# Effect of Fixation and Helper Component on the Detection of Potato Virus Y in Alimentary Tract Extracts of *Myzus persicae*

D. Lopez-Abella, T. P. Pirone, R. E. Mernaugh, and M. C. Johnson

Department of Plant Pathology, University of Kentucky, Lexington 40546. Present address of senior author: Instituto 'JAIME FERRAN' Microbiologica, C.S.I.C., Joaquin Costa 32, Madrid, Spain.

This research was supported in part by the United States-Israel Agricultural Research and Development Fund (BARD) Grant 1-27-79. Kentucky Agricultural Experiment Station Journal Series Paper 79-11-145.

Accepted for publication 31 December 1980.

## ABSTRACT

Lopez-Abella, D., Pirone, T. P., Mernaugh, R. E., and Johnson, M. C. 1981. Effect of fixation and helper component on the detection of potato virus Y in alimentary tract extracts of *Myzus persicae*. Phytopathology 71:807–809.

Aphids were given acquisition access to solutions containing equal numbers of potato virus Y (PVY) and tobacco mosaic virus (TMV) particles for 2–4 hr. The contents of the anterior alimentary tract and midgut were then examined by electron microscopy for presence of virus. The numbers of PVY particles detected were expressed as a percentage of TMV particles, which were used as a marker. When aphids imbibed untreated or control preparations, the amount of PVY present was about 2% the amount of TMV. When the virus preparation was fixed with formaldehyde or when the helper component (HC) required for transmission of purified PVY by aphids was added to the preparation prior to acquisition, PVY was about 25% of TMV. The data suggest that while HC might act by regulating adsorption of PVY by aphid mouthparts as previously suggested, HC also might have other possibly protective functions.

*Additional key words:* enzyme-linked immunosorbent assay (ELISA).

The potyvirus group is one of the largest, most widely distributed, and economically important groups of plant viruses (1); therefore, the mechanism of transmission of potyviruses by aphid vectors is of considerable practical and scientific importance.

0031-949X/81/08080703/$3.00/0


The potential for elucidating the mechanism was considerably enhanced by the finding that purified potato virus Y (PVY) cannot be transmitted unless aphids have prior or simultaneous access to the "helper component" (HC), which can be extracted from PVY-infected plants (3). This and subsequent studies (4) suggested that HC might act by binding the virus to sites in the aphid, thus rendering it transmissible. These findings suggested that electron

microscopic examination of aphids fed on HC-treated or untreated PVY might reveal differences in the condition or location of the virus in aphids, which could be correlated with transmission data. We attempted such studies using thin-sectioned material but were unable to detect, either in the anterior alimentary tract or midgut of aphids fed on HC-treated or untreated PVY, particles that were convincingly those of PVY.

Our parallel studies with thin-sectioned aphids that had been given access to suspensions of tobacco mosaic virus (TMV) also resulted in lack of reliable detection of the virus. Since it has been demonstrated that TMV is ingested by aphids and can be extracted from the dissected midgut of aphids fed on the virus (5), the fact that TMV could not be detected in thin sections suggested that thin sectioning might be inappropriate for detecting ingested virus. Therefore, we examined extracts of the contents of various parts of the alimentary canals of aphids for the presence of PVY.

## MATERIALS AND METHODS

**Virus and HC preparation.** The isolate of PVY was that used earlier (3). Virus was purified by the method of Moghal and Francki (6); final resuspension was in 0.05 M borate buffer, pH 8.1. The HC was purified by the method of Govier et al (4) except that the polyethylene glycol (PEG) used for the second precipitation was in 0.1 M potassium phosphate buffer pH 6.1 and resuspension of both the first and second PEG precipitates was in this buffer. Material to be used in place of HC, in control experiments, was prepared in the same manner, but from healthy plants. The virus suspensions and HC preparations used in these experiments were first tested in aphid transmission experiments to be certain that both were active; HC was then used for the electron microscope studies at a concentration that had given 100% transmission of PVY in the standard transmission test (4).

Unless otherwise noted, the mixture fed to aphids contained 400–600 $\mu$g of PVY per milliliter and purified TMV adjusted to equal ($\pm$10%) the number of PVY particles (determined by particle counts made by using electron microscopy [EM]), HC at concentrations determined as described above (or material prepared from healthy plants adjusted to $A_{280}$ equal to that of HC), and 20% sucrose in 15 mM borate and 50 mM phosphate (pH ~ 6.4). In some experiments brilliant blue dye was added to identify aphids that had actively ingested the test solution. The use of TMV as a marker compensated for possible differences in volumes imbibed by different aphids, and thus allowed direct comparisons among treatments.

**Aphid rearing, feeding, and dissection.** Aphids (*Myzus persicae* (Sulz.)) were removed from their mustard or turnip hosts, placed in glass vials, and fasted overnight. They were then allowed 2–4 hr acquisition access periods on the test solutions, which were contained by a stretched Parafilm 'M' membrane (4). Aphids that appeared to be actively feeding were picked off the membrane for dissection, or, if the dye was used, those showing a trace of blue color were chosen. The aphids were anesthetized by exposure to $CO_2$ for 2–3 min.

Unless otherwise noted, the method described by Kikumoto and Matsui (5) was used to remove the alimentary canal. The portion posterior to the midgut and as much of the head capsule as possible was dissected away, leaving essentially only the stylets, pharynx, foregut and midgut.

**Electron microsopy.** The parts dissected from 10–20 aphids were placed in a small (~ 20-$\mu$l) drop of distilled water, and were crushed and pulled apart with microdissecting needles. The material in the drop was deposited as three droplets in a plastic petri dish that was then covered. The debris in the droplets was allowed to settle, and a collodion-backed, carbon-coated, 48-$\mu$m (300-mesh) copper grid was placed on each droplet for about 15–20 min. The grids were then removed, and the adhering droplet was allowed to evaporate until just a thin film remained on the grid. Virus particles were negatively stained by placing a drop of 2% uranyl acetate on the grid for 3 min, after which the contents were blotted off.

A total of 40 squares was examined on each grid and, to avoid sampling bias, similar areas of the grid were examined in each experiment. Particle counts were done directly from the microscope stage; scanning was done at ~ ×15,000, with higher power used to specifically identify the particles detected. The two viruses could be readily distinguished by differences in width and staining characteristics. Practically all of the TMV particles were full-length or nearly full-length. PVY particles were counted if they exceeded twice the length of TMV particles. When end-to-end aggregates occurred, they were counted as two particles if they were twice or more the length expected for single particles.

## RESULTS AND DISCUSSION

**Effect of fixation on dectection of PVY.** In preliminary experiments, aphids were allowed to acquire the PVY-TMV mixture without HC or other amendments. Although numerous TMV particles could be detected in the extracts of the alimentary tract, PVY was rarely found. We hypothesized that ingested PVY particles were somehow being disrupted.

To attempt to stabilize the PVY, the PVY-TMV mixture was mixed with an equal volume of 2% formaldehyde in borate buffer, and, after incubation for 2 hr, was dialyzed overnight against borate buffer to remove the formaldehyde. A control PVY-TMV mixture was mixed with and dialyzed against borate buffer only. Samples of the formaldehyde-treated and untreated mixtures were applied directly to grids after appropriate dilution to obtain countable numbers of particles. The numbers of PVY and TMV particles were virtually identical in both treated and untreated preparations. Aphids were then allowed to acquire virus from these treated and untreated preparations. Formaldehyde-treated PVY was recovered from the aphids at 20–40% the concentration of TMV, while the concentration of untreated PVY was ~ 2% that of TMV (Table 1).

**Use of dye to improve efficiency of selection of viruliferous aphids.** When aphids were selected for dissection merely because they appeared to be feeding, about 20 aphids per sample were required to obtain sufficient PVY particles to detect treatment differences, when they occurred. The presence of 0.05% brilliant blue dye in the test solution allowed the selection of aphids that had imbibed sufficient solution to have a trace of blue color in the alimentary tract. This enabled the use of 10 aphids per sample. Particle ratios were similar in the presence or absence of the dye (Tables 1 and 2). The possible effect of the dye on the transmission process was tested in separate experiments. Transmission of PVY was not affected by the presence of the dye; 100% transmission was obtained with both dye-treated and control PVY (each in the presence of HC) by using the standard (3) transmission test.

**Effect of HC on detection of PVY.** The addition of HC to the PVY-TMV mixture resulted in a PVY-TMV ratio in alimentary canal extracts similar to that obtained by fixation of the viruses (Table 2). Two types of controls were used in these experiments. In the first, preparations made from healthy plants were added to the virus mixture to test the specificity of the effect. Particle ratios in extracts of aphids given access to these control preparations (Table 2) were similar to those from aphids that had received the untreated PVY-TMV mixture (Table 1).

The second type of control tested the effect of an alimentary canal extract on the ratio of PVY-TMV detected by EM. In one experiment, 1 $\mu$l of a mixture that contained 37.5 $\mu$g of PVY and 25 $\mu$g of TMV per milliliter (a particle ratio of about 1:1) was added to 20 $\mu$l of an extract of alimentary canals from 10 nonviruliferous aphids. The mixture was then prepared for EM as detailed in Materials and Methods. Treatment with the alimentary canal extract caused no decrease in the PVY-TMV ratio; 396 PVY and 298 TMV particles were counted, a PVY-TMV ratio slightly in favor of the PVY. In a second experiment, 20 $\mu$l of a mixture that contained 41 ng of PVY and 27 ng of TMV per milliliter was added to 20 $\mu$l of extract of crushed alimentary canals dissected from 10 aphids that had not had access to virus. For comparison, 20 $\mu$l of the virus mixture was added to 20 $\mu$l of 0.2% bovine serum albumin (BSA) solution (to produce a degree of spreading similar to that in the aphid extract). Particle counts for PVY and TMV were 74 and 74, respectively, for the mixture containing the aphid extracts and

TABLE 1. Effect of fixation on the detection of potato virus Y (PVY) in the contents of the alimentary tract of *Myzus persicae*

| Treatment | No. of particles[a] | | PVY as % of TMV |
|---|---|---|---|
| | PVY | TMV | |
| 20 aphids, no dye | | | |
| Fixed[b] | 45 | 197 | 23 |
| Unfixed | 8 | 402 | 1.9 |
| 10 aphids, dye[c] | | | |
| Fixed | 430 | 1,031 | 41 |
| Unfixed | 55 | 2,023 | 2.6 |

[a] The mixture fed to the aphids contained equal (± 10%) numbers of PVY and tobacco mosaic virus (TMV) particles. Values represent the total number of virus particles detected in 40 squares of each of three separate grids.
[b] Formaldehyde (2%) used as a fixative.
[c] Brilliant blue dye (0.05%) present in the virus mixture to allow identification and selection of aphids that had fed most extensively.

77 and 75, respectively, for the mixture in BSA, indicating that the alimentary canal extract per se had no effect on the PVY-TMV ratio.

Because purified PVY aphid is transmissible only by aphids that have acquired HC either prior to or simultaneously with acquisition of PVY, Govier and Kassanis (3) suggested that HC acts by enabling PVY to bind to receptor sites in the aphid from which it subsequently can be released. In our study, the very low concentration of PVY detected in aphids that had imbibed suspensions that did not contain HC, suggested several other possibilities. The first is that HC affects the ability of aphids to ingest PVY. While it is difficult to propose a mechanism by which this could occur, the possibility cannot be ruled out on the basis of the data. Another possibility is that HC has a protective effect on the virus. Such an effect could be exerted by preventing breakdown or aggregation of the particles. Still another explanation of our results is that HC may prevent PVY from being bound to parts of the alimentary tract. The control experiments in which alimentary tract extracts were added to virus indicated that if this is the mechanism, PVY, not treated with HC, is bound rather specificially and only when virus is ingested.

Unfortunately, we have been unable to determine which, if any, of these possibilities is most likely using the techniques described here. When HC was present in the preparations, the percentage of PVY particles shorter than full length was roughly similar (~20%) in the preparation ingested by the aphids and in the tract extracts. In the absence of HC there was no evidence for a greater percentage of short particles. Thus, if breakdown of PVY occurs in the absence of HC, it is probably into subunits. In some experiments, bundles of aggregated virus were found in the intact tract contents of HC-treated preparations. The mass of the bundles precluded counting of the extract ratio of TMV and PVY particles, but they were estimated to be similar to the ratio of discrete particles on the grid. Such aggregates were not found in the content of aphids that imbibed preparations that had not been treated with HC, but it seems possible that large aggregates of PVY, which could settle out with other debris when the grids are prepared, might occur in the absence of HC.

We have attempted to use enzyme-linked immunosorbent assay (ELISA) to investigate some of these possibilities, as well as to attempt to account for all of the PVY that was presumably ingested. Even with aphids given a 4-hr access to HC-treated PVY

TABLE 2. Effect of helper component on the detection of potato virus Y (PVY) in the contents of the alimentary tract of *Myzus persicae*

| Treatment | Expt. No. | No. particles[a] | | PVY as % of TMV |
|---|---|---|---|---|
| | | PVY | TMV | |
| 20 aphids, no dye | | | | |
| Helper | 1 | 54 | 211 | 26 |
| | 2 | 48 | 197 | 24 |
| | 3 | 76 | 406 | 19 |
| Control[b] | 1 | 3 | 192 | 1.5 |
| | 2 | 0 | 110 | ··· |
| | 3 | 3 | 208 | 1.4 |
| 10 aphids, dye[c] | | | | |
| Helper | 1 | 14 | 72 | 20 |
| | 2 | 136 | 632 | 22 |
| Control | 1 | 0 | 223 | ··· |
| | 2 | 31 | 1,371 | 2.3 |

[a] The mixture fed to the aphids contained equal (± 10%) numbers of PVY and TMV particles. Values represent the total number of virus particles detected in 40 squares of each of three separate grids.
[b] The control was prepared from healthy plants by the same procedure used to prepare helper component from PVY-infected plants.
[c] Brilliant blue dye (0.05%) was used to allow definite identification and selection of aphids that had imbibed the tested preparations most extensively.

as described in this paper, we were unable to obtain ELISA readings (either on whole-aphid or alimentary tract extracts) sufficiently above control readings to be confidently regarded as positive, despite repeated attempts. Similarly, we have been unable to detect PVY in aphids allowed brief probes through membranes into purified PVY or PVY-HC suspensions or in aphids allowed to probe or given access periods of up to 24 hr on PVY-infected tobacco plants. Our inability to detect PVY by ELISA may seem curious in light of the report (2) of the detection of cucumber mosaic virus (CMV) in aphids allowed brief probes on plants infected with that virus. One possible explanation may be the relative sensitivities of the ELISA systems. Our system can reliably detect purified PVY at concentrations of about 2.5 ng/ml (0.5 ng/well) while the sensitivity of the CMV system was reported to be 0.01 ng/well (2). Another possibility is that there are differences in the host-vector-virus interactions in the CMV and potyvirus systems.

## LITERATURE CITED


1. Edwardson, J. R. 1974. Some properties of the potato virus Y group. Fla. Agric. Exp. Stn. Monogr. Ser. 4:398.
2. Gera, A., Lobenstein, G., and Raccah, B. 1978. Detection of cucumber mosaic virus in aphids by enzyme-linked immunosorbent assay. Virology 86:542-545.
3. Govier, D. A., and Kassanis, B. 1974. A virus-induced component of plant sap needed when aphids acquire potato virus Y from purified preparations. Virology 61:420-426.
4. Govier, D. A., Kassanis, B., and Pirone, T. P. 1977. Partial purification and characterization of the potato virus Y helper component. Virology 78:306-314.
5. Kikumoto, T., and Matsui, C. 1962. Electron microscopy of plant viruses in aphid midguts. Virology 16:509-510.
6. Moghal, S. M., and Francki, R. I. B. 1976. Towards a system for the identification and classification of potyviruses. Virology 73:350-362.