optimum number per plant was 30 aphids) there was reduction in the percentage of transmission. It is suggested based on the results that the aphids inject an inhibitor into the leaf while feeding and there is an *in vivo* inactivation of the virus in the leaf when the quantity of inhibitor injected exceeds an optimum. It is significant that fasted aphids produced less of this effect and in the less efficient vector (*A. nerii*) this effect was produced with a small number of aphids than in the more efficient vector (*M. persicae*).

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