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Role of the helper component in vector-specific transmission of potyviruses

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Four aphid species were tested for their ability to transmit tobacco etch (TEV) and turnip mosaic (TuMV) potyviruses. *Myzus persicae* and *Aphis gossypii* transmitted both viruses efficiently from infected plants, whereas *Lipaphis erysimi* transmitted only TuMV and *Myzus ascalonicus* was a poor or non-transmitter of either virus. Similar electrically monitored probing patterns were produced by *M. persicae*, *L. erysimi* and *M. ascalonicus*, ruling out behavioural differences as the cause of differential transmission. Transmission results similar to those from infected plants were obtained when these aphids acquired homologous virus/helper component (HC) mixtures through membranes. With heterologous virus/HC mixtures, *M. persicae* remained a highly efficient vector and *M. ascalonicus* a non-vector, but *L. erysimi* became an efficient vector of TEV if acquired in the presence of TuMV HC and *A. gossypii* transmitted both viruses less efficiently when acquired with TuMV HC. Transmission was highly correlated with the retention of virus in the stylets, as determined by autoradiography of ^{125}I-labelled virions. The results show that constituent(s) of or in the food canal of different aphid species differ in their ability to interact with specific HCs, leading to qualitative or quantitative differences in ability to retain and subsequently transmit specific potyviruses.

Introduction

Vector specificity is a common phenomenon in plant virus transmission and, although transmission of potyviruses and other non-circulative viruses does not seem to be as markedly specific as it is with circulatively transmitted ones, there are numerous reports of aphid species that are non-vectors or poor vectors of non-circulative viruses which are efficiently transmitted by other species (Kennedy *et al.*, 1962). In some of these cases, failure to transmit might be explained by behavioural differences of aphids on different source or test plants, but at least one example cannot be explained on this basis. Using tobacco as both a source and test plant, Doncaster & Kassanis (1946) reported that *Myzus ascalonicus* could transmit *Hyoscyamus* virus III (henbane mosaic potyvirus; HMV) but not tobacco etch potyvirus (TEV), whereas *Myzus persicae* could transmit both viruses.

Aphid transmission of potyviruses requires, in addition to virus particles, the presence of a non-structural helper component (HC) protein (Govier & Kassanis, 1974). Current evidence supports the hypothesis that HC acts as a 'bridge', interacting with virus particles and the stylet food canal allowing the virions to be retained in the stylets, from which they can subsequently be inoculated (Pirone & Blanc, 1996; Wang *et al.*, 1996; Blanc *et al.*, 1997).

The possible role of HC in vector specificity was suggested by the experiments of Sako *et al.* (1984), although, as pointed out by the authors, their results could also have been due to inappropriate virus/HC combinations or to differences in aphid behaviour during acquisition of HC and virus from different species of infected plants. Electrical recording (Tjallingii, 1985) has shown that characteristic intracellular stylet activities are associated with potyvirus transmission by aphids (Powell, 1991; Powell *et al.*, 1995; Martin *et al.*, 1997). In the studies described here, we combine information on stylet activities and on virus uptake and retention to exclude behaviour as a possible explanation for vector specificity and provide direct evidence that the specificity and efficiency of virus transmission can be regulated by HC.

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Methods

■ **Aphids.** Two species, *Myzus persicae* and *Aphis gossypii*, were chosen for their general efficacy as potyvirus vectors. In experiments at Lexington (KY, USA), *M. persicae* was reared on mustard (*Brassica perviridis*) and *A. gossypii* on cucumber (*Cucumis sativus*). *Lipaphis erysimi* and *Myzus ascalonicus* were chosen for their potential as differential vectors and were reared on mustard and pansy (*Viola wittrockiana*),

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respectively. Procedures for rearing and handling aphids were as previously described (Raccah & Pirone, 1984); apterous adults or late instar nymphs were used in the transmission and retention studies.

■ **Procedures for electrical recording of stylet activities.** Apterous adults were used in these tests, conducted at Silwood Park (Ascot, UK). *M. persicae* and *L. erysimi* were reared on Chinese cabbage (*Brassica pekinensis*), and these two species were collected and starved in plastic Petri dishes at room temperature (23–26 °C) for 1–3 h before experiments. *M. ascalonicus* was reared on shallots (*Allium ascalonicum*); they were notably slow to initiate stylet penetration of tobacco plants after 1–3 h starvation, but probed much more readily after a 24–26 h starvation period. Individuals of this species were therefore starved overnight before experiments, contained in Petri dishes at 15 °C with damp filter paper to reduce dehydration.

Each experimental insect was attached to a fine gold wire electrode (3 cm long/20 μm diameter) using conductive silver adhesive and connected to the input of an Electrical Penetration Graph 'EPG' amplifier (Wageningen Agricultural University, The Netherlands). A second electrode, positioned in the potting soil of a tobacco 'test' plant (2–3 leaf stage), was connected to a small DC voltage supply. The insect was lowered onto the upper surface of the largest leaf and allowed to insert its stylets, thereby completing the electrical circuit and producing an amplified signal. Signals were recorded directly onto computer hard disk and analysed using STYLET 2.0 software (Tjallingii & Mayoral, 1992). Recording began the moment each insect made contact with the plant surface, and continued for up to 5 min, with a maximum of five stylet penetrations recorded from each insect. Individuals of the three species were alternated until 28–30 stylet penetrations had been recorded from each species.

■ **Viruses and helper components (HCs).** A highly aphid-transmissible strain of TEV (Pirone, 1981) was purified by the method of Murphy *et al.* (1990). The aphid-transmissible isolate of turnip mosaic virus (TuMV) was that reported by Sako (1980); the virus was propagated in mustard and purified by a combination of the methods of Murphy *et al.* (1990) and Sako (1980).

Since suitable procedures for purification of TEV HC were not available (Pirone & Thornbury, 1983), potato virus Y HC (PVY HC), which readily effects TEV transmission (Pirone & Thornbury, 1983), was used. Sucrose gradient-purified PVY HC was prepared as described by Thornbury *et al.* (1985). TuMV HC was partially purified by the method of Sako & Ogata (1981) with some modifications. Infected turnip (*Brassica rapa*) leaves (50 g) were homogenized in 100 ml 0·5 M potassium phosphate buffer (pH 8·5) and centrifuged at 8000 ***g*** for 15 min. The supernatant was centrifuged at 120000 ***g*** for 1 h to pellet the virus particles. Powdered $(NH_4)_2SO_4$ was added to the supernatant to a final concentration of 18% saturation with stirring at 4 °C for 10 min. The solution was incubated for another 20 min on ice without stirring, and then centrifuged at 8000 ***g*** for 15 min. The precipitate was dissolved in 20 ml 0·02 M potassium phosphate buffer (pH 7·5) and centrifuged at 5000 ***g*** for 10 min. Sucrose was added to the supernatant to a final concentration of 20% and the solution was divided into aliquots and frozen at −20 °C.

The activity of HC preparations was quantified by testing a series of dilutions of the HC mixed with 100 μg/ml purified TEV or TuMV in our standard *in vitro* transmission bioassay using *M. persicae* (Pirone, 1981). The amount of HC used in the actual experiments was that which produced 100% transmission in the bioassay.

Virions were radioiodinated as described by Wang *et al.* (1996). Briefly, 200 μl purified virions (adjusted to 3·5 μg/μl) was placed in an IODO-GEN pre-coated iodination tube (Pierce) and reacted for 10 min with 1 mCi $Na^{125}I$. ^{125}I-labelled virions were separated from unreacted $Na^{125}I$ by gel filtration on Sephadex G-25. The concentration of labelled virus was determined spectrophotometrically. Specific activity of labelled virus was 2640–4200 d.p.m./ng for TEV and 1140–1780 d.p.m./ng for TuMV as determined in a single-well gamma counter (Bioscan). After iodination, aphid transmissibility of the viruses was assayed.

■ **Virus acquisition and transmission by aphids.** Apterous aphids were collected and kept in glass vials for 2–3 h of pre-acquisition fasting in all experiments. In most plant-acquisition experiments, probing was observed under a dissecting microscope. Aphids that had probed for 20–30 s were transferred to test plants for overnight inoculation access. In some cases, aphids were given a 10 min acquisition access to virus-infected leaves without observation and then transferred to test plants. Procedures for acquisition of purified unlabelled or ^{125}I-labelled virus were as described elsewhere (Pirone, 1981; Ammar *et al.*, 1994). About 30 aphids were placed in a feeding chamber for a 10 min acquisition access period through a membrane of stretched Parafilm. Only those aphids that were still on the membrane at the end of the acquisition access period were selected and transferred to test plants. For both plant- and membrane-acquisition, either one or ten aphids were placed on each test plant, depending on the experiment. After overnight inoculation access, test plants were sprayed with an insecticide and placed in a growth room for symptom development.

■ **Detection of radiolabel.** For autoradiography of stylets, about 20 starved aphids were placed in each feeding chamber for a 10 min acquisition access to solutions which contained 100 μg/ml ^{125}I-labelled TEV or TuMV, and either PVY HC or TuMV HC. Those aphids that were on the membrane at the end of the acquisition access period were selected for further processing by the methods described by Wang *et al.* (1996). Results were determined using a Zeiss photomicroscope III.

For determination of comparative virus uptake, aphids were allowed acquisition access to a solution containing 200 μg/ml ^{125}I-labelled TEV plus PVY HC. Ten aphids still probing the membrane at the end of the acquisition access period were grouped, transferred to a liquid scintillation counting vial and processed as previously described (Wang *et al.*, 1996). Radioactivity was measured in a liquid scintillation counter (TRI-CARB Liquid Scintillation Analyser, Model 2200CA; Packard Instruments). Aphids that acquired unlabelled TEV plus PVY HC were used as background controls. Six vials were counted for each treatment and experiments were conducted three times. Data were subjected to analysis of variance, and means were separated by Duncan's new multiple range test (SAS Institute).

Results

Transmission of TEV and TuMV from infected plants by different aphid species

Initial experiments compared the ability of individuals of the four aphid species to transmit TEV from tobacco to tobacco and TuMV from mustard to mustard. The results (Table 1) showed that *A. gossypii* and *M. persicae* were highly efficient vectors of TEV, whereas *L. erysimi* and *M. ascalonicus* did not transmit the virus. *M. persicae* was also a highly efficient vector of TuMV, *A. gossypii* was somewhat less efficient, and *M. ascalonicus* transmitted poorly. In contrast with TEV, TuMV was transmitted quite efficiently by *L. erysimi*.

Comparison of aphid probing behaviour

Tests were conducted to determine whether the differences in ability to transmit TEV could be correlated with differences

Table 1. Comparative transmission of TEV and TuMV from infected plants by four aphid species

Data are pooled results from three tests. The source and test plant for TEV was tobacco and that for TuMV was mustard.

Aphid species	Virus transmission*	
	TEV	TuMV
A. gossypii	39/60 (65·0%)	21/70 (30·0%)
L. erysimi	0/100 (0%)†	32/70 (45·7%)
M. ascalonicus	0/140 (0%)†	4/70 (5·7%)
M. persicae	40/60 (66·7%)	64/120 (53·3%)

* Transmission is given as the number of aphids that transmitted/number tested; percentage transmission is given in parentheses. A single aphid was placed on each test plant except where noted.
† Data include experiments with ten aphids per test plant.

in probing behaviour on tobacco. Probing behaviour of the two non-transmitters was compared with that of *M. persicae*. All three aphid species readily initiated brief stylet penetrations and the duration of probes did not differ significantly from those of *M. persicae* (Table 2).

The majority of probes (around 90%) by all three species included puncture of the epidermal plasmalemma by the maxillary stylet tips, recorded as a short potential drop (pd) (Tjallingii, 1985). Each pd consists of three phases (Tjallingii, 1985) which represent stylet puncture of the membrane (I), maintenance of an intracellular stylet tip position (II), and withdrawal of the stylets to an extracellular position (III). The duration of intracellular stylet contact (phase II) was determined for each pd; this did not differ significantly between species (range for all three species was 3–11 s).

Electrically recorded intracellular stylet activities appeared to be qualitatively similar for all three aphid species. Phase II of the pd has been described in detail for *M. persicae* and *A. gossypii*, and comprises three consecutive sub-phases (Powell *et al.*, 1995; Martin *et al.*, 1997). These were observed again for *M. persicae* in the present study and were also clearly distinguishable for *M. ascalonicus* and *L. erysimi*. The duration and frequency of each sub-phase was analysed, and some inter-specific differences were found (Table 2), but there were no consistent behavioural differences large enough to account for the absolute differences in TEV transmission. In particular, the first intracellular sub-phase (II-1), apparently associated with the inoculation of non-persistent viruses (Martin *et al.*, 1997), and the final sub-phase (II-3), linked with acquisition (Powell *et al.*, 1995; Martin *et al.*, 1997), were produced by all species.

Transmission of TEV and TuMV acquired through membranes

When aphids were allowed to acquire purified virions through membranes in the presence of their homologous HCs and then transmit to tobacco (TEV) or mustard (TuMV), the

Table 2. Electrically recorded stylet activities during brief penetration of tobacco 'test' plants by three aphid species

Figures in parentheses indicate sample sizes.

	Aphid species		
	M. persicae	*M. ascalonicus*	*L. erysimi*
Median probe duration (s)	16·1 (30)	22·8 (28)	14·3 (28)
Occurrence of potential drop (pd) (%)	87 (30)	93 (28)	93 (28)
Duration pd phase II (s; mean ± SE)	5·9 ± 0·29 (26)	5·9 ± 0·32 (26)	5·2 ± 0·26 (26)
Sub-phase II-1 parameters:			
Duration (s; mean ± SE)	1·3 ± 0·05 (26)	1·2 ± 0·08 (26)	1·2 ± 0·08 (26)
Frequency (Hz; mean ± SE)	13·9 ± 0·35 (26)	13·2 ± 0·26 (24)	12·2 ± 0·47 (16)**
Sub-phase II-2 parameters:			
Duration (s; mean ± SE)	1·2 ± 0·08 (26)	1·1 ± 0·06 (26)	1·4 ± 0·13 (26)
Frequency (Hz; mean ± SE)	7·5 ± 0·16 (25)	6·5 ± 0·26 (23)**	7·7 ± 0·18 (25)
Sub-phase II-3 parameters:			
Duration (s; mean ± SE)	3·6 ± 0·25 (25)	3·6 ± 0·27 (26)	2·8 ± 0·32 (24)*
Wave frequency (Hz; mean ± SE)	8·3 ± 0·39 (20)	8·7 ± 0·41 (20)	8·0 ± 0·31 (23)
Pulse frequency (Hz; mean ± SE)	1·6 ± 0·06 (20)	1·8 ± 0·06 (24)*	1·7 ± 0·04 (12)†

*, $P < 0·05$; **, $P < 0·01$ (Student's *t*-test; comparisons with *M. persicae*).
† The phase II-3 'pulses' produced by *L. erysimi* could not always be discriminated from the underlying 'waves'. Determination of pulse frequency was therefore not always possible, and the small sample size reflects this.

Table 3. Comparative transmission of purified TEV and TuMV acquired through membranes in the presence of homologous HC by four aphid species

Virus concentration was 100 μg/ml; HC was quantified as described in Methods. The source and test plant for TEV was tobacco and that for TuMV was mustard.

	Transmission from virus/HC combinations*	
	TEV/PVY HC	TuMV/TuMV HC
A. gossypii	15/30 (50·0%)	3/30 (10·0%)
L. erysimi	0/30 (0%)	12/30 (40·0%)
M. ascalonicus	0/100 (0%)†	0/30 (0%)
M. persicae	17/30 (56·7%)	14/30 (46·7%)

* Transmission is given as the number of aphids that transmitted/number tested; percentage transmission is given in parentheses. A single aphid was placed on each test plant except where noted.
† Data from experiments with ten aphids per test plant.

results were very similar to those obtained in the plant transmission experiments. This established the suitability of the membrane acquisition system for investigating the specificity phenomenon. As shown in Table 3, TEV was transmitted at a high level by *M. persicae* and *A. gossypii* and was not transmitted by the other two aphids. *M. persicae* and *L. erysimi* transmitted TuMV at similar high levels, *A. gossypii* transmitted at a lower level, while *M. ascalonicus*, a poor transmitter from plants, did not transmit purified TuMV.

Effect of HC source on transmission of purified TEV and TuMV

The hypothesis that the source of HC was responsible for the differential ability of *L. erysimi* to transmit TEV and TuMV and for the less efficient transmission of TuMV by *A. gossypii* was tested by allowing aphids to acquire heterologous and homologous virion–HC combinations. As shown in Table 4, *L. erysimi* transmitted TEV at a level comparable to *M. persicae* when the virus was acquired in the presence of TuMV HC.

While less striking, there was also a tendency for reduced transmission of either virus by *A. gossypii* when acquired in the presence of TuMV HC. Transmission of both viruses by *M. persicae* was hardly affected by the virus/HC combination, while little or no transmission by *M. ascalonicus* occurred with any combination.

Effect of HC source on retention of virions in the stylets

Previous studies with *M. persicae* have demonstrated a high correlation between transmission and HC-mediated virion retention in the stylets (Wang *et al.*, 1996). Thus, the hypothesis that differences in retention could explain differences in transmission was tested by allowing aphids to acquire ^{125}I-labelled virions in the presence of homologous and heterologous HCs. Comparison of the results of these experiments (Table 5) reveals a good correlation with the transmission data in Table 4.

Comparative uptake of membrane-acquired virions

To test the possibility that the differences in retention shown in Table 5 were due to differences in volumes ingested during access to membranes, the overall uptake by the different species was determined by acquisition of labelled virions. Table 6 shows that there were no differences in the amount of radioactivity in the bodies of the different species.

Table 4. Effect of HC source on the transmission of purified TEV and TuMV by four aphid species

Virus concentration was 100 μg/ml; HC was quantified as described in Methods. The source and test plant for TEV was tobacco and that for TuMV was mustard.

	Transmission from virus/HC combinations*			
Aphid species	TEV/PVY HC	TEV/TuMV HC	TuMV/TuMV HC	TuMV/PVY HC
A. gossypii	49/90 (54·4%)	15/90 (16·7%)	6/60 (10·0%)	29/120 (24·2%)
L. erysimi	0/140 (0%)†	36/90 (40·0%)	27/60 (45·0%)	0/200 (0%)†
M. ascalonicus	0/300 (0%)†	2/90 (2·2%)	1/60 (1·7%)	0/200 (0%)†
M. persicae	61/90 (67·7%)	41/90 (45·6%)	23/60 (38·3%)	66/120 (55·0%)

* Transmission is given as the number of aphids that transmitted/number tested; percentage transmission is given in parentheses. A single aphid was placed on each test plant except where noted.
† Data from experiments with ten aphids per test plant.

Table 5. Effect of HC source on the retention of ^{125}I-labelled TEV and TuMV in the stylets of four aphid species

Virus concentration was 100 μg/ml; HC was quantified as described in Methods.

	Retention of ^{125}I-labelled virus in virus/HC combinations*			
Aphid species	TEV/PVY HC	TEV/TuMV HC	TuMV/TuMV HC	TuMV/PVY HC
A. gossypii	129/217 (59·4%)	23/172 (13·4%)	17/169 (10·1%)	38/82 (46·3%)
L. erysimi	8/255 (3·1%)	86/168 (51·2%)	73/164 (44·5%)	5/88 (5·7%)
M. ascalonicus	0/179 (0%)	0/159 (0%)	0/165 (0%)	1/87 (1·1%)
M. persicae	154/227 (67·8%)	79/166 (47·6%)	68/173 (39·2%)	72/93 (77·5%)

* Retention of the ^{125}I-labelled virus is given as the number of aphids with label in the stylets/number examined; percentage retention is given in parentheses.

Table 6. Total uptake of ^{125}I-labelled TEV by four aphid species as determined by liquid scintillation counting of groups of ten aphids

Results are given as mean ± SE from two trials each with five replicates. Numbers followed by the same letter are not significantly different at the 0·01 level (Duncan's new multiple range test).

Aphid species	Overall uptake of ^{125}I-labelled TEV (c.p.m.)*	Background of unlabelled TEV†
A. gossypii	139·5 ± 12·6 A	39·1 ± 5·5 B
L. erysimi	146·7 ± 11·5 A	41·7 ± 4·5 B
M. ascalonicus	137·7 ± 12·0 A	47·7 ± 3·7 B
M. persicae	142·7 ± 14·7 A	42·5 ± 4·0 B

* Aphids were allowed 10 min acquisition access to 300 μg/ml ^{125}I-labelled TEV plus PVY HC.
† Aphids were allowed 10 min acquisition access to 300 μg/ml unlabelled TEV plus PVY HC.

Discussion

The data for *L. erysimi* in Table 4 provide unequivocal evidence that HC can regulate transmission specificity. This aphid, which was unable to transmit TEV either from infected plants or from mixtures with PVY HC, was able to transmit TEV at a level comparable to *M. persicae* when the virus was acquired with TuMV HC. The data also suggest that the source of HC may regulate transmission efficiency. Transmission of either virus by *A. gossypii* was higher when PVY HC was used (Table 4). More detailed analyses will be needed to test this hypothesis.

Our results further confirm and extend our observations that retention of virions in the stylets is highly correlated with transmission (Wang *et al.*, 1996; Wang & Pirone, 1996*a*, *b*), and that a functional HC is required for such retention (Wang *et al.*, 1996). The critical importance of HC/aphid compatibility for effective virus transmission is emphasized by the behavioural suitability of *L. erysimi* as a vector; aphids of this species were able to acquire TEV with the homologous HC (Table 6) and also penetrated test plants in a manner suitable for inoculation (Table 2).

In their monumental conspectus, Kennedy *et al.* (1962) made detailed analyses of aphid/virus/host combinations that had been tested, in order to determine whether any patterns in the relations between plant viruses and aphids as vectors or non-vectors could be discerned. Among the conclusions they drew was that non-behavioural, 'intrinsic' virus/vector adaptation was most likely to arise when an aphid and virus shared host plants. Their analyses were limited to comparisons among aphid species and their adaptation to host plants and they commented that 'we have given scant attention to variation between viruses which might in some cases explain differences in vector performance that we have associated with variation between aphids'. The present study provides evidence that variations in the virus HC can be one basis for transmission specificity.

There are a number of questions that arise from our findings, the main one being how general is the phenomenon? A further question is whether the adaptation of the HC of a

primarily crucifer-infecting virus to a crucifer-adapted aphid is more than fortuitous. Tests with other aphid/virus/host combinations are needed to answer these questions. From the virus aspect, mutational analyses now under way should allow identification of the HC domain(s) that allow TuMV HC, but not PVY HC, to be retained by *L. erysimi*.

A major issue to be addressed is what makes an aphid such as *M. persicae* virtually a universal vector of potyviruses, whereas one such as *M. ascalonicus* cannot transmit, or transmits very poorly, viruses such as TuMV or TEV as reported here, or PVY, TEV, lettuce mosaic and beet mosaic potyviruses in tests by Doncaster & Kassanis (1946). The one potyvirus reported by Doncaster & Kassanis (1946) to be readily transmissible by *M. ascalonicus* is HMV. We have performed extensive tests with *M. ascalonicus* using a *M. persicae*-transmissible isolate of HMV provided by R. T. Plumb (IACR, Rothamsted, Harpenden, UK), and were unable to obtain any transmission (R. Y. Wang & T. P. Pirone, unpublished data). Whether the virus isolate used by Doncaster & Kassanis (1946) was a potyvirus or another non-persistently transmitted virus such as a cucumovirus (one of which was readily transmitted by *M. ascalonicus* in tests by Doncaster & Kassanis) must remain an open question, but the point is raised of whether *M. ascalonicus* can efficiently transmit any potyvirus. Further testing will resolve this issue and, if *M. ascalonicus* proves to be a generally poor or non-vector, comparative studies on differences in the makeup of the food canal epicuticle or the saliva of this aphid and *M. persicae* could provide the answer to what is required for a successful vector of potyviruses.

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References

Ammar, E. D., Järlfors, U. & Pirone, T. P. (1994). Association of potyvirus helper component protein with virions and the cuticle lining the maxillary food canal and foregut of an aphid vector. *Phytopathology* **84**, 1054–1060.

Blanc, S., Lopez-Moya, J. J., Wang, R. Y., Garcia-Lampasona, S., Thornbury, D. W. & Pirone, T. P. (1997). A specific interaction between coat protein and helper component correlates with aphid transmission of a potyvirus. *Virology* **231**, 141–147.

Doncaster, J. P. & Kassanis, B. (1946). The shallot aphis, *Myzus ascalonicus* Doncaster, and its behaviour as a vector of plant viruses. *Annals of Applied Biology* **33**, 66–69.

Govier, D. A. & Kassanis, B. (1974). Evidence that a component other than the virus particle is needed for aphid transmission of potato virus Y. *Virology* **57**, 285–286.

Kennedy, J. S., Day, M. F. & Eastop, V. F. (1962). *A Conspectus of Aphids as Vectors of Plant Viruses*, pp. 1–114. London: Commonwealth Institute of Entomology.

Martin, B., Collar, J. L., Tjallingii, W. F. & Fereres, A. (1997). Intracellular ingestion and salivation by aphids may cause the acquisition and inoculation of non-persistently transmitted plant viruses. *Journal of General Virology* **78**, 2701–2705.

Murphy, J. F., Rhoads, R. E., Hunt, A. G. & Shaw, J. G. (1990). The VPg of tobacco etch virus RNA is the 49-KDa proteinase or the N-terminal 24-KDa part of the proteinase. *Virology* **178**, 285–288.

Pirone, T. P. (1981). Efficiency and selectivity of the helper-component-mediated aphid transmission of purified potyviruses. *Phytopathology* **71**, 922–924.

Pirone, T. P. & Blanc, S. (1996). Helper-dependent vector transmission of plant viruses. *Annual Review of Phytopathology* **34**, 227–247.

Pirone, T. P. & Thornbury, D. W. (1983). Role of virion and helper component in regulating aphid transmission of tobacco etch virus. *Phytopathology* **73**, 872–875.

Powell, G. (1991). Cell membrane punctures during epidermal penetrations by aphids: consequences for the transmission of two potyviruses. *Annals of Applied Biology* **119**, 313–321.

Powell, G., Pirone, T. P. & Hardie, J. (1995). Aphid stylet activities during potyvirus acquisition from plants and an *in vitro* system that correlates with subsequent transmission. *European Journal of Plant Pathology* **101**, 411–420.

Raccah, B. & Pirone, T. P. (1984). Characteristics of and factors affecting helper component-mediated aphid transmission of a potyvirus. *Phytopathology* **74**, 305–308.

Sako, N. (1980). Loss of aphid-transmissibility of turnip mosaic virus. *Phytopathology* **70**, 647–649.

Sako, N. & Ogata, K. (1981). A helper factor essential for aphid transmissibility of turnip mosaic virus. *Annals of the Phytopathological Society of Japan* **47**, 68–70.

Sako, N., Yoshioka, K. & Eguchi, K. (1984). Mediation of helper component in aphid transmission of some potyviruses. *Annals of the Phytopathological Society of Japan* **50**, 515–521.

Thornbury, D. W., Hellmann, G. M., Rhoads, R. E. & Pirone, T. P. (1985). Purification and characterization of potyvirus helper component. *Virology* **144**, 260–267.

Tjallingii, W. F. (1985). Membrane potentials as an indication for plant cell penetration by aphid stylets. *Entomologia Experimentalis et Applicata* **38**, 187–195.

Tjallingii, W. F. & Mayoral, A. (1992). Criteria for host plant acceptance by aphids. In *Proceedings of the 8th International Symposium on Insect Plant Relationships*, pp. 280–282. Edited by S. B. J. Menken, J. H. Visser & P. Harrewijn. Dordrecht: Kluwer Academic.

Wang, R. Y. & Pirone, T. P. (1996*a*). Mineral oil interferes with the retention of tobacco etch potyvirus in the stylets of *Myzus persicae*. *Phytopathology* **86**, 820–823.

Wang, R. Y. & Pirone, T. P. (1996*b*). Potyvirus transmission is not increased by pre-acquisition fasting of aphids reared on artificial diet. *Journal of General Virology* **77**, 3145–3148.

Wang, R. Y., Ammar, E. D., Thornbury, D. W., Lopez-Moya, J. J. & Pirone, T. P. (1996). Loss of potyvirus transmissibility and helper component activity correlates with non-retention of virions in aphid stylets. *Journal of General Virology* **77**, 861–867.

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