Relationships Between Barley Yellow Dwarf and Beet Western Yellows Viruses

W. F. Rochow and James E. Duffus

Research Plant Pathologist, Agricultural Research Service, U.S. Department of Agriculture, also Professor of Plant Pathology, Cornell University, Ithaca, NY 14853; and Plant Pathologist, Agricultural Research Service, U.S. Department of Agriculture, U.S. Agricultural Research Station, P.O. Box 5098, Salinas, CA 93901.

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ABSTRACT

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When concentrated preparations of the MAV or PAV isolates of barley yellow dwarf virus (BYDV) were tested against antisera of four isolates of beet western yellows virus (BWYV), no reactions occurred in any of several kinds of tests. But the RPV isolate of BYDV consistently reacted with antisera (diluted 1:5) for the E-4, ST-1, and ST-9 isolates of BWYV. Reactions were detected both in antiserum absorption tests assayed by sucrose density gradient centrifugation, and in infectivity neutralization tests assayed by the serological blocking of virus transmission by aphids fed on treated inocula through membranes. Similar results occurred when treated virus preparations were assayed by injection into aphid vectors. With about 12 μg of RPV isolate, a reaction was detected in tests with antisera for the E-4 and ST-9 isolates of BWYV diluted up to 1:125. In comparative transmission tests, *Myzus persicae* occasionally transmitted the four isolates of BWYV to Coast Black oats. Transmission of the ST-9 isolate, for example, occurred in 4 of 5 experiments in which ST-9 was recovered from 11 of 23 inoculated oat plants, none of which developed symptoms. None of 128 attempts to transmit BWYV to oats by means of *Rhopalosiphum padi* or *Macrosiphum avenae* was successful, but *M. avenae* occasionally transmitted BWYV to shepherd's purse plants. None of 164 shepherd's purse plants became infected with the three isolates of BYDV. The relationship of the RPV isolate of BYDV to BWYV could have a special significance in epidemiology of these luteoviruses because of RPV's role as a helper virus in dependent virus transmission by aphids from mixed infections.

Barley yellow dwarf virus (BYDV) and beet western yellows virus (BWYV) have many features in common. Their similarities recently were recognized when both viruses were included in the new luteovirus group (17). Neither virus has been transmitted mechanically, probably because of confinement to phloem tissue of infected plants. Both are small, icosahedral particles, transmitted in the persistent or circulative manner by aphids. Both viruses induce yellows type symptoms, are widely distributed in nature, and cause diseases of much economic importance.

Two apparent differences between BYDV and BWYV are the plant host range and the aphid species that serve as vectors. In general, BYDV infects a wide range of monocotyledonous grains and grasses; whereas BWYV infects a wide range of dicotyledonous plants in many families. The other apparent difference, related to that of the divergent host range, is the aphid species that transmit each virus. *Myzus persicae* is the major vector of BWYV; it has also been reported to transmit BYDV (18), but is not known to be an important vector of that virus. Similarly, although the various aphid species that transmit BYDV are not considered to be vectors of BWYV, at least two of them (*Macrosiphum avenae*, previously called *M. granarium*, and *Acyrthosiphon dirhodum*) can also transmit BWYV (2). We think that similarities between the two viruses are much more striking than their dissimilarities.

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When antisera became available for several isolates of both BYDV and BWYV, we began a study of possible serological relationships between the two viruses. First we exchanged antisera; later we also exchanged aphid vectors and virus isolates. Here we report results of tests during a 5-yr period in New York, of preparations of three isolates of BYDV with various antisera for isolates of both viruses. We also describe the results of aphid transmission tests in New York with aphid species, virus isolates, and test plants for both the BWYV system as studied in California (2, 3, 4, 6, 7) and the BYDV system as studied in New York (1, 9, 12, 15). Preliminary reports of some of these studies have appeared (3, 14).

MATERIALS AND METHODS

Virus-free aphids were maintained in isolated rearing rooms on caged barley (*Hordeum vulgare* L.) plants under special precautions previously described (9). In most experiments the vectors used for barley yellow dwarf virus (BYDV) were *Macrosiphum avenae*

(Fabricius), the English grain aphid; and *Rhopalosiphum padi* (Linnaeus), the oat bird-cherry aphid. In some experiments *R. maidis* (Fitch) and *Schizaphis graminum* (Rondani) also were used. When *Myzus persicae* (Sulzer), the green peach aphid, was used in tests in New York, aphids were reared on radish (*Raphanus sativus* L.) under the same environmental conditions as the grain aphids. At least 40 aphids from each group of colonies used in every experiment were tested as controls.

The BYDV isolates were maintained by serial transmissions to oats (*Avena byzantina* C. Koch 'Coast Black') which also was the test plant used in all experiments. Isolates of BYDV are differentiated in part by their relative vector specificity (9). The MAV isolate is transmitted specifically by *M. avenae*; the RPV isolate is transmitted specifically by *R. padi*; the PAV isolate is transmitted by both species. Previous serological tests showed that RPV is distinct from MAV and PAV, which are related but not identical (1, 12). The isolates of BWYV were maintained by means of *Myzus persicae* in serial transfers to shepherd's purse [*Capsella bursa-pastoris* (L.) Medic.]. The four isolates of BWYV studied here (ST-1, ST-7, E-4, and ST-9) have been identified and described separately (3, 4, 6, 7).

Antisera specific for three isolates (MAV, RPV, and PAV) of BYDV were produced in New York as described previously (12). Antisera for the isolates of BWYV were produced in California (4, 5, 6), and shipped to New York for these tests. A control antiserum for the BYDV sera was made against a concentrate of healthy oats prepared in parallel with the virus isolates (12); it will be identified here as the HO antiserum. A similar control antiserum for the BWYV sera was prepared against a concentrate of healthy shepherd's purse (HSP). Serological reactions were evaluated in two ways. A biological assay of infectivity was made by means of the serological blocking of aphid transmission, a kind of neutralization-of-infectivity test previously described (8, 13). A physical assay of virions also was carried out in some experiments by layering a reaction mixture on linear sucrose gradients, centrifuging at 25,000 rpm in the SW-27 rotor of a Beckman centrifuge for 3-4 hr, and scanning the centrifuged gradients with the ISCO Model D density gradient fractionator to estimate the amount of nonreacted virus in the virus zone of the gradient (13). Sometimes the virus zone of the gradient was collected and used for infectivity tests by feeding the isolated sample to aphids through membranes (13). In a few cases, assays were also made by means of an aphid injection technique (9, 16).

In most experiments, 0.5 ml of a preparation of one of the three BYDV isolates was mixed with 0.5 ml of antiserum and incubated for 30 min at 37 C. All samples in each experiment contained the same amount of virus; the amount ranged from 5 to 50 μg in different experiments. Unless specified otherwise, antiserum was diluted 1:5 in 0.01 M potassium phosphate buffered saline (PBS), pH 7.0. In most experiments the reaction mixture

TABLE 1. Reaction of three isolates (RPV, MAV, and PAV) of barley yellow dwarf virus (BYDV) with antiserum specific for each of three isolates (ST-1, ST-7, and E-4) of beet western yellows virus, or antiserum specific for the BYDV isolates

BYDV isolate	Nonreacted virus (μg) following incubation with 1:5 dilution of antiserum or control shown[a]								
	PBS	HSP	ST-1	ST-7	E-4	HO	RPV	MAV	PAV
RPV	6	6	6	6	0	6	0	6	2
RPV	12	12	12	12	0	...[b]	0	...	...
MAV	6	6	6	6	6	6	6	0	0
MAV	11	11	11	11	11	...	...	0	...
PAV	4	4	4	4	4	4	4	0	0

[a]Amounts of virus were estimated by scanning centrifuged sucrose gradients that had been layered with virus-antiserum mixture previously incubated at 37 C for 30 min and stored at 4 C for about 18 hr. Controls were phosphate-buffered saline (PBS) and antiserum for a concentrate of healthy shepherd's purse (HSP) or healthy oat (HO) plants.

[b]Three dots mean that the combination was not tested.

TABLE 2. Reaction of three isolates (MAV, PAV, RPV) of barley yellow dwarf virus (BYDV) with antisera for each of two isolates (ST-9 and E-4) of beet western yellows virus, or antiserum for the RPV and MAV isolates of BYDV

BYDV isolate	Nonreacted virus (μg) following incubation with 1:5 dilution of antiserum or control shown[a]					
	PBS	ST-9	E-4	HO	RPV	MAV
MAV	...[b]	15	15	15	...	0
MAV	...	15	15	15	...	...
PAV	...	2	2	2	...	...
RPV	15	0	0	15	0	...
RPV	14	0	0	14	0	14
RPV	10	0	0	10	0	...
RPV	...	0	0	14	0	14

[a]Amounts of virus were estimated by scanning centrifuged sucrose gradients that had been layered with virus-antiserum mixture previously incubated at 37 C for 30 min and stored at 4 C for about 18 hr. Controls were phosphate buffered saline (PBS) and antiserum for a concentrate of healthy oats (HO).

[b]Three dots mean that the combination was not tested.

was stored for about 18 hr at 4 C before assay. The reaction mixture often was centrifuged for 10 min at 4,800 rpm in a Sorvall SP centrifuge at 4 C and the supernatant liquid used for assay. Results were the same whether or not samples were clarified by centrifugation (13). When the antigen-antibody reaction mixture was assayed by testing for the serological blocking of aphid transmission, the mixture was combined with an equal amount of 40% sucrose in 0.1 M potassium phosphate buffer (9, 12). Aphids were allowed to feed on the buffered antigen-antibody mixture through stretched Parafilm for about 18 hr at 15 C and then transferred to Coast Black oat seedlings (10 aphids per seedling) for a 5-day inoculation test feeding period at 21 C. Assays for the MAV isolate were done with *M. avenae*; *R. padi* was used for assays of the RPV and PAV isolates.

In aphid transmission tests, acquisition feeding was for 2 days at 15 C on detached leaves. Inoculation test feeding, with 10-20 aphids per plant, was for 5 days at 21 C in a growth chamber (9). When plants inoculated with BYDV were later tested for presence of virus, *R. padi* (for RPV and PAV) or *M. avenae* (for MAV) were permitted to feed for 2 days on one or more leaves from the plant to be tested, and then transferred for the 5-day inoculation test feeding to Coast Black oats. In tests for the presence of the BWYV isolates, *Myzus persicae* was the vector and shepherd's purse was the test plant.

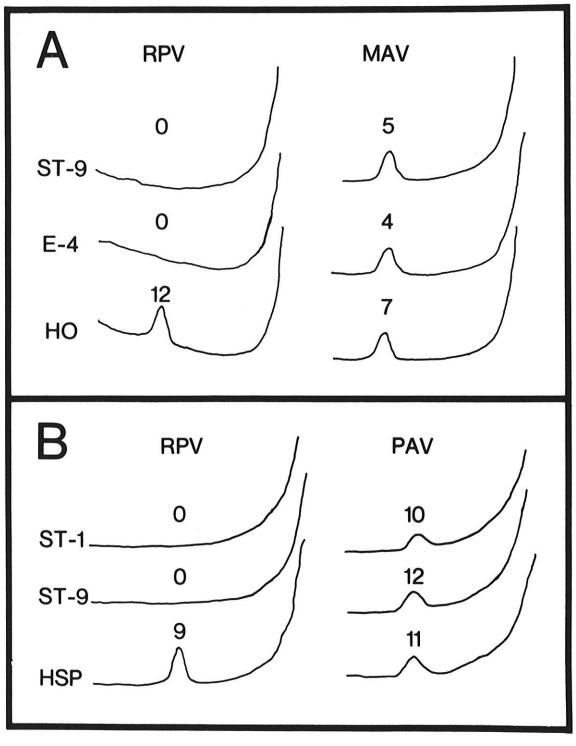

Fig. 1-(A, B). Portion of sucrose gradient scanning patterns, showing virus zones, and results of infectivity tests of the zones. Concentrates of one of three isolates (RPV, MAV, or PAV) of barley yellow dwarf virus (BYDV) had been incubated with antiserum (diluted 1:5) for the ST-1 (ST-1-2 serum of Table 3), E-4, or ST-9 isolates of beet western yellows virus for 2 hr at 37 C, kept at 4 C for 20 hr, and then centrifuged for 3.5 hr at 25,000 rpm in the SW-27 rotor of a Beckman centrifuge. Numerals above the virus zones are numbers of oat test plants that became infected, of 12 infested, following feeding by 10 aphids (*Rhopalosiphum padi* for RPV and PAV; *Macrosiphum avenae* for MAV) that had previously fed through membranes on a 2-ml sample from the virus zone of each tube. None of 48 plants infested as controls became infected. **A)** About 14 μg of RPV were incubated with each of the three antisera shown at left. Parallel tests were made with MAV at the same concentration. Antiserum prepared against a concentrate of healthy oat (HO) plants served as control. **B)** Each of the three RPV samples contained about 14 μg of virus; about 10 μg of PAV were used in the parallel tests with antisera shown at left. Antiserum prepared against a concentrate of healthy shepherd's purse (HSP) plants served as control.

RESULTS

Serological tests.—In the first series of experiments, 0.5 ml of a preparation of one BYDV isolate was mixed with 0.5 ml of each antiserum. After incubation at 37 C for 30 min, and at 4 C for 18-20 hr, one part of each virus-antiserum mixture was assayed by density gradient centrifugation. Parallel tests usually were done with antisera specific for the ST-1, ST-7, and E-4 isolates of BWYV, together with antisera for each of the three BYDV isolates. Controls included virus incubated with PBS, virus incubated with the HSP antiserum, and virus incubated with HO antiserum. None of the BWYV antisera reacted with either MAV or PAV, but RPV was no longer detectable after treatment with antiserum for the E-4 isolate of BWYV (Table 1). In agreement with past results (1, 12), antiserum for MAV did not react with RPV and that for PAV reacted slightly. Anti-MAV serum reacted with PAV and vice versa (1). Each BYDV antiserum reacted in homologous tests.

The other part of most reaction mixtures was assayed by the serological blocking of virus transmission by aphids. Transmission of MAV or PAV was not reduced by any of the three BWYV antisera. For example, in one series of six experiments with MAV, 12 test plants were infested with aphids in assays of each sample. None of the 72 plants became infected when 10-50 μg of MAV per ml had been incubated with homologous antiserum. In tests with antiserum for the ST-1, ST-7, and E-4 isolates of BWYV, the numbers of infected plants were 68, 71, and 71, respectively. Aphids fed on virus samples incubated with the control HSP antiserum transmitted MAV to 67 plants. All 72 plants infested following treatment with PBS became infected. In a similar series of four experiments with PAV, the numbers of infected plants (of 48 infested) were 31, 24, 33, 32, and 39, for treatments with HSP, PBS, ST-1, ST-7, and E-4, respectively. None of 240 plants infested with aphids as controls became infected.

Although none of the antisera for BWYV reduced infectivity of MAV or PAV, the antiserum for E-4 did appear to reduce infectivity of RPV. In the first six experiments with RPV, for example, the numbers of infected plants (of 72 infested) were 63, 69, 59, and 64, respectively, following incubation with HSP, PBS, ST-1, and ST-7 antisera. In parallel tests with E-4 antiserum, however, only 28 of 72 plants became infected. Because of the apparent reaction between RPV and antiserum for the E-4 isolate of BWYV, additional infectivity tests were carried out for RPV.

In 21 experiments the relative infectivity of RPV was estimated following incubation with antiserum for the E-4 isolate, with RPV antiserum, and with PBS as control. Although the E-4 antiserum completely neutralized RPV in only 4 of the 21 tests, infectivity of RPV treated with E-4 antiserum was lower than that of the control in 20 of the 21 comparisons. Of the total of 252 plants infested, 200 became infected when RPV had been incubated with PBS; 72, with E-4 antiserum; and only one, with RPV antiserum. None of 120 plants infested with aphids as controls became diseased. Although the reactions of RPV and antiserum for the E-4 isolate of BWYV in these infectivity tests was less striking than those in the assays made by sucrose gradient centrifugation, the consistent reduction of infectivity also indicates a relationship between E-4 and RPV.

The difference in the relationship between MAV and RPV to the E-4 antiserum also was apparent in a serological test assayed by means of aphid injection. In this test, reaction mixtures (10 μg of MAV or 12 μg of RPV, with various antisera diluted 1:5) were clarified by centrifugation and portions of the supernatant liquid were injected into *M. avenae* (for MAV) or *R. padi* (for RPV). None of the 12 plants (each infested with five injected aphids) became infected following incubation of MAV with MAV antiserum. All 12 plants became infected with MAV following treatment with the E-4 antiserum; following treatment with the HO and the HSP antisera the numbers of infected plants were 8 and 7, respectively. In parallel tests with RPV the numbers of infected plants were 9 and 3 for the HO and HSP antisera, respectively. No plants became infected when RPV had been treated with either RPV antiserum or E-4 antiserum. None of 24 plants infested with aphids as controls became infected.

We did another series of experiments with antiserum against a fourth isolate of BWYV, ST-9, and a different E-4 antiserum that had a higher homologous titer than that used in the first series of tests (5). When incubated virus-antiserum mixtures were assayed by sucrose gradient centrifugation, no reactions were detected between MAV or PAV and either of the BWYV antisera (Table 2). In all experiments with RPV, however, 1:5 dilutions of each of these two BWYV antisera completely removed 10-15 μg of RPV (Table 2). No evidence for relatedness between MAV and ST-9 or E-4 was found in several tests based on the serological blocking of virus transmission by aphids fed on reaction mixtures. In tests with RPV, antisera for both E-4 and ST-9 completely prevented or greatly reduced transmission of RPV in tests with *R. padi*. The difference between the reaction of RPV and MAV with these two antisera was clearly shown in an experiment in which the serological reaction was measured both by sucrose gradient centrifugation and by acquisition-feeding portions of the sucrose gradients collected from the virus zone to vector aphids (Fig. 1-A).

One explanation for lack of reaction between RPV and antisera for the ST-1 and ST-7 isolates of BWYV is low titer of these two sera. It seemed likely that the nonreacting BWYV sera simply had lower homologous titers than sera that did react with RPV, an observation confirmed in parallel tests in California (5). This possibility was studied in tests with two "new" antisera for the ST-1 isolate that had a higher homologous titer than the previously used isolate. Each of these two sera (ST-1-2, ST-1-3) came from a separate rabbit. These two ST-1 sera were used in tests together with the antiserum for ST-9 and E-4 (Table 3). In both sucrose gradient and membrane feeding assays, antisera for the three BWYV isolates reacted with RPV, but no clear evidence for a relationship with MAV was found. In another experiment, RPV (2 μg of virus per ml) was completely neutralized by both of the ST-1 antisera, as well as by those for E-4 and ST-9. None of 96 plants infested with *R. padi* that had fed through membranes on reaction mixtures containing either of the four antisera became infected, but 15 of 24 plants became infected when aphids had fed in parallel tests on mixtures of RPV and HSP antiserum, and 10 of 24 plants became infected following feeding on mixtures of RPV and PBS. None of 24 plants infested with control aphids became infected.

Although both "high-titer" antisera for ST-1 reacted with RPV, they did not react with PAV. The difference between the reaction of RPV and PAV was illustrated in

TABLE 3. Reaction of two isolates of barley yellow dwarf virus (BYDV) with antiserum for four isolates of beet western yellows virus assayed both by sucrose gradient centrifugation and by infectivity tests

BYDV isolate	Nonreacted virus (μg) after incubation with 1:5 dilution of antiserum or control shown, and infectivity (in parentheses) of sample collected from virus zone of sucrose gradient[a]					
	PBS	HSP	ST-1-2	ST-1-3	ST-9	E-4
RPV	12	12	0	0	0	0
	(12)	(12)	(0)	(0)	(0)	(0)
RPV	26	26	0	0	0	0
	(12)	(12)	(0)	(1)	(0)	(1)
MAV	14	9	6	12	12	7
	(12)	(12)	(12)	(12)	(12)	(12)
MAV	32	25	18	30	28	21
	(12)	(12)	(12)	(12)	(12)	(12)

[a]Numerals represent micrograms of virus estimated by photometric scanning of centrifuged sucrose gradient tube; numbers in parentheses are numbers of plants that became infected, of 12 infested, following acquisition feeding by aphids through membranes on virus zone portion of the sucrose gradient collected during scanning. About 10 aphids fed on each plant. *Rhopalosiphum padi* was the vector for all tests except the ones with MAV, for which *Macrosiphum avenae* was the vector. Controls were phosphate-buffered saline (PBS) and antiserum against a concentrate of healthy shepherd's purse (HSP) plants. None of 36 plants infested as aphid controls became infected.

an experiment that compared the two virus isolates in tests with the ST-1-2, ST-9, and HSP sera. Two kinds of assays showed the lack of reaction with PAV, and the completeness of the parallel reaction between both BWYV antisera and RPV (Fig. 1-B).

All of the tests described above were done with BWYV antisera diluted 1:5. Tests with more dilute E-4 and ST-9 antisera showed the relatively low titer of the sera for reactions with RPV, and illustrated the difference in sensitivity of the physical and biological assays. In an experiment with RPV and E-4 antiserum, for example, antiserum dilutions of 1:5, 1:25, and 1:125 each removed 10 μg of RPV, as measured by scanning centrifuged gradients. But in parallel tests with *R. padi* fed through membranes on the virus-antiserum mixtures, the numbers of infected plants (of 12 infested) for each of the three reactions were 5, 9, and 9, respectively.

We next studied reactions of six serial dilutions of both E-4 and ST-9 antisera with 4 μg of RPV. Transmission of RPV by *R. padi* was reduced by the E-4 antiserum diluted 1:5 and 1:25, but only the 1:5 dilution of ST-9 antiserum clearly reduced transmission of RPV. A similar relationship between titer of these two sera was shown in another experiment when both kinds of assay were carried out (Table 4). Parallel tests with the three isolates of BYDV and their homologous antisera showed the large difference between titers of the homologous reactions and those of the heterologous reactions between RPV and BWYV antisera (Table 4).

Because of our interest in the possibility that some treatment might alter vector specificity of an isolate of BYDV, we studied the occasional plants that became infected with virus incubated with various antisera. We were interested especially in the possibility that some selection or alteration of RPV might have occurred as a result of treatment with antisera for the BWYV isolates. We found no evidence for such change. For example, in tests of 30 oat plants infected by RPV previously incubated with various antisera, subsequent comparative transmissions by means of four aphid species had the typical RPV transmission pattern: 90 of 90 test plants became infected in tests with *R. padi*, 50 of 90 plants became infected in parallel tests with *S. graminum*, 0 of 90 plants became infected in tests with *R. maidis*, and 4 of 90 became infected in tests with *M. avenae*. Similarly, vector specificity of MAV was not altered by any of the treatments in 28 other tests. In these, *M. avenae* transmitted virus to 84 of 84 plants, *R. padi* to 2 of 84, *R. maidis* to 0 of 84, and *S. graminum* to 5 of 84 plants. None of 48 plants infested with aphids as controls became infected.

Tests of infectivity assay method.—In most experiments the complete mixture of virus and antiserum was diluted with 40% sucrose for use in membrane-feeding assays. In some cases, the reaction mixture was centrifuged to remove precipitate, and only the clarified supernatant liquid was used in the assay. In previous work such clarification did not affect results (13). It seemed possible, however, that aphids feeding through membranes on certain inocula still containing the virus-antibody complexes might somehow reverse the reaction and acquire virus that had previously reacted with antibody. To test this possibility, several experiments were done with RPV and various antisera. We divided each reaction mixture into two equal portions after incubation at 37 C; one portion was clarified by centrifugation and one was not. In three experiments the reaction mixtures were stored at 4 C for 1, 2, or 24 hr before the aphids fed on them. Results were generally similar for both clarified or nonclarified samples whether compared for each separate experiment or for all three experiments combined (Table 5).

Another kind of test carried out to study the fidelity of the membrane-feeding assay concerned storage of samples at 4 C between completion of the reaction at 37 C and use in assay. In one test, each of two concentrations of MAV (1 or 5 μg per ml) was incubated with MAV antiserum diluted 1:10, 1:50, or 1:250. A virus sample was

TABLE 4. Reactions of the RPV isolate of barley yellow dwarf virus (BYDV) with various dilutions of antisera against two isolates of beet western yellows virus (E-4 and ST-9), and reactions of three isolates (RPV, MAV, and PAV) of BYDV with their homologous sera at various dilutions, all assayed both by sucrose gradient centrifugation and by infectivity tests

BYDV isolate	Anti-serum	Nonreacted virus (μg) after incubation with antiserum at dilution shown or with phosphate buffered saline (PBS) as control; and (in parentheses) infectivity of sample collected from virus zone of gradient[a]						
		1:5	1:25	1:125	1:625	1:3,125	1:15,625	PBS
RPV	E-4	...[b]	0	2	9	12	12	12
		...	(0)	(12)	(11)	(11)	(11)	(12)
RPV	ST-9	0	2	9	12	12	...	12
		(0)	(9)	(12)	(12)	(12)	...	(12)
RPV	RPV	...	0	0	0	1	4	12
		...	(0)	(0)	(2)	(9)	(12)	(11)
MAV	MAV	...	0	0	0	7	12	16
		...	(0)	(0)	(1)	(12)	(12)	(12)
PAV	PAV	...	0	0	1	5	7	10
		...	(0)	(0)	(3)	(11)	(12)	(12)

[a]Numerals are micrograms of virus estimated by scanning centrifuged sucrose gradient tube; numbers in parentheses are numbers of plants that became infected, of 12 infested, following acquisition feeding by aphids through membranes on virus zone portion of the sucrose gradient collected during scanning. About 10 aphids fed on each plant. *Rhopalosiphum padi* was the vector for all tests except the one with MAV where *Macrosiphum avenae* was the vector. None of 60 plants infested as controls became infected.

[b]Three dots indicate that the combination was not tested.

also incubated with PBS as control. The reaction samples were thoroughly mixed at the end of the 30 min incubation at 37 C and divided into two equal portions. One portion was assayed immediately; the other was stored for 24 hr at 4 C. No virus was transmitted in tests of any treatments with antiserum diluted 1:10. When 5 μg of MAV had been incubated with antiserum diluted 1:50 and assayed immediately, 1 of 12 plants became infected. In the parallel test with the stored portion of the sample, 2 of 12 plants became infected. Results also were similar for fresh and stored samples of both virus concentrations incubated with antiserum diluted 1:250. Six of 24 plants became infected following tests of samples not stored; 7 of 24 plants became infected following tests of stored samples. All 24 plants became infected for both treatments with the PBS control.

Aphid transmission tests.—Because of the clear reaction of RPV with the antisera for the BWYV isolates, and because of the parallel reactions among the three BYDV antisera and all of the BWYV isolates being tested at the same time in California (5), we also compared the viruses in a series of transmission experiments carried out during a period of about 2 yr. We studied the ability of *R. padi*, *M. avenae*, and *Myzus persicae* to transmit four isolates of BWYV and three isolates of BYDV to the test plants regularly used for each of the separate virus groups.

Myzus persicae occasionally transmitted each of four BWYV isolates to Coast Black oats. None of 120 infested oats developed symptoms; infection was detected when *Myzus persicae* fed on the inoculated oats transmitted BWYV to shepherd's purse. The ST-1 isolate of BWYV was recovered from 9 of 26 tested oat plants (in 2 of 5 experiments); the E-4 isolate was recovered from 1 of 26 plants (in 1 of 5 experiments); and the ST-7 isolate was recovered from 1 of 26 plants (in 1 of 5 tests). In tests with the ST-9 isolate, virus was recovered from 11 of 23 oat plants in 4 of 5 experiments. Although these occasional transmissions of the BWYV isolates to oats occurred in tests with *Myzus persicae*, no transmissions occurred in parallel tests with *R. padi* or *M. avenae*. In tests with *M. avenae*, for example, none of 56 inoculated oats developed symptoms in tests with the four isolates of BWYV; no BWYV was recovered from 36 of the inoculated plants. In similar tests with *R. padi*, none of 72 inoculated oats developed symptoms; none of the four BWYV isolates was recovered from any of 52 plants tested by means of *Myzus persicae* and shepherd's purse plants.

TABLE 5. Transmission of the RPV isolate of barley yellow dwarf virus by *Rhopalosiphum padi* that had fed through membranes on clarified (centrifuged) or nonclarified virus-antiserum mixtures

Antiserum or control[a]	Plants that became infected, of 36 infested, following aphid acquisition feeding through membranes on preparation shown[b]	
	Clarified (no.)	Nonclarified (no.)
E-4	8	4
ST-9	2	3
PBS	25	21
HO	27	24
RPV	0	1
MAV	23	23
PAV	22	18

[a]Antisera were for the E-4 and ST-9 isolates of beet western yellows virus, and the RPV, MAV, and PAV isolates of barley yellow dwarf virus. Controls were phosphate buffered saline (PBS) and an antiserum against a concentrate of healthy oats (HO).

[b]None of 36 plants infested as aphid controls became infected.

In tests with the four BWYV isolates in New York, *Myzus persicae* regularly transmitted virus to shepherd's purse. For example, in one series of experiments, 159 of 167 infested plants became infected. In parallel tests with *R. padi*, none of 92 plants inoculated with the four isolates became infected. *Macrosiphum avenae* did effect occasional transmissions of some of the BWYV isolates to shepherd's purse plants. In tests with the ST-1 isolate, none of 22 plants infested with *M. avenae* became infected; but 2 of 22 plants became infected in similar tests with *M. avenae* and the E-4 isolate and the same level of transmission occurred in tests with the ST-9 isolate. In tests with the ST-7 isolate, *M. avenae* transmitted virus in 3 of 6 experiments to 7 of 24 shepherd's purse plants. Transmission of BWYV by *M. avenae* has been reported previously (2).

Although the BWYV isolates did occasionally infect oat plants, the BYDV isolates were not transmitted to those of shepherd's purse. We used *Myzus persicae, M. avenae,* and *R. padi* in various attempts to transmit RPV, MAV, or PAV to shepherd's purse. None of the infested plants developed symptoms, and no virus was recovered from any of 164 shepherd's purse plants by *R. padi* or *M. avenae* transferred to Coast Black oats. In parallel control tests with *R. padi* and the RPV isolate or the PAV isolate, almost every inoculated Coast Black oat plant became infected. Similarly, MAV was regularly transmitted in parallel inoculations by *M. avenae* to Coast Black oats. *Myzus persicae* proved to be an erratic vector of each of the three BYDV isolates in transmissions from oats to oats: with RPV, 4 of 56 infested oat plants became infected; with MAV, 6 of 46 plants developed symptoms; and with PAV, 2 of 40 plants became infected. None of 188 plants infested with control aphids in these various transmission experiments became infected.

In one series of experiments, *Myzus persicae* were injected with concentrated inoculum of either the RPV or MAV isolate of BYDV. When 5 or 10 injected aphids were placed on each oat seedling, RPV or MAV was transmitted in most of the experiments. But few plants became infected, and virus transmission was erratic compared to that by the usual vector species. In three experiments with RPV, 5 of 20 plants infested with injected *Myzus persicae* became infected. In similar experiments with MAV, 4 of 12 infested plants became infected. Tests were made of these 9 infected plants to try to determine whether any change in the vector specificity of the isolates might have occurred. No evidence for any change was found. In tests of the five RPV-infected plants, for example, *R. padi* transmitted virus from all of them (to 15 of 15 plants), but only a single transmission by *M. avenae* occurred (to 1 of 15 plants). In tests of the four MAV-infected plants, *M. avenae* transmitted from all (to

12 of 12 plants), but *R. padi* failed to transmit virus (0 of 12 plants). None of 36 plants infested as controls became infected.

These aphid transmission tests confirm previous observations about common vectors of BYDV and BWYV. The results show that oats are susceptible to BWYV, an observation of potential importance in epidemiology. Previously, BWYV was known to infect a wide range of plants in many families, but the susceptible species were all dicotyledonous plants. Susceptibility of oats extends this BWYV host range to monocotyledonous plants, the area of widespread susceptibility to BYDV. Moreover, the data also show that the RPV isolate of BYDV is not identical with BWYV despite its serological similarity.

DISCUSSION

Previous direct serological comparisons among the RPV, MAV, and PAV isolates of BYDV showed that RPV was distinct from the other two, which were related, but not identical (1, 12). The present data are in agreement with this relationship: RPV was serologically related to at least three isolates of BWYV, but MAV and PAV were not. A similar picture emerged from a separate study in which antisera for two strains of soybean dwarf virus reacted with RPV, but not with MAV or PAV (3). Results of the current companion study with BWYV antigens (5) also showed that RPV was more closely related to BWYV than MAV or PAV. But all three BYDV antisera at low dilutions neutralized BWYV (5). The data suggest at least a distant serological relationship among the three BYDV isolates, a finding supported in part by an early observation that PAV antiserum diluted 1:5 appeared to react partially with RPV in some absorption tests (1).

The data reported here confirm and extend the growing list of luteoviruses known to be serologically related (5). We think the inclusion of the RPV isolate of BYDV in this list could have special significance for epidemiology of this important group of viruses because RPV acts as a helper virus in the dependent transmission of the MAV isolate from mixed infections.

Although *R. padi* does not regularly transmit the MAV isolate from oats infected only with MAV, it often transmits MAV, together with RPV from doubly-infected plants (10, 11). The basis for this dependent transmission of MAV by *R. padi* appears to be heterologous encapsidation during simultaneous replication of RPV and MAV in the doubly-infected plant. In mixed infections, some virions apparently are formed containing the nucleic acid of MAV encapsidated with RPV protein. Such transcapsidated virus particles appear to function in *R. padi* like RPV (because of the RPV protein), but they function in the plant like MAV (because of the MAV nucleic acid). Heterologous encapsidation may be a simple mechanism responsible for variations in aphid-virus interactions (10, 11). Delivery of a virus nucleic acid to a plant host by an aphid vector that transmits virus in the circulative or persistent manner may vary with, and be controlled by, packaging of the nucleic acid. This packaging could change as a result of mixed virus infections. Similarities among protein capsids of the luteoviruses discussed here emphasize the potential of virus interactions in natural mixed infections.

The relationship between the RPV isolate of BYDV and the various isolates of BWYV suggests that the barley yellow dwarf and beet western yellows diseases may not be so distinct as previously thought. Perhaps these two diseases, and others caused by luteoviruses, are really all part of one system. The serological similarities discussed here, the susceptibility of oats to BWYV, and the known common vector species of the two virus systems all suggest that our previous view of these diseases may have been too narrowly restricted by the crop-orientation of many of us who studied those diseases.

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