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Studies on the Transmission of Benincasa Mosaic by *Myzus Persicae*

By

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Introduction

White-gourd, *Benincasa hispida* (THUNB.) COGN., is commonly grown as a vegetable crop during summer and rainy seasons throughout India. Mosaic of *Benincasa* caused by a strain of watermelon mosaic virus (SINGH & BHARGAVA 1962) is responsible for considerable loss in the yield of this important crop. Comparative transmission with prevalent aphids showed that watermelon mosaic virus (WMV) was transmitted most efficiently by *Myzus persicae* SULZ during its migration. With a view of evolving control measures, detailed investigations were undertaken to study the virus vector relationship of WMV to its vector *Myzus persicae* and the results are reported in the present paper. This is based on a thesis submitted by the author for the degree of Ph. D., University of Gorakhpur, Gorakhpur U. P., India.

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Materials and Methods

The virus (WMV) culture was maintained in *Cucurbita pepo* L. var. *Caserta* in a insect-proof chamber. Transmission tests with aphids were made from recently infected plants with systemic symptoms as virus source. Test plants were used at cotyledonary leaf stage. Condition of the insectary and the methods of culturing and handling the aphids were the same as those described by WATSON 1936, 1938. Virus free colonies of aphids, raised on the radish plants, were either transferred directly to the source of virus or after being given a preliminary fasting period. During the fasting

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period they were placed in petri-dishes covered with cellophane. When a short infection period was given, a hand-lens was used to observe the aphids when they took up a feeding position and the time was recorded by means of stop watch. The viruliferous aphids after specified treatments were placed on the test plants with the aid of a camel-hair-brush. In case of longer feeding periods, the test plants were covered with glass chimneys with their top covered with muslin cloth. After the aphids fed for the required period, they were removed from the test plants and a solution of 0.03% Folidol E 605 was sprayed. These plants were kept for one month under observation.

Experimental Results

1. Effect of preliminary fasting and infection feeding time on the transmission

Virus free aphids were starved for 0 hr., 1 hr. and 4 hrs. and each was given 2 mts., 15 mts. and 4 hrs. infection feeding time. Ten similarly treated aphids were transferred to each plant and were allowed to remain there overnight.

Table 1

Transmission of WMV by *M. persicae* with varying preliminary fasting and infection feeding time on an average of ten plants

Preliminary fasting time	Infection feeding time			Total
	2 mts.	15 mts.	4 hrs.	
0 hr.	2	3	3	8
1 hr.	5	6	4	15
4 hrs.	10	7	5	22
Total	17	16	12	—

From the observation (Table 1) it seems that preliminary fasting of the aphids increased the efficiency only when short infection time of 2 mts. was given. When the infection feeding time was prolonged, the increase in fasting time decreased the percentage of infection. Maximum infection was obtained with 4 hrs. starvation and 2 mts. feeding time. In the absence of preliminary fasting, the infection percentage slightly increased with increase in infection feeding time.

2. Effect of the number of aphids on the percentage of transmission

Experiments were performed to determine the relation between the number of aphids used and amount of infection obtained. The following treatments were given: Preliminary fasting time = 4 hrs., Infection feeding time = 2 mts., Inoculation feeding time = 24 hrs.

Table 2
Infection produced by different number of aphids

Number of aphids	Systemic infection out of ten plants
1	1
2	3
4	4
6	5
8	8
10	10
12	10

The data in Table 2 indicate, that the percentage of infection increases with subsequent increase in the number of aphids per plant upto certain limit, and that a single aphid can produce infection.

3. Minimum feeding time required to produce infection

To determine the minimum feeding time necessary for an aphid to produce infection, an experiment with the following treatments was performed: Preliminary fasting time = 4 hrs., Infection feeding time = 2 mts., Inoculation feeding time = 1, 2, 5, 10, 15 and 30 mts., Number of aphids per plant = 10.

Table 3
Infection obtained with different inoculation feeding periods

Time on test plants (in minuts)	Systemic infection out of ten plants
1	4
2	10
5	10
10	10
15	10
30	10

From the above results it is clear that the infection can be obtained by a feeding time of even one minute. The infection was maximum at 2 mts. inoculation feeding time and any further increase in the inoculation feeding time does not effect the infection much.

4. Effect of fasting after infection feeding on the transmission

An experiment was designed to find out the maximum time an aphid would remain infective after leaving the infected plant. The following treatments were given: Preliminary fasting time = 4 hrs., Infection feeding time = 2 mts., Post-infection starvation time = 0, 5, 15, 30, 60 mts., 2, 6 and 24 hrs., Inoculation feeding time = 24 hrs., Number of aphids per plant = 10.

After the aphids had fed on an infected leaf for 2 mts., they were put in petridishes covered with cellophane and were starved for given periods before being placed on the test plants. The results are given in the following table.

Table 4

Effect of fasting after infection feeding on transmission of WMV

Post-infection fasting time	Number of plants infected out of 10 plants inoculated
0 mt.	10
5 mts.	8
15 mts.	6
30 mts.	3
1 hrs.	2
2 hrs.	1
6 hrs.	0
24 hrs.	0

These results show that the infectivity of the vector was lost after 2 hrs. starvation and the capacity to produce infection decreased with the increase in starvation time.

5. Effect of transferring aphids to a series of healthy plants after infection

An experiment was performed to find out the number of plants which an individual aphid could infect by successive feedings. Twelve aphids were given an infection feeding after preliminary starvation. Each one of these aphids was fed successively on test plants for 5 mts. and 24 hrs. on five plants. The following treatments were given: Preliminary fasting time = 4 hrs., Infection feeding time = 2 mts., Number of aphids per plant = 1, Feeding time on each of the first four healthy plants = 5 mts., Feeding time on the fifth healthy plant = 24 hrs.

The results (Table 5) show that of the 12 aphids used, 6 caused infection to the first plant. The 3rd and 5th aphids could also infect the 2nd plant but not the 3rd, while the 10th aphid infected the 1st and 3rd plants only. It appears that the aphids cease to be infective very soon when fed on test plants.

Table 5

Number of plants infected in succession by each aphid. + Indicates infection, — Indicates no infection

Plants	aphids											
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
1st	+	—	+	—	+	—	—	+	—	+	+	—
2nd	—	—	+	—	+	—	—	—	—	—	—	—
3rd	—	—	—	—	—	—	—	—	—	+	—	—
4th	—	—	—	—	—	—	—	—	—	—	—	—
5th	—	—	—	—	—	—	—	—	—	—	—	—

6. Comparative efficiency of alate (winged) and apterous (non-winged) forms of aphids

Ten winged and non-winged forms of *Myzus persicae* were separately starved for four hours before feeding on an infected leaf for 2 mts. After feeding period they were transferred to healthy test plants and allowed to feed on them for 24 hrs. The results obtained are recorded in Table 6.

Table 6

Comparative efficiency of alate and apterous forms of aphids

Forms of aphids used	No. of plants infected out of 10 plants inoculated
Alate	10
Apterous	10

The results indicate that both the forms of the aphids are equally efficient in transmitting the WMV.

Discussion

The exact mechanism of aphid transmission of plant viruses is still not fully understood. Results under discussion show that the aphids acquire and transmit WMV soon and lose the infectivity rapidly. This relationship was termed as non-persistent or mechanical (Watson & Roberts 1939). Watson 1960 suggested the terms 'external' to those viruses which are born on the stylet tip and do not persist after moulting and 'internal' to those which pass through the gut-wall and return back to salivary gland and persist even after moulting. Kennedy & al. 1962 preferred the term 'stylet born' and 'circulating' for 'external' and 'internal' viruses respectively. On the basis of above definition the Benincasa mosaic strain of watermelon mosaic virus falls in to the category of 'stylet born' virus.

The preliminary fasting time given to the *Myzus persicae* increased the efficiency of the aphids only when short feeding periods were given.

These findings are similar to those of WATSON 1936, SYLVESTER 1949, 1950, BHARGAVA 1951, BRADLEY 1952 and MILLER 1952. In the present investigation the results obtained can not be interpreted with certainty but they are compatible with the inactivator hypothesis put forth by WATSON & ROBERTS 1939 and 1940. According to this hypothesis during the process of transmission from one plant to another the virus comes into contact with some substance which partially or wholly destroys its infectivity. It is presumed that this substance either ceases to be produced while the insect is starving or is produced at a much lower rate. So that, when an aphid is given a short feeding time (2 mts.) after starvation, the amount of inactivator is not sufficient to inactivate the virus taken in, hence more infections are produced. But with prolonged infection feeding time (4 hrs.) the effect of fasting disappears and the amount of inactivator increases resulting in the inactivation of a greater amount of virus. On the other hand, if the aphids are not starved, the amount of inactivator already present in insect is at its maximum and inactivates a large proportion of the virus taken in, during the short infection feeding period (2 mts.). Although a single aphid can produce infection, the percentage of infection of WMV increases with subsequent increase in the number of *M. persicae* per plant upto a certain limit. Similar results have been obtained by several workers (SEVERIN & FREITAG 1938, SEVERIN & TOMPKIN 1948, 1950 and BHARGAVA 1951). The results confirm the views of WATSON 1936 and STOREY 1939 that the infection produced by the insect vectors are local and independent and that an infection does not result from the cumulative effect of several independent sub-minimal doses of virus.

The fasting after infection feeding has shown that infectivity of the vector was lost after two hours starvation and the capacity to produce infection decreases with the increase in starvation time. Such decrease of infection is also reported by WATSON & ROBERTS 1939, 1940 and BHARGAVA 1951.

Observations in the serial transfer of aphids to healthy test plants indicate that the aphid ceases to be infective very soon while feeding on test plants. It is evident, therefore, that the virus causing Benincasa mosaic is typically of non-persistent type.

The results of the experiment on the comparative efficiency of alate and apterous forms of the aphids indicate that both the forms are equally efficient in transmitting the WMV. Similar results were also obtained with other non-persistent viruses.

Summary

Experiments conducted on the relationship of the watermelon mosaic virus and its vector, *Myzus persicae* SULZ indicate that the virus was transmitted apparently in typical 'non-persistent' manner and was 'stylet born'. A single aphid was capable of transmitting the virus. Preliminary

fasting of the aphids increased the efficiency of transmission. Maximum efficiency was attained with 4 hrs. fasting and 2 mts. infection feeding. The aphids could transmit the WMV to healthy test plants even with one minute infection feeding. The infectivity of the vector was lost after two hours starvation and the percentage of infection decreased with the increase in starvation time. In serial transfers the aphids cease to be infective very soon while feeding on the test plants. Both alate and apterous forms of the aphids were found to be equally efficient in transmitting the virus.

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