Tobacco Mosaic and Brome Mosaic Viruses in Aphids

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ABSTRACT

The movement, distribution, and biological activity of two plant viruses in aphids was studied. Upon entering the ventriculus of *Myzus persicae* or *Acyrthosiphon pisum*, tobacco mosaic virus (TMV) was enclosed in "food balls" which disintegrated during passage, releasing free virus into the gut lumen. A few TMV particles were seen in association with the microvilli, but no clear evidence for phagocytosis of virus was obtained. No TMV was seen in dissected intestines, hindguts, or in the hemolymph of aphids. With brome mosaic virus (BMV), viruslike particles were observed in the gut of *Rhopalosiphum padi*. However, their identity as BMV particles could not be established except by the fact that they were much less numerous in control aphids. Both infectious TMV and BMV were recovered from the ventriculus and honeydew of aphids, but not from the hemolymph or from aphid macerates. Infectivity of TMV was reduced by injection into the hemolymph of aphids. The total concentration of TMV in *M. persicae* was estimated to be 6×10^5 particles/aphid.

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Additional key words: inefficient vectors.

Aphids and other sucking arthropods can ingest tobacco mosaic virus (TMV) either by feeding on infected plants or through membranes on purified virus (1, 2, 3, 4, 5, 6, 8). However, little is known at present about the distribution and movement of TMV and other viruses in aphids which do not serve as efficient vectors of these viruses. We have investigated this matter (11), and now present a more detailed report. Two recent studies have yielded comparable results (2, 3).

MATERIALS AND METHODS.–*General*.–Viruses (TMV and brome mosaic virus [BMV]) and test plants were as described before except that we also used a legume strain of TMV maintained in peas (5). *Myzus persicae* Sulz., *Acyrthosiphon pisum* H., and *Rhopalosiphum padi* L. were reared on *Nicotiana tabacum Xanthi-nc, Pisum sativum,* and *Hordeum vulgare* Keystone, respectively.

Electron microscopy.–Aphids were fed on infected plants (8-12 days postinoculation) or on purified virus (usually 1 mg/ml) in 15% sucrose. The alimentary tract of adult aphids was dissected and

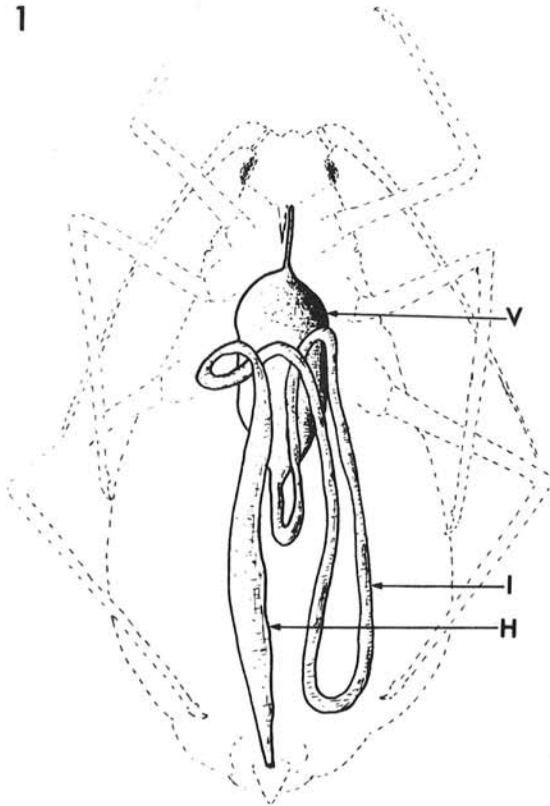

Fig. 1. Parts of the alimentary tract of an aphid (*Myzus persicae*) used for virus assay. V = Ventriculus or midgut. I = Intestine. H = Hindgut.

divided into ventriculus, intestine, and hindgut (Fig. 1). Organs were fixed in phosphate-buffered glutaraldehyde at pH 7.2, and postfixed in osmium tetroxide in the same buffer. In some experiments, fixation was in osmic acid at pH 7.2 or 4.0. Postsection staining was in uranyl acetate and lead citrate. A Zeiss EM 9s electron microscope was used for viewing the sections.

Bioassays.—Organs prepared as described above were collected in batches of 20, ground in 10 μliters of distilled water with traces of Carborundum, and inoculated to local lesion testers. Honeydew was collected by placing a glass slide underneath a leaf infested with aphids; 50 droplets were dissolved in 10 μliters of distilled water and inoculated to test leaves. Hemolymph (blood) was obtained by inserting a capillary needle drawn with a Leitz needle drawing apparatus into the coxa of a foreleg. Blood (0.01 μliters) was released into 10 μliters of buffer on the leaf surface, and the droplet was inoculated after 0.05 μliters of hemolymph had been delivered. A total of 0.05 μliters of blood in 10 μliters of buffer was inoculated to single test leaves.

In other experiments, aphids were injected with 0.01 μliters of 0.1 mg/ml TMV in saline, then bled or macerated, and the hemolymph or macerate was used for inoculation as described above.

Particle counting.—Twenty stomachs of aphids which had fed on a virus source were macerated in 10 μliters of distilled water and layered to a height of 0.4 cm on a D_2O gradient of a Strohmaier centrifugation cell (10). Separation was for 30 min at 40,000 *g* and for 90 min at 45,000 *g*. Grids from position c were used for particle counts, and five random photographs of each grid were taken.

RESULTS.—*Electron microscopy.*—In the anterior parts of the ventriculus of *M. persicae* (Fig. 1), TMV-like particles were most abundant in food balls (Fig. 2). In the posterior parts, food balls disintegrated and released TMV into the gut lumen (Fig. 3). No virus was seen in the intestine or hindgut (Fig. 1) dissected from aphids. Attempts to demonstrate entry of TMV into the gut cells were inconclusive. Although TMV-like rods were sometimes seen in close proximity to the microvilli or within phagocytotic vesicles, they were not distinct enough to be clearly identifiable as TMV (Fig. 4). Changing the method of fixation (osmium tetroxide at pH 4.0), as recently reported (3), did not improve our results. No TMV-like particles were seen in control aphids. The average concentration of TMV within an adult *M. persicae* was estimated to be 6 X 10^5 particles. Counts were based on 60 aphids, processed in three tests with four counts each. In some experiments we observed a legume strain of TMV in *A. pisum*. There was no difference from the pattern described above.

In *R. padi* feeding on BMV-infected plants or on purified virus, BMV-like particles were seen in the lumen of the ventriculus. However, their identity as BMV was difficult to establish except for the fact that spheres of similar size in control aphids were much less common. Sometimes BMV-like particles aggregated on the surface of unknown spherical bodies (Fig. 5).

Bioassays.—Infectious virus could be recovered from dissected alimentary tracts and honeydew, but not from the hemolymph or macerates of aphids (Table 1). These data also indicate that more virus could be recovered from the ventriculi of aphids having fed on purified virus (1 mg/ml) than on infected leaves.

Since no TMV was obtained from the hemolymph of aphids that had fed on a virus source, we tried to recover virus injected into the blood. Injection was into the ventral abdomen, bleeding from the coxa 2-4 hr after injection. As controls, dead aphids, killed by deep-freezing at −20 C, were treated similarly. Hemolymph (0.05 μliters diluted in 10 μliters of buffer) was inoculated to *Xanthi* tobacco leaves. Twenty lesions developed on 21 test leaves inoculated with the blood of live aphids; 55 lesions were

Fig. 2-3. 2) Food balls (FB) in anterior end of ventriculus of *Myzus persicae*. Scale = 1 μ. Inset: Tobacco mosaic virus (TMV) in food ball. Scale = 0.5 μ. 3) TMV in lumen of the posterior ventriculus. Scale = 0.5 μ.

2

FB

3

4

TMV

MV

5

BMV

TABLE 1. Infectivity of tobacco mosaic virus (TMV) and brome mosaic virus (BMV) in aphids

Virus/aphid	Ventriculus[a]		Honeydew[b]	Hemolymph[c]		Whole aphids[d]	
	Plant feeding	Membrane feeding	Plant feeding	Plant feeding	Membrane feeding	Injected	Control
	1 mg/ml				*1 mg/ml*	*1 mg/ml*	
Tobacco mosaic *Myzus persicae*	8/7	27/6	18/8	0/6	0/6	2/70	68/70
Brome mosaic *Rhopalosiphum padi*	4/9		27/6	0/6			

[a] Twenty ventriculi of aphids fed on infected plants or through membranes on 1 mg/ml TMV were ground in 10 μliters distilled water, and inoculated to single test leaves. Total number of lesions produced per number of test leaves inoculated.

[b] Fifty honeydew droplets from aphids feeding on infected plants were dissolved in 10 μliters distilled water and inoculated to single test leaves. Number of lesions produced per number of leaves inoculated.

[c] 0.01 μliter hemolymph was withdrawn from coxa of aphids having fed on infected plants or through membranes on 1 mg/ml TMV, and 0.05 μliters hemolymph diluted in 10 μliters phosphate buffer were inoculated to single test leaves. Number of lesions produced per number of leaves inoculated.

[d] Aphids were injected with 0.01 μliters of 1 mg/ml TMV, macerated 24 hr later in distilled water, and inoculated in batches of 10 to single test leaves. Number of lesions produced per number of leaves inoculated. Controls consisted of diluting 0.01 μliters of 1 mg/ml TMV in 0.2 μliters of saline (estimated to be the "wet" volume of a single aphid), and inoculating 2 μliters of this to single test leaves.

produced on 21 leaves by blood of dead aphids. Thus, live blood seems to reduce the infectivity of TMV or the number of infectious particles more than blood from dead aphids.

DISCUSSION.—Ample evidence has been presented that TMV is ingested by sucking arthropods, but little is known at present about the movement and location of the virus within the arthropod. While it is possible to trace TMV and other rod-shaped or bacilliform virus in an arthropod, polyhedral viruses such as BMV or barley yellow dwarf virus are more difficult to observe in the electron microscope because they are small, often poorly defined, and resemble nonvirus particles (7).

The first sign of TMV was noticed in the food balls of the anterior ventriculus. In a more posterior position, TMV dispersed and was seen in the lumen of midgut. This pattern is rather similar to the movement of TMV through the gut of *Tetranychus urticae* (5), except that aphids seem to ingest less virus. This perhaps is due to the different feeding habit of both arthropods: aphids feeding on the phloem; mites, on the epidermis.

Particular attention was given in this study to the passage of TMV through the gut wall. Although TMV-like rods were seen in close association with the microvilli, a distinct adsorption, attachment, or phagocytotic uptake by the microvillar membrane could not be demonstrated. It is unlikely that much TMV passes through the gut wall and into the hemolymph, because no virus could be recovered from the blood. Similar conclusions were reached in a recent study with ^{125}I TMV, where no virus was observed in the extraintestinal tissues, and an occasional intracellular label of the gut wall could not be associated with intact TMV (3). However, it has been demonstrated that rod-shaped insect viruses can be phagocytosed (12). Factors governing entry of plant or insect viruses in the gut cells are poorly understood at present, and call for more studies.

The passage of TMV from the ventriculus into the intestine and hindgut is difficult to follow. As no virus or food material was seen in these organs, their contents might have been voided prior to or after dissecting the insects. Similarly, Langenberg & Schroeder (3) were unable to observe TMV beyond the first ventriculus. However, we recovered viruses from the honeydew, indicating that at least some virus passes the alimentary tract undegraded. Pea enation mosaic virus, a circulative virus, has also been recovered from honeydew (9). These observations indicate that some plant viruses pass the digestive system regardless of their actual mode of transmission.

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Fig. 4-5. 4) Tobacco mosaic viruslike particles near the microvilli (MV) and in phagocytotic vesicle. Scale = 0.5 μ. **5)** Brome mosaic viruslike particles in lumen of the ventriculus of *Rhopalosiphum padi*. Note aggregation on spherical bodies. Scale = 0.5 μ.

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