Efficiency and Selectivity of the Helper-Component-Mediated Aphid Transmission of Purified Potyviruses

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ABSTRACT

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Aphids (*Myzus persicae*) transmitted purified potato virus Y (PVY) from suspensions that contained as little as 40 ng of virus per milliliter, in the presence of PVY helper component (HC). Transmission from the HC system is thus comparable in efficiency to mechanical inoculation and is 10^3- to 10^5-fold more efficient than aphid transmission of purified viruses that do not require HC. HC prepared from bean yellow mosiac virus (BYMV)-infected plants (BYMV-HC) effected the transmission of BYMV, as well as PVY and tobacco vein mottling virus (TVMV); but PVY-HC and TVMV-HC, although highly effective with both PVY and TVMV, were ineffective with BYMV, suggesting some specificity. Aphids consistently transmitted TVMV more often than PVY, when acquisition was from a mixture of PVY and TVMV, in the presence of TVMV-HC. In the presence of PVY-HC, however, the amount of transmission of each virus from a PVY-TVMV mixture was variable and seemed to depend on the virus preparations used. The HC-dependency system is extended to another aphid, *A. gossypii*, to two other viruses (TVMV and BYMV), and HC was obtained from plants (pepper and pea) other than tobacco. These findings further strengthen the case that HC-dependency may be general for aphid-plant-potyvirus combinations.

The transmission of purified potato virus Y (PVY) by aphids has been shown to be dependent on the presence of a helper component (HC), which is present in extracts of PVY-infected, but not healthy, tobacco plants (4). Addition of HC extracted from PVY-infected plants (PVY-HC) also has been shown to allow the aphid transmission of two other purified potyviruses, tobacco severe etch and henbane mosaic virus (4). Thus, dependence on HC for the aphid transmission of purified potyviruses may well be a general phenomenon (9).

With one recent exception (13), published studies on purified potyvirus transmission have used only PVY-HC prepared from tobacco, and the aphid *Myzus persicae*, although the seemingly related phenomenon of transmission dependency from plants has been reported with other viruses, aphids, and plants (6,8,14).

The purpose of the experiments reported here was to test other viruses, plants, and aphids for ability to function in the HC system, to test the specificity of HC prepared from different sources, and to obtain data on the efficiency of HC-mediated aphid transmission.

MATERIALS AND METHODS

The isolates of potato virus Y (PVY) and tobacco vein mottling virus (TVMV) used in this study have been described elsewhere (5,10). Unless otherwise noted, purified virus and partially purified HC were prepared from systemically infected tobacco (*Nicotiana tabacum* L. 'Burley 21') that had been mechanically inoculated 2–4 wk previously. Bean yellow mosaic virus (BYMV) isolate 204-1 (2), obtained from S. Diachun, was propagated in peas (*Pisum sativum* L. 'Dwarf Grey Sugar'); virus and HC were prepared from plants mechanically inoculated 2 wk previously. All viruses were purified by method 1 of Moghal and Francki (7); virus concentrations were determined spectrophotometrically by using an extinction coefficient $E_{261\ nm}^{0.1\%} = 2.4$. All HC preparations were made by the method of Govier et al (5) modified in that the polyethylene glycol (PEG) used for the second precipitation was in 0.1 M potassium phosphate buffer, pH 6.1, and the resuspension of the first and second PEG precipitates was in this buffer. The extract from 200 g of leaf tissue was concentrated to 5–6 ml and frozen in 0.5-ml lots. The activity of each HC preparation was assayed by adding the homologous virus (final concentration 40 μg/ml) to a series of dilutions (in 0.1 M phosphate buffer, pH 6.1) of the HC preparation and testing for aphid transmission after Parafilm membrane acquisition as previously described (5). This allowed the preparation of dilutions of HC that could be expected to result in approximately 70–90% transmission of the homologous virus by *M. persicae* in the actual experiments. In most experiments, the use of HC at this level of activity was necessary to avoid using a great excess of HC and thus to be able to detect differences in transmission between various treatments.

Myzus persicae (Sulz.), reared and handled as described previously (5), were used in all tests. The *Aphis gossypii* (Glov.) used in one series of tests were reared on cucumber (*Cucumus sativus* L.) and handled similarly. Transmission tests were done by allowing 10 groups of aphids a 10-min acquisition access to test solutions by probing through Parafilm membranes as previously described (5). Unless otherwise noted, the test solutions contained 40 μg of virus per milliliter, HC at an appropriate concentration, as determined above and 20% sucrose. Ten aphids were placed on each of 10 test plants, except in the competition tests, in which a single aphid per test plant was used. The test plant was Burley 21 tobacco for all viruses escept BYMV, for which Dwarf Grey Sugar pea was used. Aphids were allowed to remain on the test plants overnight (14–18 hr); plants were then sprayed with an insecticide and placed in the greenhouse for symptom development. Infected plants could be readily detected by symptom production, and the percentage of transmission was routinely determined on this basis when homologous virus-HC combinations or heterologous dilute HC-virus combinations were tested. In experiments that required the use of highly concentrated HC (which may contain sufficient residual virus to result in an occasional transmission) or in which more than one virus was present in the test solution, the virus(es) infecting the test plants were determined by double-diffusion tests (3) against specific antisera. Such tests were not necessary for experiments in which 10-fold or greater dilutions of HC were employed, as these have been repeatedly found to contain insufficient residual virus to allow aphid transmission.

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RESULTS

Transmission by *A. gossypii*. *A. gossypii* transmitted purified PVY, in the presence of PVY-HC, to 30, 20, and 20% of the test plants, respectively, in three experiments. Transmission by *M. persicae* was 80, 70, and 60% respectively, for these experiments. No transmission occurred with an equal number of *A. gossypii* given access to purified PVY not treated with HC.

Preparation of PVY-HC from pepper. Pepper (*Capsicum annum* L. 'California Wonder') was tested as a source of PVY-HC in two experiments. Two 200-g samples of leaves that had been infected for 18 and 25 days, respectively, were processed as described in Materials and Methods. The HC activity of these preparations was low; undiluted, the preparations resulted in 40 and 20% transmission of PVY, respectively, and had no activity at a dilution of 1/10 or higher. Preparations made from tobacco leaves infected for the same periods of time resulted in 100% transmission at a dilution of 1/10 and 30% transmission at a dilution of 1/50, for both time periods.

Quantitative studies. Results of preliminary studies indicated no stoichiometric relationship between the amounts of PVY and PVY-HC required for optimum transmission. A series of PVY concentrations ranging from 10–1,000 μg/ml was tested against a series of twofold to 100-fold dilutions of HC. There was no evidence that an excess of either virus or HC would suppress transmission or that an optimum ratio of PVY to HC existed. Further experiments were directed toward determining the efficiency of the aphid transmission.

Aphids could transmit PVY from suspensions that contained as little as 40 ng PVY per milliliter (Table 1). It should be noted that in these tests it was essential to use dilute preparations of HC, as sufficient residual virus was present in the undiluted HC preparations to give an occasional transmission. Controls in which no virus was added to the HC preparation were used in all of the experiments shown in Table 1, and no transmissions occurred. The limiting effect of the 1/50 dilution of HC can be seen at all virus concentrations above 400 ng/ml, and the limiting effect of virus concentration can be seen at 400 ng/ml and below for either HC dilution.

TABLE 1. Transmission of purified potato virus Y (PVY) acquired by aphids from suspensions that contained PVY helper component (HC) and a series of concentrations of PVY

Dilution of HC	Expt.[b]	Transmission[a] (%) at a PVY concentration (μg/ml) of: 40	4	0.4	0.04	0
1/10	1	100	100	40	0	0
	2	100	90	50	10	0
	3	···	100	80	10	0
1/50	2	40	50	30	10	0
	3	···	40	50	10	0

[a]Ten test plants with 10 aphids (*Myzus persicae*) per plant for each virus-HC combination. For controls (no virus), 20 test plants with 20 aphids per plant were used.
[b]Experiments with a common number are directly comparable.

TABLE 2. Comparison of mechanical and aphid transmission of purified potato virus Y (PVY) at a series of virus concentrations

Treatment	Method of inoculation	Expt.[a]	Transmission (%) at a PVY concentration (μg/ml) 4	0.4	0.04	0.004	0
PVY only	Mechanical[b]	1	100	40	10	0	0
		2	100	30	20	0	0
PVY + HC	Mechanical	1	100	30	20	0	0
		2	100	20	30	0	0
PVY + HC	Aphid[c]	2	90	20	10	0	0

[a]Experiments with a common number are directly comparable.
[b]Ten test plants inoculated at each virus concentration and for the control.
[c]Ten test plants with 10 aphids (*Myzus persicae*) per plant at each virus concentration. For controls (no virus), 20 test plants with 20 aphids per plant were used.

TABLE 3. Aphid transmission of three purified viruses: potato virus Y (PVY), tobacco vein mottling virus (TVMV), and bean yellow mosaic virus (BYMV), in the presence of homologous and heterologous helper component (HC)

HC from plants infected with	Expt.[b]	Transmission[a] (%) of: PVY	TVMV	BYMV
PVY	1	100	···	0
	2	100	100	0
BYMV	1	40	···	60
	2	20	10	80
TVMV	2	70	60	0

[a]Ten test plants, with 10 aphids (*Myzus persicae*) per plant used in each experiment with each virus-HC combination. Virus concentration was 40 μg/ml.
[b]Experiments with a common number are directly comparable.

TABLE 4. Aphid transmission of purified potato virus Y (PVY) and tobacco vein mottling virus (TVMV) in the presence of PVY or TVMV helper component (HC)[a]

HC from plants infected with	Dilution of HC	Transmission[b] (%) of: PVY	TVMV
PVY	Undiluted	96	63
	1:4	75	60
	1:16	9	6
TVMV	Undiluted	44	81
	1:4	24	69
	1:16	8	17

[a]Combined results of eight experiments using six different HC preparations, and four different preparations of each virus from each source.
[b]Ten test plants, with 10 aphids (*Myzus persicae*) per plant used in each experiment, with each virus-HC combination, at each HC dilution. Virus concentration was 40 μg/ml.

TABLE 5. Aphid transmission of potato virus Y(PVY) and tobacco vein mottling virus (TVMV) acquired from suspensions that contained equal amounts of each virus, and helper component (HC) from the indicated sources

Expt.[b]	HC Source	HC Prep.[c]	Virus prep. PVY	Virus prep. TVMV[d]	Transmission[a] (%) of: PVY	TVMV	Both[e]
1	PVY	Y1	Y1	T1	20	0	0
2		Y2	Y1	T1	20	0	0
3		Y2	Y2	T2	7	7	5
4		Y3	Y2	T3	20	17	0
5		Y4	Y3	T3	10	17	0
6		Y5	Y4	T4	17	7	0
1	TVMV	T1	Y1	T1	3	17	0
2		T2	Y1	T1	3	23	2
3		T2	Y2	T2	0	53	0
4		T2	Y2	T3	0	30	0
5		T3	Y3	T3	0	40	0
6		T4	Y4	T4	0	10	0

[a]Thirty test plants, with one aphid (*Myzus persicae*) per plant used in each experiment with each virus-HC combination.
[b]Experiments with a common number are directly comparable.
[c]Five different PVY-HC and four different TVMV-HC preparations were used, as indicated.
[d]Four different PVY and TVMV preparations were tested, combined as indicated. Final concentration of each virus was 80 μg/ml.
[e]Data also appear in columns for individual viruses.

The comparative efficiency of mechanical and aphid transmission and the effect of HC on mechanical transmission was tested in another experiment. A series of concentrations of PVY, with or without HC added at a dilution of 1/10, was used to mechanically inoculate Burley 21 seedlings (10 per treatment); a cheesecloth pad was used to apply the inoculum to the Carborundum-dusted leaves. Aphid transmission tests also were done with PVY, which contained HC at a 1/10 dilution. The results (Table 2) indicate that the amounts of virus required for aphid and mechanical transmission are similar and that HC has no apparent effect on the infectivity of PVY, as measured by mechanical inoculation.

Specificity studies. In initial studies, HC prepared from PVY, BYMV, or TVMV-infected plants was used in transmission tests with homologous or heterologous virus. Both PVY-HC and TVMV-HC were highly effective with either PVY, or TVMV, but did not effect transmission of BYMV. The BYMV-HC was effective with all three viruses, but somewhat less so with PVY and TVMV than with BYMV (Table 3).

More detailed studies were done with PVY and TVMV, for which a common test plant (tobacco) could be used, to eliminate effects that might be due to test plant differences. The combined results of eight experiments, in each of which the indicated HC-virus combinations were compared directly at a series of HC dilutions, are shown in Table 4. While the overall data appear to show some bias in favor of the homologous combinations, differences between homologous and heterologous combinations were not statistically significant ($P = 0.05$, t-test).

Competition studies. The potential specificity of the HC-virus interaction was further studied in a series of experiments in which aphids were allowed acquisition periods on suspensions that contained a mixture of PVY and TVMV, and either PVY-HC or TVMV-HC. The concentration of each virus was 80 μg/ml, and a single aphid was placed on each test plant, to minimize double infections. Five different PVY-HC and four different TVMV-HC preparations, and four different preparations of PVY and TVMV were used, in various combinations. The results (Table 5) with TVMV-HC were quite consistent; the transmission of TVMV was much higher than that of PVY in all cases. With PVY-HC, preferential transmission of PVY occurred in the first two experiments, but not in the subsequent ones. The transmission of TVMV mediated by PVY-HC tended to be higher in experiments 3, 4, and 5, in which TVMV transmission mediated by TVMV-HC was also high.

DISCUSSION

The results reported here extend the HC-dependency system to another aphid (*A. gossypii*), to two other viruses (TVMV and BYMV), and demonstrate that HC can be obtained from two other plants (pepper and pea) besides tobacco. Taken in conjunction the recent report on purified turnip mosaic virus (13) and with data on dependent transmission from plants (6,8,14), this further strengthens the case that HC-dependence may be general for potyviruses (9). Although the level of PVY transmission by *A. gossypii* was lower than that by *M. persicae*, this is not necessarily a reflection of relative HC efficacy in the two aphids, as the actual probing and feeding of the aphids during acquisition and transmission access was not monitored, and differences in behavior could well be responsible for differences in transmission. Similarly, differences in recovery of PVY-HC from tobacco and pepper cannot be considered significant, as we have found that modifications of the purification procedure are often necessary for the recovery of HC from different host-virus systems (*unpublished*).

The nanogram amounts of purified PVY required for efficient transmission in the HC system are 10^3- to 10^5-fold less than those required for transmission of purified cucumber mosaic virus, alfalfa mosaic virus, and carlaviruses; these viruses, which do not require a helper for transmission, must be present in concentrations ranging from several hundred micrograms to several milligrams per milliliter for transmission to occur (1,12,15). Thus, the purified potyvirus-HC system seems more likely to reflect, quantitatively, the transmission process as it occurs from plants than do these other systems. The PVY-HC system also requires considerably less virus than the poly-L-ornithine-mediated aphid transmission of viruses such as tobacco mosiac virus, potato virus X, and tobacco rattle virus, for which concentrations in the range of 250 μg/ml are required for efficient transmission (11).

The fact that highly active preparations of PVY-HC or TVMV-HC did not mediate the transmission of BYMV, while BYMV was transmitted in the presence of BYMV-HC, suggests a degree of specificity in these HC-virus interactions. However, the aphid-virus-plant combination is known to affect transmission efficiency in plant-to-plant systems, and the possibility that lack of BYMV transmission was due to an inappropriate virus-HC-plant combination cannot be ruled out.

While HC prepared from PVY- or TVMV-infected plants effected the transmission of PVY and TVMV, the bias toward more efficient transmission of the homologous virus (Table 4), and, in particular, the selective transmission of TVMV from PVY-TVMV mixtures that contained TVMV-HC (Table 5) again suggest a certain level of specificity. The competitive transmission experiments with PVY-HC (Table 5) suggest, however, that selectivity also may be a function of the virus preparation used. For example, the same virus preparations (Y1 and T1) but different HC preparations (Y1 and Y2) were used in experiments 1 and 2, and transmission was unaffected by the different HC preparations. In experiment 3, the same HC preparation (Y2) was used as in experiment 2, but different virus preparations (Y2 and T2) were used, and the transmission results differed from those in experiment 2.

Subsequently we have found that homologous HC-virus combinations are not always those that result in the highest amount of transmission, but that high levels of transmissibility may be more a function of the strain of virus used than of the HC source (Pirone and Thornbury, *unpublished*). This may have a bearing on the relative transmissibility of certain potyviruses in nature.

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