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The Role of the Helper Virus in Aphid Transmission of Potato Aucuba Mosaic Virus and Potato Virus C

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SUMMARY

Potato aucuba mosaic virus and potato virus C were transmitted by the aphid *Myzus persicae*, not only from plants also infected with a helper virus, but also from plants infected with them alone, provided the aphids fed first on plants infected with the helper virus. Several different viruses acted as helpers but all are in the potato virus Y group. Helper viruses differed in the efficiency with which they aided potato aucuba mosaic virus and potato virus C, and some potato aucuba mosaic virus isolates were transmitted more frequently than others. With potato virus Y as helper, up to 30% of the aphids transmitted potato aucuba mosaic virus. Aphids were usually fed for brief periods on plants infected with the helper virus but aphids fed for 2 days also transmitted potato aucuba mosaic virus readily. Starving the aphids for 1 to 2 hr between their acquisition feeds on plants infected with helper and aided virus decreased but did not eliminate transmission.

The helper virus need not be infective; potato aucuba mosaic virus and potato virus C were transmitted as frequently when transmission of the helper virus was prevented by exposing the source leaf to u.v. radiation as when it was not. Virus was not transmitted by aphids fed through artificial membranes on extracts of leaves infected with potato virus Y, potato aucuba mosaic virus or a mixture of the two. However, potato aucuba mosaic virus was transmitted from extracts by aphids fed through membranes when the aphids had previously fed on a potato virus Y-infected leaf. Similarly, potato virus Y was transmitted from leaf extracts by aphids fed through membranes when the aphids had previously fed on a potato virus Y-infected leaf that had been irradiated with u.v. to prevent transmission of potato virus Y from this source.

Possible mechanisms for the transmission of the helper and aided viruses are discussed.

INTRODUCTION

A few plant viruses are transmitted by aphids from plants also infected with another (helper) virus, although aphids do not transmit them from plants in which they are present alone. Potato aucuba mosaic virus and potato virus C need such a helper virus to be transmitted. Kassanis & Govier (1971) showed that aphids could also transmit potato aucuba mosaic virus or potato virus C from plants infected with them alone, provided the aphids first fed on plants infected with the helper virus (potato virus Y). Only the helper virus was transmitted when the feeding order was reversed. Because aphids fed on plants infected only with potato aucuba mosaic virus did not transmit the virus to plants already infected with potato virus Y, and aphids fed on a plant infected with both viruses transmitted potato

aucuba mosaic virus to *Datura stramonium*, which is immune to potato virus Y, we concluded that the helper virus alters conditions in the aphid in some way so that it may then acquire potato aucuba mosaic virus.

This paper describes further work on factors affecting the transmission of potato aucuba mosaic virus and potato virus C, and attempts to explain the way in which helper viruses aid aphid transmission.

METHODS

Virus isolates. Potato virus C (PVC) and potato virus Y (PVY) were obtained from Dr T. M. W. Davidson, potato virus A from Dr D. E. Richardson, tobacco severe etch virus from Dr M. Hollings and bean yellow mosaic virus, beet mosaic virus, cocksfoot streak virus, henbane mosaic virus, pepper veinal mottle virus (Brunt & Kenten, 1971) and potato aucuba mosaic virus (PAMV) from cultures maintained at Rothamsted. Bean yellow mosaic virus was multiplied in broad bean (*Vicia faba* L.), beet mosaic virus in beet (*Beta vulgaris* L.), cocksfoot streak virus in cocksfoot (*Dactylis glomerata* L.), and all other viruses in tobacco (*Nicotiana tabacum* L. var. Xanthi-nc).

Aphid transmissions. All aphid transmissions were done with *Myzus persicae* Sulz. multiplied on radish or turnip plants. Except where stated in the tables, 20 to 30 aphids were used to transmit PAMV to each test plant and 10 to transmit PVC. Aphids that had been starved for 2 to 3 hr were fed, usually for 1 to 2 min., on the helper virus source, transferred to the aided virus source for a similar period, and then left on the test plants for at least an hr before being killed by fumigating with nicotine. Some experiments tested longer acquisition feeding periods and aphids were routinely allowed 30 min. to acquire virus through membranes.

Membrane feeding. Aphids were caged in a glass tube, 3 cm. long and 2 cm. diameter, covered on the outside with black plastic tape. One open end of the tube stood on a flat surface and the other was capped with a stretched membrane (Parafilm 'M'). One or two drops of the test extract, containing 10% (w/v) sucrose, and a spacer ring cut from polythene sheet were placed on the membrane surface and covered with a glass cover slip. The spacer ring increased the depth of liquid between the cover slip and the membrane.

Test plants. Usually, 6 to 10 plants were used for each treatment. PAMV was transmitted to pepper (*Capsicum annuum* L. cv. Long Red), in which the virus causes necrotic lesions followed by systemic necrosis. PVC was transmitted to tobacco plants (*Nicotiana tabacum* var. Xanthi-nc), which were tested 2 weeks later for the presence of PVC by inoculating sap to potato plants, variety Majestic. PVC causes discrete, black, necrotic local lesions, readily distinguished from the brown lesions caused by PVY that also appear a few days later.

Irradiations. Virus source leaves were irradiated by exposing each surface in turn for 4 min. to u.v. from a 'Hanovia' low-pressure mercury lamp with a filter to eliminate radiation of wavelength shorter than 240 nm., so that most of the radiation was of the wavelength 253·7 nm. The intensity of radiation at the leaf surface 20 cm. from the lamp was about 6×10^{-4} joules/cm.^{2}/sec. Half of each leaf was covered with aluminium foil to provide an unirradiated control.

RESULTS

Helper viruses for PAMV and PVC

Table 1 shows that several different viruses helped the transmission of PAMV, and that some helpers were more efficient than others. Of five viruses that were helpers for PAMV and were tested for their ability to help PVC, only three did so, and of these beet mosaic

virus helped PVC much less efficiently than it did PAMV. Kassanis (1961) failed to transmit PAMV by aphids from plants also infected with tobacco severe etch virus. By using many more test plants, we now find that tobacco severe etch virus is an inefficient helper for PAMV, both when aphids are fed on the two sources successively (Table 1) and when the two viruses are present in the same plant (3/28 successful transmissions). Some of the viruses that did not help PAMV in our tests (bottom of Table 1) may also be inefficient helpers. Some viruses may help aphids to acquire a virus, not otherwise acquired, that then fails to infect test plants because the helper virus competes with it at the site of inoculation.

Table 1. *Aphid transmission of potato aucuba mosaic virus (PAMV) and potato virus C (PVC) assisted by different helper viruses*

Helper virus	PAMV	PVC
Potato virus Y	60/69*	14/23
Potato virus A	14/33	9/28
Beet mosaic virus	4/11	2/35
Tobacco severe etch virus	2/48	0/8
Bean yellow mosaic virus	12/17	—
Cocksfoot streak virus	7/18	—
Pepper veinal mottle virus	12/22	—
Henbane mosaic virus		
Isolate 1	2/11	—
Isolate 2	29/46	0/8
Isolate 2 (from *D. stramonium*)	6/10	—

* Number of plants infected/number tested.
— Not tested.
PAMV was not helped by PVC (0/11)*, turnip mosaic virus (0/5), lettuce mosaic virus (0/12), carnation latent virus (0/6), chrysanthemum virus B (0/5), potato virus X (0/5), sugar beet yellows virus (0/6), alfalfa mosaic virus (0/5), cucumber mosaic virus (0/11) or tobacco mosaic virus (0/4), although in parallel tests frequent transmissions were obtained using PVY as helper.

Not only do viruses differ in their efficiency as helpers, but so also do different isolates of a virus. Isolate 2 of henbane mosaic virus was a more efficient helper for PAMV than was isolate 1. Govier & Woods (1971) found that this isolate 2 was contaminated with PVY, though in such small concentration that it was not detected by serology or electron microscopy. However, this isolate was not a more efficient helper for PAMV because of its contamination with PVY, because it was equally efficient after PVY was eliminated by passing the culture through *D. stramonium*.

Although viruses from several different groups were tried as helpers for PAMV, transmissions were obtained only with viruses of the PVY group. A possible exception is henbane mosaic virus, which was recently shown to differ in particle length from members of the PVY group (Lovisolo & Bartels, 1970; Govier & Woods, 1971). However, henbane mosaic virus resembles members of the PVY group in many other respects and its ability to act as a helper virus for PAMV should probably be regarded as one further property relating it to the PVY group.

Kassanis (1961) found that strains of PAMV differed in the readiness with which they were transmitted by aphids from plants also infected with PVY. Early in our work the frequency of transmission diminished and only occasional pepper plants became infected, possibly because a mutant was favoured by serial mechanical transfer from systemically infected tobacco leaves. However, when leaves from the original culture that had been kept in the deep-freeze were used as the source of inoculum and aphid transmissions attempted from the inoculated leaves, transmissions were again frequent. Sap from one of the

few pepper plants infected with PAMV by aphids when transmission was infrequent was inoculated to tobacco plants; aphids transmitted PAMV from these plants as frequently as from the original culture, provided the aphids were fed on inoculated leaves (Table 2). It seems that one aphid transfer reselected the aphid-transmitted component of the original isolate, and that it competed successfully with the mutant in inoculated leaves. Inoculated leaves were better virus sources for aphids than systemically infected leaves, but using them did not improve transmission from the mutated culture. We therefore maintained PAMV by transfer from inoculated leaves and used inoculated leaves as the virus source for aphids.

Table 2. *Aphid transmission of different potato aucuba mosaic virus (PAMV) isolates helped by potato virus Y*

	Experiment		
	1	2	3
PAMV isolate	Sys†	Sys	Loc
Original	5/5*	3/5	8/10
Mutant from tobacco	0/5	0/5	0/10
Tobacco mutant after one aphid transmission	—	1/5	8/10

* Number of plants infected/number tested.
† The PAMV source was either inoculated (Loc) or systemically infected (Sys) leaves.

Table 3. *Efficiency of transmission of potato aucuba mosaic virus by aphids when helped by potato virus Y*

No. of aphids/plant	Proportion of plants infected	Calculated percentage (P)* of aphids transmitting
3	14/21	31
10	12/14	18
30	4/4	—

* $P = 100\,(1-\sqrt[n]{q})$, where q is the proportion of test plants not infected when n aphids were placed on each.

Strains of cucumber mosaic virus differ in the readiness with which they are transmitted by *M. persicae*, the yellow strain only rarely and the green strain frequently. The frequency with which the yellow strain was transmitted was not increased in two tests involving a total of 20 test plants, in which aphids were first fed on plants infected with the green strain.

Efficiency of transmission

Although in most experiments 20 to 30 aphids were placed on each test plant, large numbers are not necessary to achieve transmission. Table 3 shows the results of experiments to find what proportion of aphids transmitted PAMV after they had first fed on PVY-infected leaves. The greater percentage of aphids that transmitted when fewer aphids were placed on each plant is probably explained by the fact that the aphids were more carefully handled when fewer were transferred. It is the opposite result to that expected if the effects of individual aphids were accumulative, and shows that, as with other aphid-transmitted viruses, each infection is an independent event.

In the field, aphids usually feed for longer than the 1 to 2 min. period given in most of our experiments. However, aphids that had fed for 2 days on PVY-infected leaves also readily transmitted PAMV, and experiments in which aphids were starved for different periods between acquisition feeds showed that the ability of aphids carrying PVY to acquire and transmit PAMV persisted at least as well when they had fed for a long period on the PVY

source as when they had fed briefly (Table 4). PAMV may well be transmitted in the field by aphids that feed first on a source of PVY and then on a source of PAMV because, in other experiments, some aphids transmitted PAMV when they were starved for 1 hr after feeding on the source of PVY before they were fed on the source of PAMV, and for a further hr before they were transferred to the test plants. In aphids fed for 2 days on the PVY source, the ability to acquire PAMV seemed to persist longer than the ability to transmit PVY (Table 4). A similar comparison was not made with aphids that had fed for a short period on the PVY source.

Table 4. *Transmission of potato aucuba mosaic virus (PAMV) by aphids† after a short or long feeding period on the potato virus Y (PVY) helper, and the effect of starving*

Time on PVY	Starved between feeds (min.)	Transmission of PAMV	Transmission of PVY
2 min.	0	6/6*	5/5
	30	2/6	—
	60	2/6	—
	120	1/6	—
2 days	0	3/6	3/5
	30	2/6	1/5
	60	3/6	0/5
	120	1/6	0/5

* No. of plants infected/number tested.
† Ten aphids were used for each test plant.

Table 5. *Aphid transmission of potato aucuba mosaic virus (PAMV) and potato virus C (PVC) assisted by irradiated potato virus Y (PVY)*

First feed (helper virus)	Second feed (aided virus)	Transmission of PVY†	Transmission of PAMV§	Transmission of PVC†
Control PVY leaf	PAMV	3/4*	17/21	—
Irradiated PVY leaf	PAMV	0/4	21/27	—
Control PVY leaf	PVC	6/8	—	4/8
Irradiated PVY leaf	PVC	0/8	—	4/8

* Number of plants infected/number tested.
† Ten aphids were used for each test plant.
§ Twenty aphids were used for each test plant.

Irradiated helper virus

If the ability to acquire PAMV persisted in aphids after they had lost the ability to transmit PVY, it seemed possible that the helper virus need not be infective to enable the aphid to acquire PAMV, or that the helping agent was a component of PVY-infected cells other than the virus itself. So, aphids that had been fed on PVY-infected leaves, previously irradiated with u.v., were tested for their ability to acquire and transmit PAMV or PVC (Table 5). Irradiating the leaves prevented aphids from transmitting PVY, but did not prevent them from acquiring and transmitting PAMV or PVC. Indeed, they transmitted as often as did aphids that had fed on unirradiated half leaves. Hence, aphids need not acquire infective helper virus to be able to acquire and transmit PAMV or PVC.

Acquisition through membranes

Pirone & Megahed (1966) failed to transmit turnip mosaic virus, a member of the PVY group, with aphids fed through membranes on purified virus solutions, although two other non-persistent viruses were readily transmitted in this way. Using similar techniques, we failed to transmit PVY from infective sap or purified virus solutions. If the virus particle or other agent that enables the aphid to transmit PAMV and PVC were acquired by aphids

Table 6. *Transmission of potato aucuba mosaic virus (PAMV) by aphids fed on plant extracts through membranes or on infected leaves, using potato virus Y (PVY) as helper*

First feed on PVY source	Second feed on PAMV source: Leaf	Second feed on PAMV source: Membrane
Leaf	15/18*	14/32
Membrane	1/20	0/8†

* Number of plants infected with PAMV/number tested.
† Aphids fed through membranes on sap from PVY- and PAMV-infected leaves mixed in equal volumes.

Table 7. *Transmission of potato virus Y (PVY) by aphids* fed on plant extracts through membranes, using irradiated PVY-infected leaves as helper*

First feed	Second feed	Transmission of PVY
Irradiated leaf	Membrane	11/30†
Irradiated leaf	—	2/30
—	Membrane	0/10

* Ten aphids were used for each test plant.
† Number of plants infected/number tested.

feeding through membranes, this technique would be a valuable method for characterizing the helper agent. For this reason, we fed aphids first either through a membrane or on a leaf source of PVY and then on a membrane or leaf source of PAMV (Table 6). Aphids first fed on a PVY-infected leaf acquired PAMV through membranes and transmitted it, although less readily than when they fed on a leaf infected with PAMV. By contrast, only one test plant was infected by aphids that had fed first on a PVY extract through a membrane and then on a PAMV-infected leaf. Aphids fed through membranes on an extract containing PVY and PAMV did not transmit PAMV.

Having transmitted PAMV from plant extracts by aphids that had first fed on a PVY-infected leaf, we thought aphids fed on plant extracts through membranes might acquire PVY if they were first fed on PVY-infected leaves previously irradiated to inactivate the virus. Table 7 shows that they did, and that PVY was transmitted about as frequently as PAMV was when similarly treated aphids transmitted virus acquired through membranes. In this experiment, irradiating the PVY-infected leaf did not entirely prevent aphids from acquiring infective virus but they transmitted much less frequently than aphids fed additionally on extracts containing PVY.

DISCUSSION

It is widely accepted that non-persistent transmission of plant viruses by aphids is essentially a mechanical process and that the transmissible virus is carried near the stylet tips (Pirone, 1969). We shall not be concerned here with where the virus is carried by the aphid and shall use the term mouth surface to include the pharynx and stylets. We shall confine

our discussion to considering what factors are responsible for an aphid transmitting some viruses and not others.

Pirone (1969) selects three hypotheses representative of the more recent attempts to explain the known specificities between vector and virus in non-persistent aphid transmission. We shall redefine these hypotheses with some modifications. They are: the *specific inactivation* hypothesis, in which transmission specificities are determined by differential susceptibility of viruses to salivary components; the *host cell resistance* hypothesis, in which inactivators in the saliva act on the host cell to render it resistant to infection by some viruses, which are therefore not transmitted by the aphid; and the *specific adsorption hypothesis*, in which differences in the structure of aphids' mouth surfaces and of viruses account for differential adsorption to, and elution from, the mouth surface.

Our results show that the assistance given to the aided virus by the helper virus does not depend on an interaction between the two viruses either in the source plant (because an aphid can acquire the two viruses from different plants), or in the test plant (because aphids previously fed on an irradiated source of the helper virus can acquire and transmit the aided virus). The helper, therefore, modifies conditions prevailing in the aphid during acquisition of the aided virus, but we cannot see how the presence of a helper virus during acquisition can protect the aided virus from the action of an inactivator in the aphid's saliva. For these reasons, the specific adsorption hypothesis seems to fit our results best. According to this hypothesis, the helper viruses are transmitted by *M. persicae* because they adsorb specifically to the aphid mouth surface, and PAMV and PVC are not transmitted because their surface structure does not permit them to be adsorbed. We can suggest two possible mechanisms that would enable PAMV and PVC to attach to the mouth surface of aphids previously fed on a source of helper virus. First, particles of the helper virus, already adsorbed to the mouth surface, may aggregate with and so retain particles of the aided virus. Secondly, particles of both the helper and aided viruses may adsorb to a component, produced in plant cells during multiplication of the helper virus, that is itself specifically adsorbed to the mouth surface. We shall call this cell component a helper agent.

End-to-end and side-to-side aggregation of particles of elongated plant viruses is well known but there are no reports of this occurring between particles of unrelated viruses. The forces responsible for aggregation are not fully understood, but for aggregation to occur between particles of PAMV and those of several different helper viruses, it cannot be a very specific process. It is therefore difficult to see why some isolates of henbane mosaic virus should be more efficient helpers than others that are similarly concentrated in plants, and why some isolates of PAMV should be more efficiently aided than others. However, adsorption of a virus particle to a helper agent produced during virus multiplication could be very specific, and small differences in the structure of virus proteins or in the helper agents produced by different helper viruses would account for the different efficiencies of helper viruses. Specific adsorption to similar helper agents produced in infected plant cells could also explain the vector-virus specificities of some of the many viruses that are transmitted in a non-persistent manner without the aid of a helper virus.

Many viruses of the PVY group induce the formation of pinwheels in cells of infected plants. Recently, A. J. Gibbs & R. H. Turner (personal communication) demonstrated pinwheels in cells of plants infected with PVC so, if these structures are concerned in aphid transmission of viruses, those found in PVC-infected cells must be non-functional. Their large size and the fact that they are attached to the endoplasmic reticulum excludes the possibility that pinwheels are the helper agents but they may in some way be concerned in their production.

Perhaps, our most important result is the transmission of PVY from virus extracts by aphids fed through membranes, only when they had first fed on PVY-infected leaves previously irradiated with u.v. to prevent transmission of PVY from this source. This suggests that, during extraction of the sap, either the virus surface or the helper agent is changed, so preventing adsorption to the mouth surface concerned with transmission. Extracting sap in a way that will prevent these changes will, we hope, shed some light on the mechanism of transmission of viruses in the PVY group.

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