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Potyvirus transmission is not increased by pre-acquisition fasting of aphids reared on artificial diet

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Aphids (*Myzus persicae*), fasted after removal from healthy rearing plants, transmitted tobacco etch potyvirus (TEV) more efficiently than unfasted aphids whether virus acquisition was from infected leaves or through membranes. There was no difference in uptake of ^{125}I-labelled TEV by fasted or unfasted aphids as measured by liquid scintillation counting. When aphids acquired ^{125}I-labelled TEV, label was retained in the stylets (as determined by autoradiographic light microscopy) by 51 % of 272 fasted aphids, as against 7·8% of 258 unfasted aphids. There was a close correlation between virus transmission by aphids and virion retention in stylets. The effect of pre-acquisition fasting disappeared when aphids reared on an artificial diet were used in virus transmission tests. The transmission rates obtained with such aphids were similar to the rates with fasted aphids reared on healthy plants. Our results support the hypothesis that fasting eliminates plant component(s) which interfere with the retention of virions in the food canal of aphid stylets.

Increased transmission as a result of fasting (also termed starving) *Myzus persicae* prior to allowing the aphids to acquire *Hyoscyamus* virus 3 (henbane mosaic potyvirus) was first reported by Watson (1938). Since then, there have been reports of the fasting effect with a variety of aphid vectors and non-persistently transmitted viruses (Day & Irzykiewicz, 1954; Kassanis, 1941; Powell, 1993; Swenson, 1960; Sylvester, 1954, 1955; Taylor & Robertson, 1974; Watson & Roberts, 1939).

The mechanism by which pre-acquisition fasting enhances transmission has yet to be explained satisfactorily. Non-persistently transmitted viruses have a non-circulative relationship with the vector, and various lines of evidence indicate that the virions retained in the food canal of the stylets (and perhaps other parts of the foregut) are those involved in the transmission process. Watson & Roberts (1939) proposed that the aphid transmission of plant viruses is reduced by a virus-inactivating substance(s) secreted by aphids during feeding and that secretion is arrested or reduced in fasting aphids, thus increasing transmission. There is no direct evidence to support this hypothesis although the saliva of aphids does contain virus-inactivating substances (Pirone, 1970).

Another hypothesis for the pre-acquisition fasting effect is that starvation alters aphid probing and/or feeding behaviour which in turn affects virus acquisition. Although behavioural differences between fasted and non-fasted aphids have been observed by several researchers using different virus–aphid combinations (Bradley, 1952; Day & Irzykiewicz, 1954; Nault & Gyrisco, 1966; Swenson, 1960; Taylor & Robertson, 1974), recent studies by Powell (1993) and Powell *et al.* (1995) using electronic monitoring showed that fasting does not affect the stylet punctures of cell membranes which are correlated with virus transmission, and concluded that the higher transmission efficiency of fasted aphids is caused by non-behavioural factor(s).

In recent studies comparing aphid-transmissible and non-transmissible variants of tobacco etch virus (TEV) Wang *et al.* (1996) found a close correlation between aphid transmission and virion retention in the stylets by using immunogold labelling and light microscopic autoradiography. In another study, mineral oil was found to reduce aphid transmission of TEV by interfering with the retention of virions in the stylets (Wang & Pirone, 1996). The evident importance of stylet retention suggested that pre-acquisition fasting might increase virus transmission by increasing virion retention in the stylets. In this study, the retention of ^{125}I-labelled TEV ingested by fasted and non-fasted aphids was compared to test this hypothesis. The effect of fasting *per se* on transmission efficiency was also determined.

The highly aphid-transmissible strain of TEV, methods for virion purification, quantification and radio-iodination, the source and quantification of the helper component (HC) which was used in all experiments with purified TEV, and procedures for liquid scintillation counting and autoradiographic detection of label in the stylets were as described previously (Wang *et al.*, 1996).

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Table 1. Comparative transmission of TEV acquired from infected plants or as purified virions by fasted or non-fasted *Myzus persicae*

Purified virions were acquired from a virion–HC mixture; virion concentration 200 μg/ml.

	Virus source			
	Infected plants		Purified virions*	
	Transmission	%	Transmission	%
Fasted aphids				
Experiment 1	19/30*	63·3	23/30	76·7
Experiment 2	17/30	56.7	18/30	60.0
Total ...	**36/60**	**60·0**	**41/60**	**68·3**
Non-fasted aphids				
Experiment 1	2/30	6·7	5/30	16·7
Experiment 2	3/30	10·0	4/30	13·3
Total ...	**5/30**	**8·3**	**9/60**	**15·0**

* Numerator, no. of infected test plants; denominator, total no. of test plants. A single aphid was placed on each test plant.

Myzus persicae was reared on *Brassica perviridis* cv. Tendergreen under previously described conditions (Raccah & Pirone, 1984). Only fourth or fifth instar apterae were used in the experiments. Non-fasted aphids were those in the feeding position on the rearing host at the time of removal with a camel's hair brush. They were used immediately for virus acquisition. Aphids that had not initiated probing within 3 min after removal from the rearing plant were discarded, to preclude fasting effects which can occur within 5 min (Swenson, 1960). Fasted aphids were those kept in glass vials for 2–3 h after removal from the rearing plant. Aphids were given a 30 s acquisition feed on an infected leaf or through a Parafilm membrane. Only aphids observed, under a dissecting microscope, to probe for 30 s were used in the experiments.

For transmission tests, aphids were placed on tobacco (*Nicotiana tabacum* L. cv. Kentucky 14) seedlings (one aphid per plant). Aphids were allowed to remain on the test plants overnight (14–18 h) after which plants were sprayed with an insecticide and placed in a growth room for symptom development. Aphids used for label detection were plunged into liquid nitrogen immediately after removal from the feeding membrane and processed as previously described (Wang *et al.*, 1996).

Fasted aphids transmitted TEV more efficiently than plant-reared non-fasted aphids, and similar results were obtained using either infected plants or purified virions plus HC as the source inoculum (Table 1). Some behavioural differences were observed between fasted aphids and non-fasted aphids. When placed on a piece of leaf or a feeding membrane, fasted aphids spent little time wandering around and usually made no, or only a brief, exploration of the surface with the labium before making a probe, whereas non-fasted aphids usually spent some time wandering about and then spent a few seconds exploring the surface with the labium before probing. These results are in agreement with earlier reports using different virus–aphid combinations (Bradley, 1952; Day & Irzykiewicz, 1954; Lopez-Abella *et al.*, 1988; Nault & Gyrisco, 1966; Powell, 1993; Swenson, 1960; Taylor & Robertson, 1974).

To test whether the differences in transmission efficiency could be a reflection of differential uptake of TEV, fasted and non-fasted aphids were allowed to acquire for 30 s from solutions containing 400 μg/ml ^{125}I-labelled virions (specific activity 3790–4970 d.p.m./ng virus) mixed with HC. Groups of 20 entire aphids were processed for liquid scintillation counting. There was no significant ($P = 0·01$) difference in total labelled virion uptake between groups of 20 fasted ($125·7 \pm 6·4$ c.p.m.) and 20 non-fasted aphids ($122·1 \pm 7·1$ c.p.m.), [background (20 aphids with unlabelled virions) $45·2 \pm 2·9$ c.p.m.]. This indicated that similar amounts of virions were ingested by fasted and non-fasted aphids during the 30 s acquisition probing period.

When aphids acquired ^{125}I-labelled TEV from solutions containing 200 μg/ml virions in the presence of HC, 51·1% of 272 fasted aphids retained label in the stylets whereas only 7·8% of 258 non-fasted aphids retained label in the stylets, as determined by autoradiography. There was a close correlation between the number of aphids that transmitted TEV and the number that retained label in the stylets (Table 2). There was no apparent difference in label distribution within the stylets of fasted and non-fasted aphids (data not shown).

Table 2. Effect of preacquisition fasting on transmission of purified (TEV) and retention of ^{125}I-labelled TEV in stylets of *Myzus persicae*

Purified virions were acquired from a virion–HC mixture; virion concentration 200 μg/ml.

	Transmission	%	Retention	%
Fasted aphids				
Experiment 1	15/40*	37·5	68/127†	53·5
Experiment 2	19/40	47·5	71/145	48·9
Total ...	**34/80**	**42·5**	**139/272**	**51·1**
Non-fasted aphids				
Experiment 1	3/40	7·5	12/136	8·6
Experiment 2	2/40	5·0	8/122	6·6
Total ...	**5/80**	**6·2**	**20/258**	**7·8**

* Numerator, no. of infected test plants; denominator, total no. of test plants. A single aphid was placed on each test plant.
† Numerator, no. of stylets positive by autoradiography; denominator, total no. of stylets examined.

Table 3. Transmission of TEV by fasted and non-fasted *Myzus persicae reared on temporary artificial diet*

Purified virions were acquired from a virion–HC mixture; virion concentration 200 μg/ml.

	Fasted		Non-fasted	
Virus source	Transmission	%	Transmission	%
Infected tobacco				
Aphids from diet	60/100*	60·0	59/100	59·0
Aphids from rearing plant	44/80	55·0	10/80	12·5
Purified virions				
Aphids from diet	65/100	65·0	63/100	63·0
Aphids from rearing plant	49/70	70·0	11/70	15·7

* Totals of two experiments. Numerator, no. of plants infected; denominator, total no. of test plants. A single aphid was placed on each test plant.

To distinguish between the effect of fasting *per se* and potential effects of residual plant constituents in the food canal on transmission, aphids were reared on an artificial diet. The temporary artificial diet (TA-diet) consisted of 10% sucrose, 0·5% BSA and 1% NaCl in deionized water. Aphids fed and sustained themselves well on this diet for 2 days. Aphids were removed from a rearing plant and placed in a feeding chamber containing the TA-diet solution enclosed between two layers of stretched Parafilm for overnight feeding (16–20 h). At the end of this period aphids that were microscopically observed to be feeding on the diet were removed and then divided into two groups. One group of aphids was transferred to glass vials for 2–3 h pre-acquisition fasting. The other group of non-fasted aphids was used directly for transmission tests.

The effect of pre-acquisition fasting disappeared when the aphids reared on the TA-diet were used in virus transmission tests. Similar results were obtained when either TEV-infected tobacco leaves or purified TEV plus HC was used as virus source (Table 3). In comparison, there was a large difference in virus transmission rate between fasted and non-fasted aphids when aphids were taken from healthy rearing plants. Moreover, the virus transmission rates obtained with aphids reared on TA-diet were close to the transmission rates with fasted aphids reared on healthy plants.

Taken together, our data show that the effect of pre-acquisition fasting is to allow increased retention of virions in the food canal which in turn results in increased transmission. We hypothesize that non-fasted aphids have substances in the food canal that interfere with virion retention. During the fasting period these materials presumably are egested or ingested clearing the food canal and thus allowing increased retention. Based upon the lack of a fasting effect with aphids reared on artificial diet, the interfering substances seem likely to be of plant origin, rather than aphid secretions as suggested by Watson & Roberts (1939).

Since the fasting effect has been observed with aphids other than *M. persicae* and for non-persistently transmitted viruses other than potyviruses, identification of the material(s) that interfere with retention could be the basis of control strategies that might be broadly effective in preventing plant-to-plant spread of viruses with this transmission characteristic.

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