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Polyamino Acid Induced Aphid Transmission of Plant Viruses

By T. P. PIRONE* AND B. KASSANIS
Rothamsted Experimental Station, Harpenden, Hertfordshire, AL5 2JQ

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SUMMARY

Aphids transmitted poly-L-ornithine (PLO)-treated tobacco mosaic virus (TMV) when given acquisition and inoculation access periods as brief as 30 s and 2 min, respectively; the ability to transmit was lost within 90 min. Aphids without claws were able to transmit the virus. Transmission thus seems similar to that of non-persistent viruses.

The ratio of virus to polyamino acid, as well as the KCl concentration, markedly affected transmission. Transmission was best from mixtures which contained 250 μg/ml TMV, 2·5 μg/ml PLO (mol. wt. 120000) and 0·6 M-KCl. A similar mixture favoured transmission when poly-L-lysine (mol. wt. 85000) was substituted for PLO, but with poly-L-lysine (mol. wt. 30000) it was necessary to decrease the KCl to 0·3 M to obtain transmission. Less KCl (0·08 to 0·24 M) also favoured aphid transmission of PLO-treated potato virus X and tobacco rattle virus. PLO-treated TMV ultracentrifuged in the presence of, and resuspended in, 0·6 M-KCl remained aphid transmissible while PLO-treated virus in 2 M-KCl, which favours greater dissociation of the virus-PLO complex, was transmissible neither before nor after sedimentation by ultracentrifuging, and resuspension in 0·6 M-KCl. These results show that transmissibility is not due to a permanent alteration of the virus by PLO and indicate that the formation of a TMV-PLO complex is required for transmission. Sequential acquisition experiments suggest that PLO may act by binding TMV to receptor sites in aphids. However, the possibility that PLO affects the infection process was not ruled out.

INTRODUCTION

Transmission of non-persistent or stylet-borne viruses by aphids has been considered to be essentially a mechanical process. However, recent work with potato virus Y (Govier & Kassanis, 1974) and cauliflower mosaic virus (Lung & Pirone, 1974) has shown that, when purified, these viruses can be transmitted only if aphids have simultaneous or prior access to a helper agent.

The report by Pirone & Shaw (1973) that tobacco mosaic virus (TMV) becomes aphid transmissible when mixed with poly-L-ornithine (PLO) suggested that more detailed studies on this system might be useful in gaining an insight into the mechanisms involved in aphid transmission of plant viruses. In this paper we describe conditions under which purified TMV is consistently transmitted with an efficiency comparable to that of purified non-persistent viruses. This made possible detailed studies on the transmission characteristics of PLO-treated TMV and other viruses. We also report the results of experiments designed to determine how PLO acts to make TMV aphid transmissible.

* Present address: Department of Plant Pathology, University of Kentucky, Lexington, Kentucky, 40506.

METHODS

Virus sources. Purified virus preparations were used in all experiments. Tobacco mosaic virus (TMV) was purified from *Nicotiana tabacum* L. cv. White Burley by differential centrifugation. Purified potato virus X (PVX) was supplied by W. S. Pierpoint; potato virus Y (PVY) by D. A. Govier; and tobacco rattle virus (TRV, strain CAM) by S. Kubo.

Aphids. Apterous *Myzus persicae* (Sulz.), reared on turnip or radish plants, were used in all experiments after fasting in glass vials for 3 to 5 h.

Chemicals. Poly-L-ornithine HBr, mol. wt. 120000; poly-L-lysine HBr, mol. wt. 85000. 30000 and 15000; spermidine trihydrochloride; and L-ornithine HCl were obtained from Sigma. The cationic polyacrylamides Magnafloc 140 and Magnafloc 292 (both mol. wt. 8×10^6) were a gift from Allied Colloids, Bradford.

Transmission tests. The standard mixture was that found by Pirone & Shaw (1973) to give optimum transmission, and contained 250 μg/ml TMV, 2·5 μg/ml PLO and 0·6 M-KCl. The mixture contained 20% sucrose as this stimulated aphid feeding to a greater degree than the 5% used by Pirone & Shaw. Except in the buffer experiments, all preparations were in 0·05 M-tris-HCl, pH 7·1. Aphids (15 to 30 per feeding chamber) were allowed 10 min access to this mixture in a membrane feeding chamber as described by Kassanis & Govier (1971). Aphids on the membrane at the end of this acquisition access period were removed and placed on *Nicotiana tabacum* cv. Xanthi-nc plants, on which they were allowed to remain for 2 to 3 h before fumigation with nicotine. These conditions were used in all experiments unless otherwise indicated.

RESULTS

Effect of pH and buffer

Several buffer systems were compared to try to improve aphid transmission compared with that reported by Pirone & Shaw (1973). In the first test, 0·05 M-tris-HCl buffers at three pH values were compared with an unbuffered system. The final pH and transmission (no. of lesions/80 aphids tested) was: pH 7·1, 12 lesions; pH 8·0, 5 lesions; pH 9·0, 2 lesions; unbuffered control (pH 6·5), 5 lesions. A second experiment compared tris-HCl and potassium phosphate buffers, both 0·05 M, pH 7·1. Transmission (no. of lesions/100 aphids tested) was 20 from the tris buffer and 7 from the phosphate buffer. All subsequent experiments were done with constituents suspended in 0·05 M-tris, pH 7·1.

Acquisition access period

Transmission by aphids which had 'probed' the parafilm membrane containing the standard TMV–PLO mixture for 30 s was compared with that by aphids given acquisition access periods of 10 or 20 min. Transmission was, respectively, 10, 15 and 11 lesions per 100 aphids for the 30 s, 10 min and 20 min periods.

Inoculation access period

Aphids placed on test plants after 10 min access to the standard TMV–PLO mixture were either removed manually after 2 or 10 min or fumigated after 2 h (control). Transmission was, respectively, 5, 6 and 12 lesions per 100 aphids.

Retention period

Aphids were given a 10 min acquisition access period on the standard TMV–PLO mixture and were then placed on test plants on which they were given a 90 min inoculation access

Table 1. *Effect of the relative concentrations of virus and poly-*L*-ornithine on aphid transmissibility of TMV*

Concentration (μg/ml)*		Aphid transmission†						
		Experiment						
TMV	Poly-L-ornithine	1	2	3	4	5	6	Mean
2500	250	—	—	3	—	—	—	3
	25	13	15	—	27	—	—	18
	2·5	1	—	—	4	—	—	2·5
250	25	—	—	1	—	—	—	1
	2·5	14	15	22	18	20	15	16
	0·25	—	—	—	7	—	—	7
25	25	—	0	—	—	—	—	0
	2·5	5	—	3	—	—	6	4·7
	0·25	—	2	—	11	—	5	6
2·5	2·5	0	—	—	—	—	—	0
	0·25	—	—	—	—	1	1	1
	0·025	—	—	—	—	0	0	0

* All preparations contained 0·6 M-KCl and 20 % sucrose in 0·05 M-tris-HCl buffer, pH 7·1.
† Number of local lesions per 100 aphids tested; —, not tested.

period. The aphids were then transferred, with a camel hair brush, to a second set of test plants, on which they were allowed to remain for 2 days. Eighteen lesions were produced by 100 aphids placed on the first set of test plants, while no lesions were produced on the second set.

Transmission by declawed aphids

Aphid transmission of purified TMV sprayed onto leaf surfaces is not due to probing or feeding (Pirone, 1972) but rather to clawing (Bradley & Harris, 1972). Pirone & Shaw (1973) produced several lines of indirect evidence that PLO-treated TMV acquired through membranes was not transmitted by clawing, but this has been questioned (K. F. Harris, personal communication) so direct evidence seemed desirable.

Aphids were anaesthetized with CO_2 and their claws removed with micro-dissecting scissors. The aphids were then placed in glass vials and fasted for 2 to 3 h, before their ability to transmit was tested by the standard procedure. Using 80 aphids per treatment, we obtained 7 lesions with declawed aphids; 12 with anaesthetized but not declawed aphids, and 20 with non-anaesthetized control aphids. Sufficient time for recovery from the effects of anaesthetization was found to be essential because aphids tested immediately after their apparent recovery from the effects of CO_2 rarely transmitted even if not declawed.

These results show conclusively that transmission occurs by a means other than clawing. Although the reduced transmission observed with declawed aphids might indicate that transmission occurs by claws as well as by mouthparts, a more probable explanation is that injury and manipulation has affected the aphids' behaviour. Declawed aphids were less mobile and made fewer probes on the test plants than did control aphids of either group. The fact that anaesthetization alone reduces transmission is further evidence that behaviour was affected.

TMV–PLO ratio

Pirone & Shaw (1973) tested only one concentration of TMV (250 μg/ml) and found that aphids transmitted TMV better from suspensions containing 2·5 μg/ml PLO than from

Table 2. *Effect of KCl concentration on the aphid transmissibility of poly-L-ornithine-treated TMV*

Concentration*			Aphid transmission† Experiment			
TMV (μg/ml)	Poly-L-ornithine (μg/ml)	KCl (M)	1	2	3	4
250	25	0·6	—	1	—	—
		1·2	—	0	—	—
250	2·5	0	3	—	—	5
		0·6	20	7	—	16
		1·2	—	0	—	—
250	0·5	0	—	—	—	0
	0·1	0	—	—	—	0
	0·02	0	—	—	—	0
2·5	0·25	0	—	—	1	—
		0·6	—	—	0	—
2·5	0·025	0	1	—	0	—
		0·6	0	—	0	—

* All preparations contained 20 % sucrose in 0·05 M-tris-HCl buffer, pH 7·1.
† Number of local lesions per 100 aphids tested; —, not tested.

those which contained 25 or 0·25 μg/ml PLO. Table 1 shows the results of a series of experiments testing whether this ratio was optimal over a range of TMV concentrations. The level of transmission from the standard solution (250 μg/ml TMV; 2·5 μg/ml PLO) was consistent for all experiments, so overall comparisons seem valid.

Frequencies of transmission comparable to standard solution were obtained with 2500 μg/ml TMV, provided that the PLO concentration was increased to 25 μg/ml. When the TMV–PLO ratio was greater or less than 100:1, decreased transmission resulted. The optimum TMV–PLO ratio at this virus concentration is thus similar to that for 250 μg/ml TMV.

At a TMV concentration of 25 μg/ml, transmission was somewhat lower than that of the standard solution, and similar levels of transmission were obtained with preparations in which the TMV–PLO ratios were 10:1 and 100:1. Transmissions rarely occurred from preparations which contained 2·5 μg/ml TMV.

Effect of KCl concentration

Aggregation and consequent loss of infectivity of TMV increases with increasing concentrations of polylysine or polyornithine (Stahmann *et al.* 1951; Pirone & Shaw, 1973). Such aggregation can be reduced by the presence of salt (Burger & Stahmann, 1951). This suggested that aphid transmissibility of TMV–PLO mixtures at ratios of 10:1 or less might be increased by KCl concentrations greater than 0·6 M. Conversely, the presence of 0·6 M-KCl in mixtures at ratios greater than 100:1 or in those which contained low concentrations of virus might cause excessive dissociation of the TMV–PLO complex and thus inhibit aphid transmission. Transmission might thus increase in the absence of KCl in the second case.

The results of a series of experiments at differing KCl concentrations are presented in Table 2. The data show that aphid transmission from suspensions containing 250 μg/ml TMV and 2·5 μg/ml PLO is reduced if KCl is omitted and show that increasing the KCl to 1·2 M in such preparations also reduces transmission. Increasing KCl to 1·2 M did not

increase transmission from preparations which contained excess PLO, nor did omitting KCl increase transmission from preparations which contained less than 2·5 μg/ml PLO. Further data on the effects of KCl are given in subsequent sections.

Effect of dissociation on transmissibility

To determine whether PLO alters TMV in such a way as to make it transmissible, or whether attachment of PLO to TMV is required for transmission, an experiment was done which compared the transmissibility of PLO-treated TMV under conditions favouring greater dissociation of PLO from the virus.

TMV (250 μg/ml) and PLO (2·5 μg/ml) were mixed in tris buffer. The suspension immediately became quite turbid, indicating the formation of a precipitate. The suspension was then divided and KCl added either to 0·6 M or 2·0 M. The suspension which contained 0·6 M-KCl remained very slightly turbid, while that in 2·0 M-KCl became clear. Transmission was 12 lesions per 100 aphids from an aliquant of the preparation containing 0·6 M-KCl, while no transmission occurred from that containing 2 M-KCl.

Each preparation was then centrifuged at high speed to pellet the TMV. Each pellet was rinsed and resuspended, by gentle stirring for 3 h, in tris buffer containing 0·6 M-KCl, and centrifuged at low speed to remove insoluble material. Spectrophotometric assay of the supernatant fluids showed that 80 to 90 % of the virus originally treated with 0·6 M-KCl did not resuspend, while all of the virus treated with 2·0 M-KCl resuspended. The TMV concentration of each preparation was adjusted to 250 μg/ml with 0·6 M-KCl in tris buffer, and sucrose was added to 20 %. Transmission averaged 8 lesions per 100 aphids from the preparations originally treated with 0·6 M-KCl, while again no transmission occurred from the preparation treated with 2 M-KCl. When 2·5 μg/ml PLO was added to a sample of the latter preparation, transmission was 8 lesions per 100 aphids.

These results indicate that some PLO remains bound to TMV centrifuged from, and resuspended in, 0·6 M-KCl, whereas 2 M-KCl dissociates the PLO from the virus, which then loses its transmissibility. They also indicate that PLO treatment does not induce transmissibility by permanently altering the virus because while the resuspended virus from the 2 M-KCl treatment was not transmissible, it became so if treated a second time with PLO.

Other compounds

Preliminary experiments showed that aphids transmitted TMV readily when polylysine, mol. wt. 85000 was substituted for PLO in the standard mixture, but not when polylysine of lower mol. wt. was used.

Tests with mixtures of 250 μg/ml TMV and 25 or 2·5 μg/ml polylysine showed that the amount of KCl required to prevent precipitation of TMV (as measured by loss during low speed centrifuging) decreased with decreasing mol. wt. of polylysine. Since excess KCl had been found to prevent aphid transmission of PLO-treated TMV, aphid transmission tests with polylysine were done using KCl concentrations which were slightly lower than those required to prevent complete loss of TMV during low speed centrifuging. Table 3 shows that TMV treated with polylysine (mol. wt. 30000) can be transmitted by aphids at KCl concentrations lower than 0·6 M.

Aphid transmission tests were also done with TMV treated with ornithine and spermidine, both with 0·6 M-KCl and without KCl, and with the cationic polyacrylamides Magnafloc 140 and 292, using KCl concentrations (0·05 or 0·2 M) just below those required to prevent loss of TMV during low speed centrifuging. No transmissions occurred with any of these, although 200 to 300 aphids were tested with each.

Table 3. *Effect of mol. wt. and KCl concentration on the aphid transmissibility of poly-L-lysine-treated TMV**

Mol. wt. of poly-L-lysine	KCl concentration (M)	Aphid transmission†
85000	0·6	24/300
30000	0·6	0/200
	0·3	7/200
	0·1	2/200
15000	0·6	0/200
	0·3	0/200
	0·06	0/200

* All preparations contained 250 μg/ml TMV, 2·5 μg/ml poly-L-lysine, and 20 % sucrose in 0·05 M-tris buffer, pH 7·1.

† Number of local lesions/number of aphids tested. Totals of 2 to 3 experiments, 100 aphids per experiment.

Table 4. *Effect of the sequence of acquisition of virus and poly-L-ornithine on the aphid transmissibility of TMV*

Acquisition sequence* (concentration, μg/ml)			
First	Second	KCl†	Aphid transmission‡
TMV (250)	Poly-L-ornithine (2·5)	Both	0/200
TMV (250)	Poly-L-ornithine (250)	Both	0/500
Poly-L-ornithine (2·5)	TMV (250)	Both	0/300
Poly-L-ornithine (250)	TMV (250)	Both	5/500
Poly-L-ornithine (25)	TMV (250)	Both	1/100
Poly-L-ornithine (250)	TMV (2500)	Both	1/100
Poly-L-ornithine (250)	TMV (250)	Neither	0/100
Poly-L-ornithine (250)	TMV (250)	TMV only	0/100

* Aphids allowed 10 min acquisition access period on each. All preparations were in 0·05 M-tris-HCl, pH 7·1 and contained 20 % sucrose.

† Denotes the presence or absence of 0·6 M-KCl in respective preparations.

‡ Number of local lesions/number of aphids tested. Totals of 1 to 3 experiments, 100 to 200 aphids per experiment.

Sequential acquisition

Aphids have transmitted purified PVY only when preparations contained a 'helper component' or when they were allowed to acquire the helper component before the virus (Govier & Kassanis, 1974). This indicates that the helper component is required for aphid acquisition of the virus. Similarities between the effects of the helper component on PVY transmission and those of PLO on TMV transmission prompted investigation of sequential acquisition in the latter system.

Aphids were given a 10 min acquisition access period on either virus or PLO and then 10 min to acquire the other component. Table 4 shows that transmission occurred only if the aphids acquired PLO before acquiring TMV. In this respect the data are similar to those for helper and PVY. However, transmissions were far fewer than from TMV–PLO mixtures, and they occurred only with PLO concentrations inhibitory to TMV transmission from mixtures (Table 1; Pirone & Shaw, 1973).

In another experiment we investigated the extent to which aphids retain the TMV–PLO complex. Aphids were allowed 10 min to acquire the standard TMV–PLO mixture and were then allowed 10 min access to a 20 % sucrose solution in water, before they were placed on

Table 5. *Aphid transmissibility of purified potato virus X, potato virus Y and tobacco rattle virus*

Virus*	Poly-L-ornithine†	KCl (M)	Aphid transmission‡	No. of aphids per plant
PVX	+	0·6	3/10	20
	+	0·08	7/10	20
	−	0·08	0/10	20
PVY	+	0·6	0/10	20
	+	0	0/10	20
TRV	+	0·24	2/5	15
	+	0·05	0/5	15
	+	0·24	2/10	10
	−	0·24	0/10	10

* All preparations contained 250 μg/ml virus and 20 % sucrose in 0·05 M-tris-HCl, pH 7·1.
† + indicates 2·5 μg/ml poly-L-ornithine; − indicates no poly-L-ornithine.
‡ Number of plants systemically infected/total number of tobacco test plants.

test plants. Two hundred aphids were tested in this manner, and no transmission was obtained.

Assay of released virus

The consecutive acquisition experiments suggested that PLO might act by binding TMV to aphid 'receptor sites' from which the virus could subsequently be released during the probing or feeding activities of the aphid on a test plant. If this were the case, one might expect to find a greater amount of TMV released into buffer solutions by aphids which acquired virus from TMV–PLO mixtures than by those which acquired untreated TMV.

Aphids (20 to 30 per feeding chamber) were allowed 10 min acquisition access on mixtures of 2500 μg/ml TMV, 0·6 M-KCl, and 20 % sucrose in 0·05 M-tris buffer, pH 7·1, which contained either 25 μg/ml or no PLO. The aphids were then transferred for 20 min to feeding chambers which contained 2 drops of a 20 % sucrose solution in 0·01 M-potassium phosphate buffer, pH 7·1. This buffer was then assayed for TMV by mechanical inoculation of carborundum dusted Xanthi-nc tobacco. Extreme care was taken in preparation and handling of all materials to exclude contamination by extraneous TMV.

Approx. 1000 aphids were used for each of the two treatments in five experiments. Buffer exposed to aphids which had first been given access to untreated TMV produced 14 lesions, while 58 lesions were produced from buffer exposed to aphids given access to PLO-treated TMV. Despite the difference in totals, the results were variable, with untreated TMV producing more lesions in 2 of the 5 experiments.

Transmission of other viruses

The effect of PLO on aphid transmission of PVX, TRV and PVY was also tested. The concentration of KCl required to reduce the turbidity of mixtures of 250 μg/ml virus and 2·5 μg/ml PLO to approximately equal that of TMV and PLO mixed in the same concentrations varied with the virus. PVY required 0·6 M-KCl, while TRV and PVX required less. Table 5 shows that PLO-treated PVX and TRV were transmitted by aphids, while PVY was not. No decrease in virus infectivity due to PLO treatment was detected, when the suspensions used for aphid transmission experiments were diluted to produce 20 to 50 lesions/half leaf and compared with untreated virus by mechanical inoculation of *Chenopodium amaranticolor*.

Sequential acquisition tests were made with PVY to test whether PLO could function as a 'helper component'. Aphids were allowed 10 min to acquire solutions of 1, 10 or 100 μg/ml PLO and then 10 min to acquire a 1 mg/ml suspension of PVY. None of 100 aphids/treatment transmitted PVY to tobacco test plants.

DISCUSSION

The brief acquisition, inoculation and retention periods, and the fact that clawless aphids transmit the virus, indicate that PLO-treated TMV is transmitted by aphids in a manner similar to non-persistent viruses. The transmission of TMV is also similar to that of purified non-persistent viruses in that relatively high concentrations of virus, in the order of several hundred μg/ml, are required to obtain consistent transmission. The efficiency of transmission is higher than that for purified alfalfa mosaic virus (Pirone & Megahed, 1966) or cauliflower mosaic virus (Lung & Pirone, 1974) and equal to that of cucumber mosaic virus (Pirone & Megahed, 1966) and PVY (Govier & Kassanis, 1974) if the fact that 10 aphids per test plant were used in the latter system is taken into account.

Potato virus X and TRV, which are not normally transmitted by aphids, also become transmissible when treated with PLO, but not PVY, which can be transmitted in the presence of its helper agent. This may indicate that there are basic differences in the conditions required for these viruses, but a wider range of experimental conditions must be tested with PVY before firm conclusions can be drawn about the inability of PLO to promote its transmission.

Aphids can acquire non-transmissible TMV from plants (Kikumoto & Matsui, 1962; Matsui, Sasaki & Kikumoto, 1963) and by feeding through membranes (Pirone, 1967). Pirone & Shaw (1973) suggested three ways in which PLO might make TMV transmissible by aphids: (1) PLO treatment might alter the virus, for example, by partial uncoating (virus alteration hypothesis). (2) PLO may permit TMV infection as it does in protoplast systems (Takebe & Otsuki, 1969; virus-cell interaction hypothesis). (3) PLO might affect the interaction between TMV and the aphid stylets (virus-aphid interaction hypothesis). The last hypothesis will be expanded, for the purpose of this discussion, to include the alimentary canal, in view of evidence (Harris & Bath, 1973) that regurgitation occurs during the periods used in our experiments and hence parts other than the stylets may be involved in transmission.

The data on dissociation of the TMV–PLO complex (ultracentrifugation experiments), the fact that a salt concentration (0·6 M-KCl) which is not inhibitory to aphid transmission of TMV prevents transmission of TMV treated with polylysine (mol. wt. 30000) and several more indirect lines of evidence indicate that the mechanism involves something other than an alteration of TMV particles by these polyamino acids. Some complexing between virus and polyamino acid seems necessary for aphid transmission. For 250 μg/ml TMV, the optimum amount of PLO is around 2·5 μg/ml. However, this amount of PLO causes severe aggregation of virus particles and hence a loss of infectivity (Pirone & Shaw 1973). The interaction between virus and polyamino acid can be regulated by the salt concentration to a level which allows sufficient binding to the virus but which prevents excessive aggregation of virus particles.

The evidence against the virus alteration hypothesis seems more conclusive than that concerning the other hypotheses. Transmission of untreated TMV by aphids to leaves infiltrated with PLO would support the virus-cell interaction hypothesis, but such experiments have given negative results (Pirone & Shaw, 1973). Such results cannot be regarded

as conclusive evidence against this hypothesis, however, because leaves infiltrated by injection with a TMV–PLO mixture which gives 50% infection of tobacco protoplasts (Kassanis & White, 1974) do not become infected. This suggests that infiltration of PLO into intercellular spaces does not facilitate entry of virus particles into a cell.

Comparison of the relative amounts of TMV and PLO required to obtain infection in the protoplast system and transmission in the aphid system reveals both similarities and differences. In the protoplast system, optimum infection occurs with a PLO concentration of 2 μg/ml. Infection is markedly reduced by decreasing the concentration to 0·4 μg/ml and no infection occurs at 0·08 μg/ml; concentrations above 2 μg/ml are toxic to the protoplasts (B. Kassanis & R. F. White, unpublished data). The optimum concentration is thus similar to that in the aphid system. However, in the protoplast system, the TMV concentration can vary between 1 and 0·01 μg/ml with little effect on infection, provided the optimum PLO concentration is used. This is in contrast to the aphid system, in which virus concentrations greater than those of PLO are required, and the ratio of virus to PLO appears to be quite critical. Because the means of virus delivery and assay are very different for the two systems, the virus-cell interaction hypothesis cannot be resolved on the basis of these comparisons.

To transmit TMV, aphids must acquire PLO before or with the virus. This is similar to the helper component–PVY system and would thus seem to favour for both viruses an explanation based on adsorption to aphid mouthparts or to sites in the alimentary canal. The polycation might act by binding TMV to sites from which it could be eluted during subsequent feeding or probing by the aphid on test plants.

The infrequency of transmission in the sequential acquisition experiments and the fact that a 10 min feeding on a sucrose solution given after acquisition of PLO-treated TMV abolishes transmission indicate that if PLO acts by binding TMV to aphid receptor sites, it does so much less firmly than helper component binds PVY. Aphids which acquire helper component before PVY transmit as frequently as aphids which acquire a helper–PVY mixture (Govier & Kassanis, 1974), and aphids fed on sucrose solutions for 5 to 10 min after acquiring a helper–PVY mixture retain the ability to transmit PVY (D. A. Govier, B. Kassanis & T. P. Pirone, unpublished data).

Two points possibly weaken the case for polycation mediated binding of virus to aphid receptor sites. The first is the small difference in the amount of infective TMV released into buffered sucrose by aphids which have acquired PLO-treated and untreated TMV. This may not reflect the situation *in vivo*, however. The patterns of ingestion, salivation and regurgitation may differ on sucrose solutions and on plants, and the material ingested, which could affect release of the virus, obviously differs. It is thus conceivable that bound TMV might be released into plants but not into sucrose solutions. The second point is the high concentration of PLO needed for sequential acquisition and the infrequent transmission which resulted. The somewhat narrow limits of the ratio of TMV to PLO for optimum transmission may provide an explanation for this. When TMV is mixed with PLO, a large number of PLO molecules may bind to each particle, and these could efficiently bind the virus to aphid receptor sites. When aphids acquire PLO before TMV, PLO may also bind to aphid receptor sites, but fewer PLO molecules are available than *in vitro*, and thus fewer TMV particles are bound and less transmission occurs.

The evidence obtained in these studies thus seems to favour the virus-aphid interaction hypothesis, but more evidence is needed, such as can be obtained by electron microscopy of thin sections of aphids.

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