Transmission Interference Between Two Isolates of Barley Yellow Dwarf Virus in *Macrosiphum avenae*

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ABSTRACT

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Fewer aphids (*Macrosiphum avenae*) transmitted the PAV isolate of barley yellow dwarf virus (BYDV) if they had first acquired the MAV isolate, than if they had previously fed on healthy oats or on oats infected with other BYDV isolates. The reduction in transmission of PAV by previous acquisition of MAV was consistent in each of 30 experiments done over a period of 3 yr, regardless of varying length of feeding times, temperatures, or age of aphid used. When aphids acquired virus by feeding, the reduction in transmission was 66% in 22 experiments that utilized 905 aphids per treatment. No interference in transmission of PAV by *M. avenae* occurred in tests with any of three other BYDV isolates, nor was PAV transmission by *Rhopalosiphum padi* reduced by previous acquisition of other BYDV isolates. The interference by MAV in the transmission of PAV also occurred in *M. avenae* when MAV was injected into aphids at a concentration of at least 170 μg/ml. When MAV and PAV were injected simultaneously into *M. avenae*, transmission of PAV decreased as the MAV concentration was increased. A possible mechanism to explain these data, based on competition between the MAV and PAV isolates for virus specific receptor sites on aphid salivary glands, is discussed.

In a review of interactions between plant pathogens in insect vectors, Freitag (6) described several cases of cross protection or transmission interference between two pathogens within a vector. We now know that the pathogens discussed were spiroplasmas or mycoplasmalike organisms (MLO) and not viruses. Little is known of similar interactions between plant viruses in insect vectors. Several reports (3,9,14,27) describe a lack of interaction between unrelated viruses or between isolates of the same virus when transmitted simultaneously by aphids or by leafhoppers. MacKinnon (14) found no evidence of interference between the persistently transmitted potato leaf roll virus (PLRV) and turnip latent virus when aphids acquired both viruses either independently or simultaneously. Likewise, Sylvester (27) reported no interference between beet yellows virus and beet yellow net virus in aphids. No interference between mild and severe isolates of PLRV in *Myzus persicae* was found by Harrison (9), or between beet curly top virus isolates in leafhoppers by Bennett (3). This lack of virus interference is not surprising since cross protection by MLO occurred between disease agents which replicate in their leafhopper hosts. There is no evidence for replication of the viruses studied in their vectors.

This paper describes transmission interference in an aphid vector between two isolates of barley yellow dwarf virus (BYDV), an isometric luteovirus 20–24 nm in diameter (23). Five vector-specific isolates, which also differ in virulence and serological properties, have been described (11,19). The virus is transmitted in a persistent-circulative manner by aphids, but there is no evidence of virus replication in the vector (18,20). In this report we describe interference in transmission of the PAV isolate of BYDV by the MAV isolate in the aphid vector, *Macrosiphum avenae*. The MAV and PAV isolates are serologically related but not identical, induce similar cytopathological symptoms in host plant cells, and cross protect against each other in plants (1,7,25).

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MATERIALS AND METHODS

The New York clones of the English grain aphid, *Macrosiphum avenae* (Fabricius), and the oat bird-cherry aphid, *Rhopalosiphum padi* (Linnaeus) were used (22). Virus-free stock colonies of aphids were reared on caged barley plants, *Hordeum vulgare* (L.), under controlled conditions to prevent BYDV contamination and mixing of aphid species (19,22). Five isolates of BYDV were used: the MAV isolate, transmitted specifically by *M. avenae;* the RPV isolate, transmitted specifically by *R. padi;* the PAV isolate, transmitted by both *M. avenae* and *R. padi;* and the RMV and SGV isolates, which are not transmitted regularly by the two vector species studied here (11,19). Virus isolates were maintained by serial transmissions to oats, *Avena byzantina* Koch 'Coast Black', the test plant used in all experiments.

In virus transmission tests, aphids acquired virus in one of three ways. For acquisition periods of 1 or 2 days, aphids were fed on detached leaves in plastic dishes at 15 C in the dark. For acquisitions longer than 2 days, aphids were caged on oat plants in a growth chamber at 21 C with a 16-hr photoperiod. For acquisition by injection into the hemolymph, aphids received approximately 0.02 μl of a partially purified virus preparation (16,25). Except where mentioned, aphids used were fourth instar alatoid nymphs. Each seedling was infested with a single aphid for the inoculation test feeding period of 5 days on 7-day-old oat seedlings in the growth chamber at 21 C. Aphids were removed from plants by fumigation with DDVP (0,0-dimethyl-2,2-dichlorovinyl phosphate). Plants were then grown in a greenhouse for 4 wk during which they were scored as infected or not infected on the basis of symptoms.

Most experiments were done with *M. avenae* allowed a first acquisition feeding on oats infected with MAV (or other tissue as controls) and a second acquisition feeding on PAV-infected oats. Since *M. avenae* transmits both of these viruses, further tests were necessary to determine which isolate (or isolates) had been transmitted by an aphid. It was possible to detect PAV in the presence of MAV by making an index test with *R. padi,* which transmits PAV but not MAV (19). Thus, plants infected by PAV

were identified by allowing *R. padi* to feed for 2 days on a leaf from an infected test plant, then transferring 10 of these aphids to each of three seedlings. If all three plants became infected, then PAV was judged to be present in the original test plant. In tests of interactions between RPV and PAV in *R. padi,* a similar index test was done by using *M. avenae,* which transmits PAV, but not RPV.

Possible interference in MAV transmission by previously acquired PAV was not tested because of limitations of the bioassay used to differentiate the two BYDV isolates. At the time these experiments were done no simple method existed for identifying MAV in the presence of PAV. We are currently testing for PAV interference in MAV transmission by utilizing the recently developed enzyme-linked immunosorbent assay procedures for identifying MAV and PAV in single plants.

All experiments included some aphids fed only on healthy plants or leaves as controls. Other controls included aphids transferred directly from virus source plants to test seedlings to confirm identity of the BYDV isolate being tested.

RESULTS

Interactions in *M. avenae*. *Feeding tests.* In preliminary tests, fewer aphids (*M. avenae*) transmitted the PAV isolate, if they had first acquired MAV, than if they previously had fed on healthy oats. To study consistency of this reduction in PAV transmission, six experiments were done in which aphids were given a 2-day acquisition feeding either on detached leaves of healthy oats, on oats infected with MAV, or on oats infected with the RMV isolate. Aphids from all three groups were given a second acquisition feeding of 1 or 2 days on PAV-infected oat leaves or on healthy leaves. Infested plants subsequently were indexed to determine how many aphids had transmitted PAV.

In all six experiments, interference occurred regularly only between MAV and PAV (Table 1). Transmission of PAV by 168 aphids fed first on healthy, on RMV-infected, or on MAV-infected oats was 55%, 45%, and 10%, respectively.

In seven other experiments we studied MAV-PAV interference in aphids that acquired the first virus from intact plants over a longer feeding period, and also investigated the possibility of interference by other BYDV isolates. Aphids were given a 5-day acquisition feeding on healthy oats, or on oats infected with the MAV, RPV, RMV, or SGV isolates of BYDV. This was followed by a 2-day second acquisition on detached leaves from PAV-infected or from healthy oats. Results (Table 2) were similar to those of the first six experiments. Only 28% of the 266 aphids previously exposed to MAV transmitted PAV. But 71% of the aphids previously exposed to one of the other three BYDV isolates transmitted PAV, and 72% of those from healthy oats transmitted PAV. These data show that the MAV-PAV interference is consistent, that the interference occurs following acquisition of MAV from intact plants as well as from detached leaves, and that the interference is specific for the interaction between MAV and PAV.

Tests for MAV-PAV interference in plants. One possible explanation for the interference by MAV in transmission of PAV is that both isolates are transmitted to the plant but MAV suppresses replication or infection by PAV in the test seedling and thus reduces the likelihood of recovering PAV in index tests. That this is unlikely is suggested by previous work that demonstrated cross protection between MAV and PAV in oats, but only when the protecting isolate had been inoculated to the plant 4 days prior to challenge inoculation with the second isolate (1).

To determine whether MAV-PAV interference occurred in test seedlings following inoculation, oats were inoculated simultaneously with MAV and PAV, and recovery of PAV from these plants by aphids was compared to that from plants inoculated with PAV alone. In four separate experiments, 60 oat seedlings were inoculated with MAV by single *M. avenae,* another 60 seedlings were inoculated with PAV by single *M. avenae,* and 60 seedlings were inoculated simultaneously both with MAV by single *M. avenae* and with PAV by either *M. avenae* or *R. padi.*

The presence of MAV had no effect on the probability of recovering PAV from doubly infected plants. Of 240 plants inoculated in each group, the number of plants that became infected following inoculation by aphids exposed to MAV, PAV, or to both viruses was 196, 173, and 230, respectively. The PAV isolate subsequently was recovered in index tests by *R. padi* from all 173 plants inoculated only with PAV, and from 167 of 230 plants inoculated with MAV and PAV. None of 72 plants infested with aphids fed only on healthy oats as controls became infected. When inoculated into plants simultaneously with PAV, the MAV isolate did not reduce the chance of recovery of PAV. These data support the view that the interference between MAV and PAV takes place in the aphid.

Aphid injection tests. Tests were performed to determine whether MAV-PAV interference occurred when MAV was injected into aphids. In the first set of experiments, fourth instar alatoid

TABLE 1. Transmission of the PAV isolate of barley yellow dwarf virus (BYDV) by *Macrosiphum avenae* allowed a first acquisition feeding on healthy or infected oat leaves, before a second acquisition on PAV-infected leaves

Experiment	No. of aphids (of 28) that transmitted PAV following first acquisition as shown[a]		
	RMV	MAV	HO
1	19	1	16
2	11	4	18
3	11	3	17
4	12	1	12
5	18	2	16
6	4	5	13

[a]Aphids were first allowed a 2-day acquisition feeding on detached leaves of healthy oats (HO) or of oats infected with the MAV or RMV isolate of BYDV, followed by either a 1-day (exp. 1–3) or 2-day (exp. 4–6) second acquisition feeding on leaves from healthy or PAV-infected oats. Single aphids were then allowed a 5-day inoculation test feeding on Coast Black oat seedlings. None of 36 plants infested with aphids as controls became infected. In tests with MAV and PAV, 154 of 168 plants became infected. Since *M. avenae* transmits both MAV and PAV, plants infected with PAV were identified in indexing tests with *Rhopalosiphum padi* as described in the text. In these tests *R. Padi* transmitted PAV from 16 of the 154 infected plants (to 48 of 462 plants).

TABLE 2. Transmission of the PAV isolate of barley yellow dwarf virus (BYDV) by *Macrosiphum avenae* allowed a first acquisition feeding on healthy oat plants or on oats infected with one of four other BYDV isolates, before a second acquisition feeding on PAV-infected oats

BYDV isolate for first acquisition[a]	No. of aphids (of 40) that transmitted PAV following first acquisition as shown[b]		
	BYDV isolate at left	MAV	HO
RMV	15	3	17
RMV	25	11	31
RMV	38	17	28
RPV	25	17	30
RPV	23	10	23
SGV	35	9	35
SGV	29	8	29

[a]Aphids were allowed a first acquisition feeding of 5 days on healthy oat plants (HO) or oats infected with 1 of 4 BYDV isolates (MAV, RMV, RPV, or SGV), followed by a 2-day acquisition on PAV-infected or healthy oats. Single aphids were then allowed a 5-day inoculation test feeding on Coast Black oat seedlings.

[b]None of 114 plants infested with aphids as controls became infected. Controls for RMV, RPV, and SGV indicated *M. avenae* did not transmit these isolates; but 137 of 141 aphids exposed only to MAV did transmit the MAV isolate. In tests with MAV and PAV, 251 of 266 plants became infected. Since *M. avenae* transmits both MAV and PAV, the plants infected with PAV were identified in index tests with *Rhopalosiphum padi* as described in the text. In these tests *R. padi* transmitted PAV from 75 of the 251 plants (to 225 of 762 plants). In tests with PAV and RMV, RPV, or SGV, all infected plants proved to be infected with PAV. In indexes of these plants *R. padi* transmitted PAV to 171 of 171 plants.

nymphs of *M. avenae* were injected with phosphate-buffered saline (PBS) or with MAV before 1-day acquisition feeding on PAV-infected leaves or on healthy leaves. Single aphids were then placed on oat seedlings. All plants infested with MAV-injected aphids became infected; they were subsequently tested to determine how many aphids had transmitted PAV.

In three experiments involving a total of 120 aphids per treatment, no difference occurred in PAV transmission among groups injected with PBS, or with MAV at 50 or 100 μg/ml; PAV transmission among the three groups was 72%, 69%, and 79%, respectively. In three additional experiments aphids were injected with PBS or with MAV at 170 μg/ml, before they fed on leaves of PAV-infected or healthy oats. From a total of 180 aphids per treatment, 64% transmitted PAV following injection of PBS, but only 18% transmitted PAV following injection of MAV at the high concentration. None of 83 plants infested as controls became infected.

In a second set of experiments, MAV and PAV were injected simultaneously into aphids. In these experiments PAV concentration was constant, and MAV concentration was varied. In three experiments, involving a total of 60 aphids per treatment, transmission of PAV by single aphids injected with PAV at 20 μg/ml and MAV at 0, 20, or 40 μg/ml was 18, 8, and 2%, respectively. In two additional experiments, with 40 aphids per treatment, PAV transmission by aphids injected with PAV at 20 μg/ml and MAV at 0, 70, or 140 μg/ml was 80, 10, and 5%, respectively. None of 20 plants infested as controls became infected.

These data show that PAV transmission was reduced when virus was injected into aphids as well as when acquired by feeding. This result suggests that MAV-PAV interference occurs after virus enters the hemocoel of the aphid, since injection by-passes the route of virus in the feeding apparatus and gut. The data also suggest that the level of interference depends on the amount of MAV present since PAV transmission decreased as MAV concentration was increased.

Factors that might affect MAV-PAV interference. Tests were carried out to study the effects of length of virus acquisition period, temperature during acquisition, and age of aphid on MAV interference in PAV transmission. Such information could be useful in improving experimental techniques, or in providing clues to possible mechanisms of interference. However, no consistent differences in MAV-PAV interference occurred. Reproducibility of results under all conditions tested illustrated the stability of the MAV-PAV interference.

To study short PAV acquisition feeding times, aphids (*M. avenae*) were allowed a 5-day first feeding on healthy oats, or RMV-infected oats, or on MAV-infected oats. The second feeding was on healthy oats or on PAV infected oats for 12, 24, or 48 hr. The MAV-PAV interference was consistent following 12-, 24-, or 48-hr feedings. The percentage transmission by 40 aphids fed on healthy oats, RMV-infected oats, or MAV-infected oats before a 12-hr feeding on PAV-infected leaves was 63%, 65%, and 23%; before a 24-hr feeding, 58%, 45%, and 13%; and before a 48-hr feeding, 43%, 38%, and 8%; respectively. At all feeding times transmission of PAV was reduced 50–80% in the presence of MAV.

A second set of experiments tested the effect of various inoculation feeding times on the MAV-PAV interference. Aphids were allowed a 2-day first acquisition on leaves of healthy oats, or MAV-infected oats, followed by a 2-day second acquisition feeding on healthy or PAV-infected leaves. Single aphids were transferred daily to new oat seedlings during the 5-day inoculation test feeding period. Each plant was subsequently tested to determine how many *M. avenae* had transmitted PAV. Of 15 aphids fed first on healthy oats, 7 transmitted PAV by day 3 of the inoculation feeding, but only 1 of 15 aphids fed first on MAV-infected oats transmitted PAV, and this only on the fifth day of inoculation feeding. None of eight plants infested as controls became infected. Most of the aphids molted during the inoculation feeding; this had no apparent effect on virus transmission.

In two experiments, aphids were allowed a 2-day acquisition on detached oat leaves infected with the MAV or RMV isolate, followed by a 1-day feeding on leaves of healthy or PAV-infected oats. The aphids were then placed individually on oat seedlings for inoculation feedings of 1, 3, or 5 days. Transmissions of PAV by 56 aphids in the RMV control group after 1, 3, and 5 days of inoculation test feeding were 5%, 29%, and 66%, respectively. In the MAV group corresponding PAV transmissions were 0%, 18%, and 30%. These data show that maximum PAV transmission did not occur until late in the 5-day inoculation feeding. There was no evidence that PAV "overcame" the MAV interference with increased inoculation-feeding time.

Two experiments involving 56 aphids per treatment were conducted to determine stability of the MAV-PAV interference when *M. avenae* acquired virus under different temperature regimes. Temperature can affect aphid transmission of some BYDV isolates (19). Aphids were allowed to feed 2 days on leaves of healthy or MAV-infected oats at either 15 or 25 C before a second feeding on leaves of healthy or PAV-infected oats at 15 or 25 C. For all temperature treatments PAV transmission was reduced by about 50% in the presence of MAV.

All prior experiments utilized fourth instar alatoid nymphs because of the ease with which this developmental stage could be identified, thus eliminating age of aphid as a variable among and within experiments. Usually these nymphs molted to adults during the first 24–48 hr of the inoculation test feeding. Newly emerged winged adults were found occasionally on the cage and had to be returned to the plant. Use of younger, less-mobile nymphs alleviated this problem. To determine whether MAV-PAV interference occurred in first and second instars, nymphs were compared with adults. Adult *M. avenae* were allowed to produce nymphs for 24 hr on detached leaves of healthy or MAV-infected oats. Adults were removed from the leaves and the nymphs were allowed to feed an additional 48 hr. At the same time fourth-instar alatoid nymphs were given a 48-hr feeding on leaves of healthy or MAV-infected oats. Each group of aphids was then given a 1-day second acquisition feeding on healthy or PAV-infected oats.

Results of three experiments (Table 3), indicated no difference in PAV transmission or in MAV-PAV interference between the age groups. Fourth instar nymphs, molting to adults during the first 24 hr of the inoculation test feeding, fed first on healthy or MAV-infected oats, transmitted PAV to 46% and 15% of the seedlings; young nymphs transmitted PAV to 57% and 16% of the test seedlings, respectively. None of 12 plants infested as controls became infected.

TABLE 3. Transmission of the PAV isolate of barley yellow dwarf virus (BYDV) by adults and nymphs of *Macrosiphum avenae* allowed a first acquisition feeding on healthy or MAV-infected oat leaves, before a second acquisition feeding on PAV-infected leaves

Experiment	No. of *M. avenae* adults or nymphs (of 28) that transmitted PAV when first acquisition feeding was as shown[a]			
	Adults		Nymphs	
	HO	MAV	HO	MAV
1	14	5	23	6
2	14	8	9	4
3	11	0	16	4
Total	39	13	48	14

[a]Fourth instar nymphs molting to adults during the first day of the inoculation test feeding were selected as adults; aphids 1- to 24-hr-old were selected as nymphs. Both groups were given 2-day acquisition feedings on detached leaves of healthy oats (HO) or oats infected with the MAV isolate of BYDV, followed by a second acquisition feeding of 1-day on leaves of healthy or PAV-infected oats. Single aphids were then placed on Coast Black oat seedlings for a 5-day inoculation test feeding. None of 33 plants infested with aphids as controls became infected, but 84 of 96 aphids exposed only to MAV transmitted the virus. In tests with MAV and PAV, 159 of 168 plants became infected. Since *M. avenae* transmits both MAV and PAV, plants infected with PAV were identified in index tests with *Rhopalosiphum padi* as described in the text. In these tests *R. padi* transmitted PAV from 59 of the 72 infected plants (to 185 of 216 plants). In plants).

Test for virus interference in *R. padi*. The possibility that interference between PAV and other BYDV isolates could occur in another aphid species was investigated in a series of experiments with *R. padi,* which regularly transmits both RPV and PAV. Aphids were allowed a 2-day first acquisition feeding on detached leaves from healthy oats, or oats infected with the MAV, RMV, or RPV isolate, followed by a second, 1-day feeding on PAV-infected or healthy oats. No appreciable differences in PAV transmission among treatments were found in four experiments (Table 4). From a combined total of 160 aphids in each treatment, the transmission of PAV by *R. padi* that had first fed on oats infected with RMV or RPV, on oats infected with MAV, and on healthy oats was 80%, 85%, and 78%, respectively. These data indicate no interference in *R. padi* between MAV and PAV, between RMV and PAV, or even between RPV and PAV, both of which are transmitted by *R. padi.*

DISCUSSION

At least two general mechanisms might explain the observed MAV-PAV interference in *M. avenae.* One is cross protection, a common virus interaction in plants that involves interference in replication of one virus by another virus previously established in the host. The PAV and MAV isolates represent related isolates that do show cross protection in plants, if the protecting isolate is allowed several days to become established before the challenge inoculation is made. No interference was found between RPV and PAV in *R. padi*; these two isolates are not serologically related and do not protect against each other in plants (1). The fact that the MAV-PAV interference occurred after simultaneous injection of MAV and PAV into aphids does not support a cross-protection type of mechanism. Moreover, there is no evidence for replication of these viruses in their aphid vectors (18,20). It is possible, however, that limited BYDV replication occurs with continuous excretion of virus by feeding aphids, thus preventing increase in virus titer within the vector (20).

We favor a second possible mechanism based on competition between virus isolates for receptor sites on membranes of aphid salivary glands. Recent work on the role of cell receptors in enterovirus infection provides examples of how such competition could occur (5). Attachment to and penetration of susceptible mammalian cells by enteroviruses was shown to be dependent upon genetically determined virus-specific receptors (15). Virus attachment is a cell-surface phenomenon involving recognition of the virus capsid by cell surface receptor proteins. Cells that lack the appropriate receptor are resistant to viral penetration. Attachment interference studies have demonstrated that serologically similar isolates compete for attachment to receptors specific for homologous serotypes of that virus (4). For example, when HeLa cells were first exposed to high levels of coxsackie virus B-3, the cells subsequently were unable to adsorb particles of coxsackie virus B-1, but attachment of poliovirus T-2 was unaffected. Similarly, poliovirus T-2 interfered with attachment of poliovirus T-1, but not with coxsackie B-3. Virus-receptor interactions of the same type were demonstrated for isolates of rhinoviruses (12).

There is some evidence that the salivary glands in various aphid species act as a barrier conferring vector specificity and regulating BYDV transmission (20,24). Perhaps only certain aphid species have the appropriate cell receptors on the salivary gland to recognize specific BYDV isolates and allow attachment, penetration, and eventual virus transmission. If this is the case, then MAV and PAV might share common receptors. In such a system, if most receptors were saturated with MAV, attachment and penetration by PAV would be inhibited and expressed as decreased PAV transmission. This mechanism is consistent with our data for MAV-PAV interference.

A similarity in coat-protein structure between MAV and PAV could be recognized by cell receptors, just as antibodies to MAV recognize, to a limited extent, the PAV isolate (1). The MAV-PAV interaction occurs only in *M. avenae* which transmits both viruses, and not in *R. padi* which transmits PAV but not MAV. *Macrosiphum avenae* may have common receptors for MAV and PAV, but *R. padi* may have receptors for PAV which do not recognize MAV. There is no reason to assume that receptors in different aphid species would recognize the same determinate factors on the virus capsid. Since no interaction occurs between RPV and PAV in *R. padi,* these serologically distinct isolates could be recognized by independent receptors and competition for sites would not occur.

The concept of specific receptor sites in insect vectors for nonpropagative plant viruses may help explain the nature of persistent transmission. In mammalian systems each cell possesses a limited number of virus-specific receptors that can be saturated by excess virus (13). If the number of salivary gland receptor sites is limited, the flow of virus through the salivary system of an aphid vector could be restricted, thus conserving virus in the hemocoel. This would prolong the time the vector remained viruliferous and help explain the ability of vectors to transmit nonpropagative viruses for long periods following a single acquisition feeding.

Results of transcapsidation studies are compatible with a receptor mechanism for specificity (21). *Rhopalosiphum padi* does not transmit the MAV isolate of BYDV when fed on MAV-infected plants, but frequently transmits MAV from plants infected with both MAV and RPV. The RNA of MAV apparently becomes incorporated into a capsid of RPV coat protein during simultaneous replication of the two viruses. If *R. padi* possesses receptors for RPV, all particles with RPV capsids could be recognized and transmitted regardless of their RNA content.

The receptor concept might also explain results of Harris et al (8), in which virus particles were found associated with the accessory glands of pea aphids that had acquired a transmissible isolate of pea enation mosaic virus, but not in aphids fed or injected with a nontransmissible virus isolate. It is now known that the nontransmissible isolate lacks a minor coat protein that is present in the transmissible isolate (2,10). Receptors may not recognize the nontransmissible isolate because of differences in coat protein structure.

Genetically determined cell receptors that regulate transmission of persistently borne viruses also could explain differences in transmission efficiency among biotypes or clones of aphids. These differences have been reported for a number of vectors, including several for BYDV (17,22,26). In a study of BYDV transmission by forms of *R. fitchii* (Sanderson) Orlob and Arny (17) presented evidence that virus-vector specificity was genetically fixed. Differences also occur among clones of *R. maidis* (26). Rochow and Eastop (22) reported differences between a New York clone

TABLE 4. Transmission of the PAV isolate of barley yellow dwarf virus (BYDV) by *Rhopalosiphum padi* allowed a first acquisition feeding on leaves of healthy oats (HO) or on leaves infected by one of three BYDV isolates before a second acquisition feeding on PAV-infected leaves

BYDV isolate tested[a]	No. of aphids (of 40) that transmitted PAV following first acquisition as shown[b]: BYDV isolate at left	MAV	HO
RMV	34	36	37
RMV	36	38	30
RPV	34	37	30
RPV	25	25	28

[a]Aphids were allowed a 2-day acquisition feeding on detached leaves of healthy oats (HO) or oats infected with the MAV, RMV, or RPV isolate of BYDV before a 1-day second acquisition feeding on PAV-infected or healthy oats. Single aphids were then allowed a 5-day inoculation test feeding on Coast Black oat seedlings.

[b]None of 63 plants infested with aphids as healthy controls became infected. When *R. padi* was exposed only to RPV as a control, virus was transmitted to 34 of 40 plants; in similar parallel controls, *R. padi* transmitted MAV to 1 of 60 and RMV to 1 of 12 plants. In tests with RPV and PAV, 72 of 80 plants became infected. Since *R. padi* transmits both RPV and PAV, plants infected by PAV had to be identified in index tests with *Macrosiphum avenae* as described in the text. In these tests *M. avenae* transmitted PAV from 59 of the 72 infected plants (to 185 of 216 plants). In tests with PAV and MAV or RMV all infected plants proved to be infected only with PAV.

and a Kansas clone of *R. padi* in transmitting the RMV isolate of BYDV. In parallel tests, the Kansas clone transmitted the RMV isolate more readily than did the New York clone. Slight morphological differences between clones indicated a degree of genetic difference. It was assumed that the difference in ability to transmit RMV represented a genetically controlled physiological trait. It may be that the Kansas clone had more receptors capable of recognizing RMV.

Genetically determined, virus-specific cell receptors that regulate transmission of persistent, circulative, nonpropagative plant viruses should be considered as a possible basis for the aphid-virus interaction.

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