Vector Relations

Barley and Oats as Reservoirs for an Aphid Virus and the Influence on Barley Yellow Dwarf Virus Transmission

F. E. Gildow and C. J. D'Arcy

Assistant professor, Department of Plant Pathology, The Pennsylvania State University, University Park 16802; and associate professor, Department of Plant Pathology, University of Illinois, Urbana 61801.
Contribution 1650, Department of Plant Pathology, the Pennsylvania Agricultural Experiment Station. Authorized for publication 21 August 1987 as Journal Series Paper 7752.
Accepted for publication 7 January 1988 (submitted for electronic processing).

ABSTRACT

Gildow, F. E., and D'Arcy, C. J. 1988. Barley and oats as reservoirs for an aphid virus and the influence on barley yellow dwarf virus transmission. Phytopathology 78:811-816.

The role of small grain cereals in horizontal transmission of the isometric *Rhopalosiphum padi* virus (RhPV) among aphid populations of *R. padi* and *Schizaphis graminum* was examined. Results of four types of RhPV transmission studies suggested that aphids acquired RhPV from plant tissues during feeding. RhPV was detected in 65 of 120 *S. graminum* when uninfected *S. graminum* were reared 5 days on plants coinfested with RhPV-infected *R. padi*. When uninfected aphids were given a 24-hr feeding on washed leaf tissue previously fed on by RhPV-infected aphids, 41 of 80 aphids became infected. Transport of RhPV through plants was suggested by aphid acquisition of RhPV from plant tissues not directly fed on by RhPV-infected aphids. When uninfected *S. graminum* or *R. padi* and RhPV-infected *R. padi* were fed on opposite ends of leaves for 7 days, 36 of 80 uninfected aphids became infected. Transmission tests indicated that virus in the plant tissue did not remain available for acquisition indefinitely. Results of enzyme immunoassays for RhPV in four barley cultivars and dsRNA analysis of oats and barley used to rear RhPV-infected *R. padi* failed to detect evidence of RhPV replication in plants. RhPV infection of *R. padi* or *S. graminum* had no effect on barley yellow dwarf virus (BYDV) transmission efficiency or vector-specificity when tested with the RPV, RMV, MAV, and PAV isolates of BYDV. Similarities in the virus-vector-plant interactions between RhPV and BYDV are discussed.

Isometric viruslike particles (VLP) have been repeatedly observed and described in aphid species that are vectors of plant viruses (10,15,16). With one exception, these VLP have not been identified. Only one isometric aphid-infecting virus, *Rhopalosiphum padi* virus (RhPV), has been purified, partially characterized (1,2), and shown to infect and replicate in aphid

tissues (8). First identified infecting laboratory-maintained colonies of *Rhopalosiphum padi* (L.), particles of RhPV were determined to be 27–30 nm in diameter and to have a capsid consisting of three major proteins containing one single-stranded RNA (2). Reduced longevity of *R. padi* was associated with RhPV infection.

RhPV was found to be transovarially transmitted from parent aphids to 28% of the next generation nymphs (vertical transmission). Enzyme immunoassays (EIA) of randomly selected individual adult aphids from mature RhPV-infected colonies, however, indicated an 87% incidence of infection within the population (1). These results suggested that other mechanisms occurred for RhPV transmission from aphid to aphid within a single generation (horizontal transmission).

RhPV was also identified by EIA in colonies of *R. padi* from North Dakota, and in *R. rufiabdominalis* (Sasaki) and *Schizaphis graminum* (Rondani) maintained at the University of Illinois. These species are important vectors of barley yellow dwarf virus (BYDV). Because RhPV may reduce aphid longevity, the potential effect of RhPV on aphid population dynamics and BYDV epidemiology has obvious importance for potential use in biological control. RhPV has also been implicated as a possible component of a complex of viruses associated with a poorly understood disease of small grains (19).

The objectives of our work were to elucidate mechanisms of RhPV transmission and to determine what role host plants could play in horizontal spread of RhPV through an aphid population. Because aphids feed specifically on host plant phloem tissues and are not known to ingest surface-related foliage components, the virus would have no other obvious means of ingress into the aphid other than feeding.

MATERIALS AND METHODS

Aphids. Clones of uninfected *R. padi* and *S. graminum* were initiated parthenogenetically from single apterous adults obtained from previously characterized clones (18) maintained by W. F. Rochow (Cornell University, Ithaca, NY). Colonies were reared on caged barley (*Hordeum vulgare* L. 'Barsoy') maintained at 20 C with a 24-hr photoperiod. The RhPV-infected *R. padi* used as a virus source were from a previously described colony of infected aphids from Illinois (1). Infected aphids were reared as described but were kept in a separate building from the uninfected aphids.

Virus detection. Methods for RhPV purification, antibody production, and enzyme immunoassay were as previously described (1,2). The method used for RhPV detection in single aphids was immunospecific electron microscopy (ISEM) using Protein A enhancement (20). Formvar-carbon coated grids were incubated 10 min on 20 μl of Protein A at 50 μg/ml in 0.01 M phosphate buffer, pH 7, followed by incubation for 10 min on 10 μl of anti-RhPV polyclonal rabbit IgG at 20 μg/ml. Grids were then rinsed in distilled water and incubated for 30 min on a 20-μl drop of aphid homogenate, rinsed twice in distilled water, and stained 3 min in 2% aqueous uranyl acetate. Aphid samples were made immediately before use by homogenizing each aphid in 20–30 μl of 0.01 M phosphate buffer, pH 7, in a 2-ml disposable glass homogenizer. Control treatments for ISEM tests consisted of grids incubated on buffer alone or on antibodies made against the RPV or PAV isolates of barley yellow dwarf virus (17). Grids were examined and photographed in an electron microscope at a magnification of 10,000 times. RhPV virions were identified in infected aphid tissues after fixation and thin-sectioning by their size (30 nm), staining characteristics, and by in vivo indirect labelling with ferritin-conjugated antibodies microinjected into feeding aphids, as previously described (6).

Horizontal RhPV transmission. Four types of tests were done to study the potential role of plants in transmission of RhPV from aphid to aphid. The first consisted of rearing mixed colonies of uninfected and RhPV-infected aphids. Ten RhPV-infected *R. padi* were placed on a caged 7-day-old barley seedling for a 7-day feeding to initiate these tests. Ten uninfected second instar nymphs of *Schizaphis graminum* were then added to each seedling for an additional 5-day feeding. After the 5-day feeding, the *S. graminum* were removed and placed on healthy seedlings for an additional 10-day feeding before testing for RhPV-infection by ISEM. Test aphids were transferred to fresh healthy seedlings during the virus incubation period whenever necessary to prevent confusing test aphids with subsequently produced offspring.

In a second type of test, nymphs of uninfected aphids were given a 24-hr acquisition feeding on detached leaves from barley plants previously used for 3 wk to rear RhPV-infected *R. padi*. Leaf tissue was removed from the colony plant and rinsed 10 min under running tap water, washed for 1 min in a dilute detergent solution (Alconox), and rinsed an additional 5 min in tap water and distilled water before initiation of the 24-hr acquisition feeding in dishes with tight-fitting lids at 20 C in the dark. Virus was not detected on the tissue by EIA or ISEM after this protocol. The tissue remained turgid and green during use. After the acquisition feeding, aphids were reared on healthy oat seedlings for 10 days before ISEM testing for RhPV.

A third transmission test consisted of giving 20 RhPV-infected or uninfected *R. padi* a 24-hr feeding on stretched Parafilm membrane sandwiches containing 20% sucrose in 0.01 M phosphate buffer. These aphids were then removed with a small brush and the sucrose withdrawn from the center of the sandwich with a sterile syringe. The sucrose, which had been previously fed on by infected aphids, or uninfected aphids as controls, was then used to construct new membrane units with fresh Parafilm. This was done to reduce the likelihood of virus contamination from the surface of the Parafilm. Uninfected *R. padi* and *S. graminum* were then given a 24-hr feeding on the membranes, followed by a 10-day feeding on healthy oat seedlings. Aphids were then tested by ISEM to determine whether or not RhPV had been acquired from the sucrose.

The fourth type of test, referred to as the split-cage test, consisted of rearing RhPV-infected *R. padi* on the top half of a barley seedling leaf and uninfected *R. padi* or *S. graminum* on the lower half of the same leaf. Healthy 14-day-old Barsoy barley seedlings at the two-leaf stage were grown in 15-cm clay pots covered by a cylindrical transparent plastic cage 2.5 cm wide and 30 cm long. The first (oldest) leaf was removed and the second leaf allowed to extend through the length of the cage. The cage had been previously split lengthwise and 4-cm-long pieces of soft foam rubber attached to the inside of each cage half at its midpoint. When the cage halves were brought together around the barley leaf, the leaf was able to extend undamaged through the center of the foam pad, but aphids were prevented from moving past the barrier. Thus, the leaf from a single seedling extended through two separate chambers in the cage. Twenty first instar uninfected aphids were placed in the chamber at the base of the seedling and 50–100 RhPV-infected aphids were placed in the upper chamber containing the top half of the leaf. Seedlings with feeding aphids were kept at 20 C with a 24-hr photoperiod for 7 days. After this time, infected aphids were collected from the top chamber and tested for RhPV to verify infection. The uninfected aphids in the lower chamber were then transferred to healthy oat seedlings for an additional 7-day feeding before ISEM testing for RhPV acquisition.

Tests for RhPV replication. Double-stranded RNAs (dsRNAs) were purified from aphid and plant tissue by cellulose chromatography fractionation (13). DsRNA was extracted from 1 g of aphids powdered in liquid nitrogen as previously described (7). The ethanol precipitated dsRNA samples were resuspended in 90 μl of electrophoresis buffer (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.5) and 10 μl of 50% glycerol containing bromophenol blue as a marker. Usually a 1–10-μl sample was adequate for electrophoretic analysis. Plant tissues were processed in a similar manner, except that 20 g fresh weight of leaf tissue samples was homogenized in 40 ml of STE to begin the procedure, and the final dsRNA sample was resuspended in a volume of only 50 μl.

DsRNA samples were electrophoresed in Tris-acetate-EDTA buffer in either 6% polyacrylamide or 1% agarose gels. Gels were stained with ethidium bromide (20 μg/ml) and photographed over

an ultraviolet light source. The single- or double-stranded nature of the extracted RNA was determined by post electrophoresis incubation of ethidium bromide stained 1% agarose gels with RNAse A (Sigma type III A) at 25 μg/ml in either 0.3 or 0.03 M sodium chloride.

The RhPV virion nucleic acid was extracted by incubating 50 μg of density gradient purified RhPV suspended in 1 ml of 0.01 M phosphate buffer in 0.2 ml of Protease K (5 mg/ml) and 0.01 ml of 10% SDS at 37 C for 30 min. Then 2 ml of double-strength STE buffer, 0.1 ml of bentonite, and 0.2 ml of SDS were added and the sample heated to 60 C for 3 min. An emulsion was formed by adding 5 ml of phenol and 3 ml of chloroform and shaking. The aqueous phase containing the RNA was recovered by low-speed centrifugation; RNA was precipitated overnight in 2 volumes of ethanol at −20 C. The RNA precipitate was recovered by centrifugation, resuspended in 50 μl of sterile distilled water, and stored at −20 C. Molecular weight estimations were made by analysis of samples on 1% agarose gels using formaldehyde denatured samples (11). The BRL-RNA ladder (Bethesda Res. Lab., Gaithersburg, MD) and lambda phage DNA *Hind*III and *Bst* EII fragments (New England Biolabs, Beverly, MA) were used as standards after denaturation.

Barley cultivars Barsoy, Clipper, Hudson, and Robust were grown as previously described (3). Twelve days after planting, half of the plants of each cultivar were infested with RhPV-infected *R. padi* as the test treatment, and half were kept aphid-free as controls. Aphids were allowed to feed and reproduce for 10 days on the infested plants in a growth chamber at 23 C with a 14-hr photoperiod (8,000 lx). All plants were then fumigated with DDVP (0,0,-dimethyl-2,2-dichlorovinyl phosphate) and maintained in a greenhouse until harvested. Plants of each cultivar from infested and noninfested treatments were harvested at 0, 4, 8, 13, 18, and 24 days after fumigation, and stored at −80 C. Roots and shoots were tested by EIA as previously described (3). Tissues were cut into fine pieces with razor blades, then homogenized in phosphate-buffered saline-Tween 20 (1 g/3 ml) for 15–30 sec in a Tekmar Tissumizer (Tekmar Co., Cincinnati, OH). EIA plates (Immulon 1, Dynatech Lab. Inc., Alexandria, VA) were coated with antibody (10 μg/ml) for 1 hr at 37 C. Samples were incubated overnight at 4 C, phosphatase-conjugate (10 μg/ml) for 4 hr at 20 C, and substrate for 2–4 hr at 20 C. Absorbance at 405 nm was recorded. Values greater than twice the mean absorbance of control wells were considered positive. Extracts of RhPV-infected *R. padi* were included in each plate as positive controls.

BYDV transmission tests. Uninfected and RhPV-infected aphids were given parallel 48-hr acquisition feedings at 20 C on detached leaves from healthy oats or from oats infected with the New York RPV, RMV, MAV, or PAV isolates of BYDV (17,18). Aphids were then transferred singly or in groups of 5 or 10 to individually caged 7-day-old seedlings of California Red oats for a 5-day inoculation feeding. Plants were fumigated and the seedlings transferred to the greenhouse where they were observed for symptoms of BYDV infection over a 4-wk period.

For direct comparisons of uninfected and RhPV-infected New York clones of *R. padi* and *S. graminum*, aphids of each species were fed 24 hr on 5 μg/ml of RhPV suspended in 20% sucrose in Parafilm membranes. These aphids were then used to initiate infected colonies maintained by transfers to healthy barley seedlings every 3 wk. Tests of these colonies by ISEM indicated 90–100% infection among aphids sampled. Healthy aphid colonies were sampled periodically by ISEM to verify that they remained virus-free. Infected colonies were reared in a separate building from healthy colonies.

RESULTS

RhPV transmission through plants. To determine whether or not RhPV could be spread through plants, mixed colonies of RhPV-infected *R. padi* and uninfected *S. graminum* were initiated on barley. Results of four experiments (Table 1) indicated that 30–70% of the *S. graminum* became infected after feeding on plants coinfested with RhPV-infected *R. padi*. These results suggested mechanisms for RhPV transmission within a population by direct aphid to aphid contact, acquisition from contaminated plant surfaces, or acquisition of virus from plant tissues during feeding. Experiments were therefore designed to test these hypotheses.

Uninfected aphids were given a 24-hr acquisition feeding on washed leaves of barley plants previously used to rear RhPV-infected colonies. These aphids were tested 14 days later for virus infection. Virus was not detected by ISEM or EIA on the leaf tissues following extensive washing. This procedure reduced the probability of virus acquisition from contaminated leaf surfaces. Results of three experiments (Table 2) indicated that RhPV could be readily acquired by 20–80% of the uninfected aphids fed on leaves previously fed on by infected aphids. Virus was consistently acquired from all plants tested. These data suggested that RhPV was acquired from within the plant leaf tissue and that virus transmission did not require direct contact or simultaneous feeding with infected aphids. Similar results were not obtained if the leaf tissue was not used within several days of removing the infected aphids. In one detached leaf experiment, the leaves were washed and stored at 4 C for 72 hr before infesting with uninfected aphids. None of 20 uninfected aphids fed on the tissue was infected when tested 14 days later. In another test, uninfected *R. padi* were allowed to feed 24 hr on leaves from each of three Barsoy barley plants that had been used to rear RhPV-infected *R. padi* for 3 wk. The plants were used immediately after washing off the infected aphids. After the 24-hr feeding, the uninfected aphids were reared 10 days on healthy oats and then tested for RhPV. In the meantime, the three plants were fumigated to kill remaining aphids and maintained 3 wk in the greenhouse. At the end of 3 wk the plants were again used to feed uninfected *R. padi*. After 3 wk, representative adult aphids were selected at random from each

TABLE 1. Number of *Schizaphis graminum* (SG) that became infected with *Rhopalosiphum padi* virus (RhPV) after a 5-day acquisition feeding on barley coinfested with RhPV-infected *Rhopalosiphum padi*

Experiment	Plants infested[a] (no.)	SG's tested per plant (no.)	SG's infected (of 30) (no.)
1	6	5	10
2	6	5	17
3	3	10	17
4	3	10	21

[a]Ten RhPV-infected *R. padi* were allowed to feed on each seedling for 7 days before infesting each plant with 10 uninfected *S. graminum*. None of 10 *S. graminum* fed on healthy barley in the absence of RhPV-infected *R. padi* in each experiment tested positive for RhPV when tested by immunospecific electron microscopy.

TABLE 2. Number of *Schizaphis granimum* (SG) and *Rhopalosiphum padi* (RP) that became infected with *Rhopalosiphum padi* virus (RhPV) after a 24-hr acquisition feeding on detached leaves of barley plants used to rear RhPV-infected aphids

Experiment[a]	Plant replicate	Aphid tested	Number tested	Number infected
1	1	SG	5	3
	2	SG	5	2
	3	RP	5	3
	4	RP	5	4
2	1	SG	5	2
	2	SG	5	1
	3	RP	5	3
	4	RP	5	4
3	1	SG	10	2
	2	SG	10	5
	3	RP	10	7
	4	RP	10	5

[a]RhPV was not detected by immunospecific electron microscopy by using RhPV-specific polyclonal IgG in any of 10 aphids from colonies of each species used as sources at the beginning of the experiments or in any of 10 aphids of each species reared in parallel on healthy oats as controls.

plant and tested for RhPV infection. RhPV was detected in 12 of 18 aphids fed 24 hr on the plants at the start of the experiment, but in none of 18 aphids fed on the same three plants after a 3 wk aphid-free period in the greenhouse. These results suggested that aphids acquired virus from within the plant tissue, but that the virus did not remain infective or available for acquisition indefinitely. The data do not support the idea that RhPV replicates in Barsoy barley.

Data from three sucrose membrane experiments indicated that 60–80% of the uninfected *R. padi* and *S. graminum* acquired RhPV from sucrose previously fed on by infected aphids (Table 3). Virus was not detected in any of 24 aphids fed on sucrose previously fed on by uninfected aphids. These results clearly demonstrated that aphids were capable of transmitting RhPV through the salivary canal or regurgitating virus through the food canal during stylet probes into plant tissue.

To determine whether or not RhPV could be transported through the plant after virus introduction into the vascular system by feeding aphids, 100 RhPV-infected *R. padi* and 20 healthy *R. padi* or *S. graminum* were allowed to feed simultaneously on opposite ends of healthy barley seedlings separated by a 4-cm-wide barrier. In order for the uninfected aphids to acquire RhPV, the virus would have to move through a 4-cm length of leaf tissue not directly accessible to feeding aphids. The results of two experiments (Table 4) supported the idea that RhPV could be transported through plant tissue. The data indicated that although virus transport occurred from the top of the leaf, where the infected-aphids were feeding, to the basal portion of the leaf, where uninfected aphids fed, only 36 of 80 aphids tested (45%) had acquired virus. These results must take into account local spread of the virus among initially uninfected aphids once one of them became infected. Therefore, the amount of virus transported and the probability of infection are probably lower than suggested by the data. Of more significance is the fact that aphids on seven of eight plants acquired virus while feeding on tissue never directly in contact with infected aphids. In two preliminary experiments similar to those described, only 10 to 20 infected aphids were used to act as virus sources at the top of the plants. In those experiments none of 40 uninfected aphids became infected. This suggested that increased numbers of infected aphids feeding on plants resulted in increased virus availability and possibly an increased number of vascular bundles becoming contaminated. In four treatments using infected *R. padi* and uninfected *S. graminum*, *R. padi* were never found contaminating the *S. graminum* colony at the end of the experiments. This indicated that infection of the uninfected aphids did not result from contamination or mixing of aphid treatments.

Test for RhPV replication in plants. RhPV was not detected by ISEM in leaf homogenates of any of six California Red oat or six Barsoy barley plants used to rear RhPV-infected *R. padi* for 3 wk, after extensive washing of the plant surface to remove any possible contaminating virus and infected aphids. Approximately 20 plants of each of four barley cultivars (Barsoy, Clipper, Hudson, and Robust) were used to rear RhPV-infected colonies of *R. padi*, washed to remove infected aphids, and then tested by EIA for RhPV in root and leaf tissue. Of 82 plants tested, only four plants tested weakly positive for RhPV. One positive sample was identified for Clipper and Robust and two positive samples for Barsoy. It is possible these positives could have resulted from incomplete washing of infected aphids from the leaf surface, or that we were detecting very low concentrations of the virus in the tissue of a few plants. We know the virus occurs in the tissue, because it is acquired by feeding aphids. No increase in virus concentration was detected in plants over the 24-day sampling period. These results did not support the theory that RhPV replicated in plant tissue. No increase in virus titer was noted.

When RhPV was purified from infected *R. padi* and the virion nucleic acid analyzed by electrophoresis on 1% agarose-formaldehyde denaturing gels, one single-stranded RNA with a molecular weight (MW) of approximately 3.8×10^6 was detected. This suggested that a full-length genomic replicative form of dsRNA with a molecular weight of about 7.6×10^6 should occur in tissues in which RhPV was replicating.

To test for RhPV replication in plants infested with RhPV-infected aphids, tissues of uninfected *R. padi*, RhPV-infected *R. padi*, and uninfected or BYDV-infected oats and barley used to rear infected aphids were processed for dsRNA analysis. Results (Fig. 1) indicated two dsRNAs of about 7.6 and 1.7×10^6 MW occurred specifically in extracts from RhPV-infected *R. padi* (lane 2), but not in uninfected aphids (lane 1). The smaller RNA may represent a subgenomic replicative form, but this remains to be tested. No dsRNAs suggestive of RhPV replication were detected in healthy California Red oats (lane 3), or Pennrad barley (lane 7) used to rear RhPV-infected *R. padi* for 3 wk. The sensitivity of the method was demonstrated by detection of the two major dsRNAs associated with BYDV-infection (7) of Coast Black oats (lane 6) and Barsoy barley (lane 8). These plants were used to rear colonies of RhPV-infected *R. padi* that acquired the PAV isolate of BYDV. A large dsRNA (12.5×10^6 MW) of unknown origin, which appears to be seedborne, also occurred consistently in all Barsoy barley samples. These results further suggested that the RhPV virus did not infect and replicate in small grain plants used to rear RhPV-infected aphids and that replication was not associated with the ability of aphids to acquire the virus during feeding.

Effect of RhPV on BYDV transmission. When uninfected and RhPV-infected clones of *R. padi* from Illinois and New York were studied for their ability to transmit vector-specific isolates of BYDV, no differences were detected (Table 5). RhPV-infected aphids from both colony sources efficiently transmitted the RPV and PAV isolates, but not the MAV or RMV isolates, which are transmitted by *Sitobion avenae* and *R. maidis*, respectively. No reduction in vector efficiency for either isolate was detected between uninfected or infected aphids.

In another comparative study, uninfected and RhPV-infected *S. graminum* and *R. padi* from New York were allowed a 48-hr acquisition feeding on healthy oats or oats infected with MAV or

TABLE 3. Number of *Rhopalosiphum padi* (RP) and *Schizaphis granimum* (SG) that became infected with *Rhopalosiphum padi* virus (RhPV) after a 24-hr acquisition feeding on 20% sucrose previously fed on by RhPV-infected aphids

Experiment	Aphid	Sucrose treatment	No. RhPV-infected no. aphids tested[a]
1	RP	RhPV	16/20
		Control	0/8
2	RP	RhPV	15/24
		Control	0/8
3	SG	RhPV	12/20
		Control	0/8

[a] Individual test aphids were homogenized in 20 μl of 0.01 M phosphate buffer and incubated 30 min on RhPV-sensitized grids for ISEM examination.

TABLE 4. Number of uninfected *Rhopalosiphum padi* (RP) and *Schizaphis graminum* (SG) that became infected with the *Rhopalosiphum padi* virus (RhPV) when reared 7 days on the same barley seedling with RhPV-infected *R. padi* (RP-RhPV), but without direct contact between aphid treatments[a]

Seedling number	Aphid in top cage	Aphid in bottom cage	Healthy aphids infected with RhPV (of 10) (no.) Exp. 1	Exp. 2
1	SG	SG	0	0
2	RP-RhPV	SG	0	7
3	RP-RhPV	SG	8	4
4	RP	RP	0	0
5	RP-RhPV	RP	5	6
6	RP-RhPV	RP	5	1

[a] Each seedling was allowed to extend through a foam rubber pad, which prevented aphid movement between the top and bottom halves of the leaf.

PAV. After a 5-day inoculation feeding on 7-day-old oats using five aphids per plant, the seedlings were fumigated and observed for symptoms. Twelve plants were infested for each virus treatment. None of eight plants infested with aphids fed on healthy oats as controls became infected. Uninfected and infected *S. graminum* transmitted PAV to 10 and 12 plants, respectively, but did not transmit MAV to any of 24 infested plants. Uninfected and infected *R. padi* transmitted PAV to five and six plants, respectively, but did not transmit MAV to any of 24 plants tested. These results indicated that vector-specificity and efficiency were unaffected by RhPV-infection of *S. graminum*. ISEM tests of aphids selected at random from the RhPV-infected colonies used in the above transmission tests indicated that about 90% of the aphids were infected.

DISCUSSION

Uninfected *R. padi* and *S. graminum* consistently acquired RhPV by feeding on barley and oats previously fed on by RhPV-infected aphids. Although RhPV was transported through leaf tissue, apparently in the vascular system, EIA and dsRNA analysis failed to detect evidence of RhPV replication in the plant. In addition, aphids were unable to acquire RhPV from previously infectious plants after the plants were maintained free of infected aphids for 3 wk. These results suggest that RhPV is injected into plants by feeding aphids and that virus can survive in an infectious form for a short time in the plant, but that RhPV did not replicate in the plant. The aphid host plant functions as an intermediate source for the aphid virus and facilitates horizontal transmission of RhPV from aphid to aphid.

When parasites are detrimental to the survival of their infected host, as RhPV is for *R. padi*, vertical transmission alone cannot maintain the pathogen in the host population, and some mechanism for horizontal transmission is necessary (4). It is not surprising, therefore, that plants function in horizontal transmission of RhPV, because aphids ingest only from internal plant tissues with their stylets and may not provide other avenues for virus ingress. Some natural selection for tolerance to RhPV may have been noted during this study. Colonies of newly infected *R. padi* from New York were much slower to develop and had higher mortality rates than uninfected colonies or chronically infected *R. padi* from Illinois.

Our results are similar to those reported by Ofori and Francki (14) for leafhopper A virus transmitted through maize, and the data provide detailed experimental evidence to substantiate the hypothesis that plants can act as reservoirs for insect viruses.

Infection of BYDV vectors with RhPV had no obvious effect on BYDV transmission efficiency or on vector-specificity. Ultrastructural examinations of RhPV-infected aphids failed to detect virus or any cytopathological abnormalities in the accessory salivary glands, which are considered to be key sites regulating BYDV transmission (6,8). RhPV infection could be expected to influence BYDV epidemiology, however, because the virus does have detrimental effects on aphid survival and fecundity (1). RhPV was found to be host tissue specific and rapidly infected the midgut epithelium, followed by infection of the hindgut. How this might affect BYDV acquisition through the hindgut (6) by RhPV-infected aphids is unknown.

The taxonomy of RhPV and other isometric ssRNA insect viruses is uncertain (12). Previous characterization studies (2) indicated that RhPV might be associated with other isometric insect viruses grouped with the picornaviridae because of its size, density, and coat proteins. Electrophoretic analysis of formaldehyde denatured virion nucleic acid reported here indicated a ssRNA genome of about 3.8×10^6 MW. This size range was supported by dsRNA analysis of RhPV-infected aphid tissues that repeatedly yielded a large dsRNA of 7.6×10^6 MW, and a smaller dsRNA of 1.7×10^6 MW. The larger RNA is presumed to be the full-length replicative form of RhPV since it is twice the size of the ssRNA isolated from purified virions. These data suggest that the RhPV genomic RNA is larger than the expected size range for picornavirus associated insect viruses and may have a different replication strategy involving subgenomic RNAs. These data suggest that further work will be necessary before RhPV can be appropriately classified.

Evidence suggesting relationships among insect and plant viruses has been discussed (9). It is interesting, therefore, to compare the association of BYDV, a plant virus, and RhPV, an insect virus, with their common aphid vector. Both viruses are transmitted to plants by feeding aphids, are apparently transported in the plant vascular system, are acquired from plants by feeding aphids, and show aphid species specificity in their ability to penetrate vector cells. For instance, the RPV isolate of BYDV is

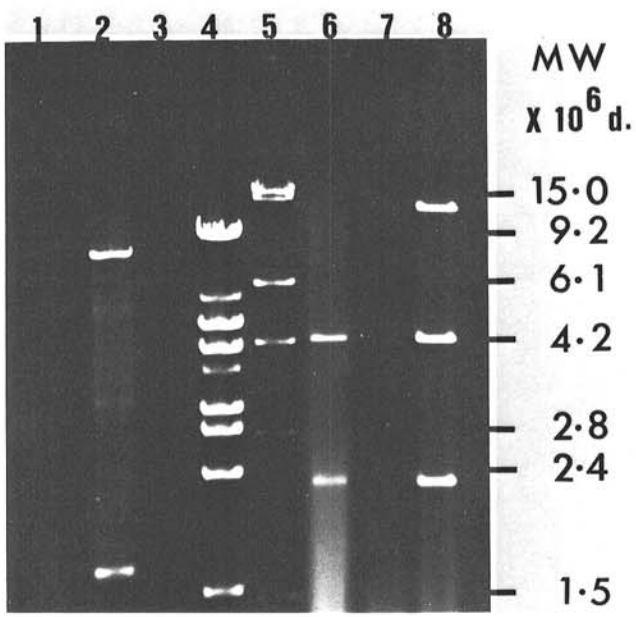

Fig. 1. Electrophoretic profiles of dsRNAs purified from uninfected *Rhopalosiphum padi* (lane 1), RhPV-infected *R. padi* (lane 2), healthy Coast Black oats (lane 3), and Pennrad barley (lane 7) used to rear RhPV-infected *R. padi*, and oats (lane 6) and Barsoy barley (lane 8) used 3 wk to rear RhPV-infected *R. padi* previously acquisition fed on oats infected with the PAV isolate of barley yellow dwarf virus. Lambda DNA *Bst* EII (lane 4) and *Hind* III (lane 5) restriction endonuclease fragments (New England Biolabs, Beverly, MA) were used as molecular weight standards. Agarose gels (1%) were run 6 hr at 25 V followed by ethidium bromide staining.

TABLE 5. Comparison of uninfected and *Rhopalosiphum padi* virus (RhPV)-infected *R. padi* from Illinois and New York for their ability to transmit vector-specific isolates of barley yellow dwarf virus[a]

Aphids per plant (no.)	Aphid source	Aphid treatment	Plants per isolate (no.)	Plants infected with BYDV isolates indicated (no.)			
				RPV	RMV	MAV	PAV
1	NY	uninfected	12	10	0	0	7
1	NY	RhPV	12	9	0	0	8
1	IL	uninfected	12	9	0	0	8
1	IL	RhPV	12	9	0	0	6
1	IL	RhPV	24	19	0	0	12
5	NY	uninfected	15	15	0	0	15
5	NY	RhPV	15	14	0	0	15
5	IL	uninfected	12	12	0	0	12
5	IL	RhPV	12	12	0	0	12
10	NY	uninfected	8	8	0	0	8
10	NY	RhPV	8	8	0	0	8

[a]Uninfected aphids were from colonies initiated from single 24-hr-old nymphs produced by apterous adults kept overnight on moist filter paper. Infected colonies were from chronically RhPV-infected *R. padi* from Illinois or from uninfected New York *R. padi* inoculated by allowing aphids to feed 24 hr on 5 μg/ml RhPV in 20% sucrose in Parafilm membranes. All colonies were indexed by ISEM to verify the infected or uninfected condition of the colony. In 3-wk-old RhPV colonies, 90% of the aphids tested positive for RhPV infection.

transmitted by *R. padi* but not by *Sitobion avenae* (F.), because RPV is recognized and transported through the accessory salivary gland of *R. padi* but not *S. avenae* (6). The Illinois isolate of RhPV infects *R. padi* but not *S. avenae*, because it is capable of penetrating and replicating in midgut cells of *R. padi* but not *S. avenae* (8). This comparison is useful in speculating on how circulative virus transmission mechanisms evolved to allow insect cells to recognize specific plant virus isolates and then to use highly organized cell membrane systems to transport the viruses across two distinct aphid organ systems associated with virus acquisition (hindgut) and transmission (salivary gland). Perhaps small isometric ssRNA viruses, such as the plant luteoviruses, evolved from an aphid-infecting virus that became attenuated in the aphid after developing the ability to infect plant hosts of its vector. This might explain how virus-specific receptors, believed to be necessary for virus transmission (6), could have evolved in aphids. If RhPV were to mutate and develop the ability to replicate in plants acting as RhPV reservoirs, then its ability to replicate in its aphid host might be selected against, because the virus is deliterious to aphid survival. The ability of the aphid to continue transmitting the virus might be retained and selected for, however, because virus-infected host plants can favor aphid survival compared with healthy plants (5). Survival of the virus would also require selection for isolates that retained the ability to be aphid transmitted. Direct evidence to support this hypothesis is lacking, but continued study of plant and insect viruses may reveal currently undetected associations and relationships.

LITERATURE CITED

1. D'Arcy, C. J., Burnett, P. A., and Hewings, A. D. 1981. Detection, biological effects, and transmission of a virus of the aphid *Rhopalosiphum padi*. Virology 114:268-272.
2. D'Arcy, C. J., Burnett, P. A., Hewings, A. D., and Goodman, R. M. 1981. Purification and characterization of a virus from the aphid *Rhopalosiphum padi*. Virology 112:346-349.
3. D'Arcy, C. J., Hewings, A. D., Burnett, P. A., and Jedlinski, H. 1983. Comparative purification of two luteoviruses. Phytopathology 73:755-759.
4. Fine, P. E. M. 1981. Epidemiological principles of vector-mediated transmission. Pages 77-91 in: Vectors of Disease Agents. J. J. McKelvey, Jr., B. F. Eldridge, and K. Maramorosch, eds. Praeger Publishers, New York. 243 pp.
5. Gildow, F. E. 1984. Aphid vector biology and the effect of barley yellow dwarf virus on aphids. Pages 28-33 in: Barley Yellow Dwarf, A Proceedings of the Workshop. CIMMYT, Mexico.
6. Gildow, F. E. 1987. Virus-membrane interactions involved in circulative transmission of luteoviruses by aphids. Curr. Top. Vect. Res. 4:93-120.
7. Gildow, F. E., Ballinger, M. E., and Rochow, W. F. 1983. Double-stranded RNA's associated with barley yellow dwarf virus infection of oats. Phytopathology 73:1570-1572.
8. Gildow, F. E., and D'Arcy, C. J. 1985. Effect of an aphid-infecting virus on barley yellow dwarf virus transmission. (Abstr.) Phytopathology 75:1348.
9. Goldbach, R. W. 1986. Molecular evolution of plant RNA viruses. Annu. Rev. Phytopathol. 24:289-310.
10. Kitajima, E. W. 1976. Isometric, virus-like particles in the green peach aphid, *Myzus persicae*. J. Invert. Pathol. 28:1-10.
11. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 545 pp.
12. Moore, N. F., Reavy, B., and King, L. A. 1985. General characteristics, gene organization and expression of small RNA viruses of insects. J. Gen. Virol. 66:647-659.
13. Morris, T. J., and Dodds, J. A. 1979. Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. Phytopathology 69:854-858.
14. Ofori, F. A., and Francki, R. I. B. 1985. Transmission of leafhopper: A virus, vertically through eggs and horizontally through maize in which it does not multiply. Virology 144:152-157.
15. Parrish, W. B., and Briggs, J. D. 1966. Morphological identification of virus-like particles in the corn leaf aphid, *Rhopalosiphum maidis* (Fitch.). J. Invert. Pathol. 8:122-123.
16. Peters, D. 1965. The purification of virus-like particles from the aphid *Myzus persicae*. Virology 26:159-161.
17. Rochow, W. F., and Carmichael, L. E. 1979. Specificity among barley yellow dwarf viruses in enzyme immunosorbent assays. Virology 95:415-420.
18. Rochow, W. F., and Eastop, V. F. 1966. Variation within *Rhopalosiphum padi* and transmission of barley yellow dwarf virus by clones of four aphid species. Virology 30:286-296.
19. Rybicki, E. P., and von Wechmar, M. B. 1982. Characterization of an aphid-transmitted virus disease of small grains. Phytopathol. Z. 103:306-322.
20. Shukla, D. D., and Gough, K. H. 1979. Use of protein A, from *Staphylococcus aureus*, in immune electron microscopy for detecting plant virus particles. J. Gen. Virol. 45:533-536.