Importance of Virus Source Leaves in Vector Specificity of Barley Yellow Dwarf Virus

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ABSTRACT

The age of leaf from which aphids acquired virus affected virus transmission in tests with two isolates of barley yellow dwarf virus, but not with a third. Although the vector *Macrosiphum avenae* transmitted the MAV isolate efficiently from all leaves, *Rhopalosiphum padi* was much more likely to effect an occasional transmission of MAV from young leaves than from old. In tests with the PAV isolate, *R. padi* transmitted virus efficiently from all leaves, but *M. avenae* transmitted PAV regularly only from young leaves. When concentrated preparations were made from the two kinds of leaves, virus titers were higher for both MAV and PAV in preparations of young leaves than in those of old. The average yield of MAV (per 100 g of tissue) was 89 μg from young leaves and 9 μg from old. Corresponding yields of PAV were 20 μg from young and 6 μg from old leaves. When fed through membranes, *M. avenae* more frequently transmitted PAV acquired from preparations of young leaves than of old ones; the relationship was similar for occasional transmission of MAV by *R. padi*.

In corresponding tests with the RPV isolate, *R. padi* transmitted the virus regularly from all leaves, but *M. avenae* was no more likely to transmit RPV from young than from old leaves. Differences were not consistent in virus titer of preparations made from each kind of leaf. The average yield of RPV was 29 μg from 100 g of young leaves and 23 μg from old ones. When aphids were fed on concentrates of each kind of leaf, *M. avenae* was no more likely to effect an occasional transmission from one kind than the other.

Virus concentration affected vector specificity, but the amount of virus needed to alter specificity varied with each virus isolate and aphid species.

In tests to evaluate use of liquid nitrogen in extraction of BYDV from plants, no more virus was found in RPV preparations made from tissue extracted with liquid nitrogen than in those from parallel preparations made without it. Preparations of the MAV isolate made without liquid nitrogen contained about 47% more virus than parallel preparations made with it.

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Barley yellow dwarf virus (BYDV) is an aphid-transmitted, circulative, persistent plant virus for which vector specificity is pronounced (5, 10). Since this specificity in virus-vector relationships is stable and consistent, isolates of BYDV have been differentiated by selective transmission by particular aphid species. For example, one isolate (MAV) is transmitted specifically by *Macrosiphum avenae* (F.), a second isolate (RPV) is transmitted specifically by *Rhopalosiphum padi* (L.), and a third (PAV) is transmitted by both species (9). Because many factors can influence any of the three biological systems that interact when a virus is transmitted to a plant by an aphid, it is not surprising that the specificity is relative and not absolute (10). Studies of these factors facilitate an understanding of the specificity and provide a basis for study of its mechanism. Major factors previously found to influence BYDV specificity include length of time aphids feed on plants, temperature during acquisition and inoculation test feeding, variation among clones of an aphid species, and the presence of more than one virus isolate in source plants.

Length of the acquisition feeding period often influences vector specificity. For example, when *R. padi* were reared on MAV-infected plants, the aphids were more likely to effect an occasional transmission of MAV than when 2-day acquisition feeding periods were used (10). The effect of temperature is pronounced for certain virus-vector combinations. In one study (9), the RMV isolate which is transmitted efficiently by *R. maidis* (Fitch), was transmitted by *M. avenae* rarely at 15 or 20 C, but regularly at 30 C. But other vector species were not able to transmit RMV at the high temperature, and transmission of RPV by *M. avenae* was not enhanced by the high temperature.

Variation among clones of an aphid species can be a critical factor. Studies on variations among clones have included work with *Schizaphis graminum* (Rondani) (8), *R. maidis* (15), and *R. padi* (7, 14). Sometimes clones are active or inactive as vectors of all isolates of BYDV tested; in other instances, transmission abilities among aphid clones varied with the virus isolate tested. The presence of more than one isolate of BYDV in source plants also determines which virus isolate can be transmitted by some aphids (11). Although *R. padi* rarely transmits MAV from singly-infected plants, it regularly transmits MAV, together with RPV, from plants infected by both viruses. A similar instance of dependent virus transmission occurs for *R. maidis*. *R. maidis* almost never transmits MAV alone, but it often transmits MAV, in combination with RMV, from doubly infected plants. Use of such interactions has especially helped us study the mechanism of vector specificity (11).

The source leaf from which vectors acquire virus is important for many virus-vector systems (6, 16, 17). The likelihood of virus transmission by vectors is often increased when vectors acquire virus from young leaves with high virus titer. Gill (4) has shown the importance of the interval between inoculation of plants and their use as sources of BYDV in transmission experiments. But little is known about interactions among age of leaf, isolate of virus, and vector species. In some preliminary work, *M. avenae* was more likely to transmit an isolate of BYDV from young leaves than from old ones (12), an observation that originated in earlier work (7).

The purpose of this study was to evaluate the importance of the age of leaf from which aphids acquire virus in relation to the specificity in virus-vector relationships among three isolates of BYDV.

MATERIALS AND METHODS.—Stock colonies of the aphid species *R. padi* and *M. avenae* were maintained on caged barley (*Hordeum vulgare* L. 'Catskill') by special precautions to minimize the possibility of accidental contamination by BYDV or aphid parasites (9). The clone of each aphid species used was the same as that of previous studies in this laboratory. Some aphids from each colony used were always tested as controls in every experiment.

The virus isolates were maintained by serial transfer to oats (*Avena byzantina* C. Koch 'Coast Black'), the test plant used in all experiments. Two of the virus isolates, MAV and RPV, are vector specific; they are transmitted specifically by *M. avenae* and *R. padi*, respectively (9). Since the specificity is relative, occasional transmissions of MAV by *R. padi* and of RPV by *M. avenae* do occur, but they are rare and inconsistent. We will refer to *M. avenae* as the "nonvector" of RPV and to *R. padi* as the "nonvector" of MAV. The third virus isolate, PAV, is transmitted efficiently by *R. padi* and inefficiently by *M. avenae*. In one study, about 60% of *R. padi* and about 20% of individual *M. avenae* transmitted PAV (9). Considering the two aphid species and the three virus isolates together, their relationship to each other will be described in this study as follows: *M. avenae* is the vector of MAV, the "nonvector" of RPV, and the inefficient transmitter of PAV; *R. padi* is the vector of RPV, the "nonvector" of MAV, and the efficient transmitter of PAV.

Virus source leaves were considered to be either young or old. Young leaves, representing either the fourth or fifth ones on the major shoot of BYDV-infected Coast Black oats, 5-6 weeks old, were the youngest fully expanded leaves that had not yet developed symptoms. Old leaves were from either the second or third position on the same shoots; they had clear symptoms of infection. In most comparisons, 23 leaves of each age were collected in the greenhouse, rinsed in tap water, and placed in plastic dishes for acquisition feeding by "nonvector" aphids. A few corresponding leaves were infested with vectors as controls in each experiment. For tests with PAV, each leaf was divided in half to permit parallel testing with both aphid species on all leaves. Acquisition feeding was for 2 days at 15 C in the dark. In most tests 10 aphids were transferred to each of four oat test seedlings in a 10-cm pot for an inoculation test feeding of 5 days at 21 C. Plants were then fumigated and grown in a greenhouse as described previously (9).

To estimate virus concentration in extracts of leaves, we extracted 100-150 g of each kind of leaf, usually immediately after leaves were harvested. In a few experiments, harvested leaves were stored in a freezer before processing. Preparations made from stored samples contained fewer impurities than those made from fresh samples, but no other differences were observed. Most virus preparations were made by extracting virus from tissue frozen with liquid nitrogen. Leaves were frozen in liquid nitrogen and, while still frozen, ground in a hand-operated meat grinder. The ground tissue was then refrozen in liquid nitrogen in a large mortar and

TABLE 1. Virus transmission by *Macrosiphum avenae* and *Rhopalosiphum padi* following feeding on young or old leaves infected by barley yellow dwarf virus (BYDV)

BYDV isolate[a]	Age of source leaf	Transmission[b] by aphid species shown, following feeding on detached leaves	
		M. avenae	*R. padi*
MAV	young	24/24	63/462
	old	24/24	4/462
PAV	young	106/123	123/123
	old	33/123	115/123
RPV	young	17/462	18/21
	old	8/462	21/21

[a]BYDV isolates MAV and RPV are transmitted specifically by *Macrosiphum avenae* and *Rhopalosiphum padi*. Isolate PAV is transmitted efficiently by *R. padi* and inefficiently by *M. avenae*.

[b]Numerator is number of plants that became infected; denominator is number of plants each infested with 10 aphids for a 5-day inoculation test feeding period at 21 C. None of the 144 control plants infested in these experiments became infected. Data are the totals from eight experiments with MAV, five with PAV, and seven with RPV.

TABLE 2. Barley yellow dwarf virus (BYDV) yields from young and old leaves, and aphid transmission of virus from concentrated preparations

BYDV isolate[a]	Age of virus source	Virus yield[b]	Transmission[c] by aphid species shown, following acquisition feeding through membranes on virus concentrates	
			M. avenae	*R. padi*
MAV	young	83	36/36	5/72
	old	9	36/36	1/72
PAV	young	20	105/132	89/95
	old	6	12/107	65/94
RPV	young	29	49/168	67/72
	old	23	61/168	70/72

[a]BYDV isolates MAV and RPV are transmitted specifically by *Macrosiphum avenae* and *Rhopalosiphum padi*. Isolate PAV is transmitted efficiently by *R. padi* and inefficiently by *M. avenae*.

[b]Numbers are micrograms of virus per 100 g of source tissue. Data are averages from 10 experiments with MAV, seven with PAV, and eight with RPV.

[c]Numerator is number of plants that became infected; denominator is the number of plants each infested with 10 aphids for a 5-day inoculation test feeding period at 21 C. None of the 144 control plants infested in these experiments became infected.

pulverized with a pestle. The sample was transferred to a Waring Blendor and combined with a volume of 0.1 M neutral potassium phosphate buffer (hereafter called buffer) equal to the fresh weight of the tissue. After the tissue thawed in the buffer for about 20 minutes at room temperature, Triton X-100 was added to a final concentration of 1% by volume, and the sample was blended for 5 minutes at room temperature. The juice was then squeezed through four layers of cheesecloth, and clarified with chloroform and normal amyl alcohol (13). Virus was concentrated by two or more cycles of differential centrifugation. In the final high speed centrifugation, virus was often centrifuged through a 2-ml cushion of 20% sucrose in buffer for 3 hours in the 40 rotor of the Beckman centrifuge. Virus titer of the final preparation (0.5-1.0 ml) was estimated by scanning centrifuged sucrose gradient columns in the ISCO density-gradient fractionator (1, 13). Recent studies of nucleic acid content of the virus isolates suggest that absolute values of some of the estimates are high (3). Virus zones were collected from the gradients for use in bioassays by membrane feeding; these gradients were either similar to those used previously (13) or linear-log (3).

Because of the importance of virus extraction in purification of viruses such as BYDV, we did one series of experiments to compare yields of virus in preparations made by use of liquid nitrogen, as described above, with yields in preparations made without liquid nitrogen. In these experiments we harvested 200 g of infected leaves, and divided the tissue into two comparable 100 g samples. One sample was processed by the extraction method described above. The corresponding sample was ground in the meat grinder, transferred to the Waring Blendor, and processed as described above, except that the manipulations in liquid nitrogen were omitted. Virus from the two parallel preparations was concentrated, partially purified, and the virus concentration of each was estimated from scanning patterns in the ISCO density-gradient fractionator.

RESULTS.—First, we studied virus transmission by "nonvectors" after acquisition feeding on either young or old detached virus-infected leaves. In each of eight experiments where *R. padi* fed on MAV-infected leaves, transmissions by this "nonvector" were more frequent from young leaves than from old ones (Table 1). MAV was transmitted by *R. padi* from young leaves to 63 of 462 test plants, but to only 4 of 462 plants from old leaves. *M. avenae* transmitted MAV consistently from all leaves. Leaf age was important also for transmission of the PAV isolate (Table 1). *M. avenae* transmitted PAV more often from young leaves than from old ones. *R. padi* transmitted PAV consistently from both kinds.

In contrast to results of tests with MAV and PAV, *M. avenae* was no more likely to transmit RPV from young leaves than from old ones. In each of seven experiments, the likelihood of *M. avenae* transmitting RPV from either kind of leaf was very low (Table 1). *R. padi* transmitted RPV consistently from leaves of either age.

Because of the marked differences in virus transmission from young and old leaves, experiments were carried out to study virus titer in leaf extracts. Virus was extracted from comparable 100 g samples of young and old leaves, the yield of virus from each kind of leaf was estimated, and infectivity assays were made with both *M. avenae* and *R. padi*. Much more MAV was purified from young leaves than from old leaves (Table 2). In 10 experiments, the difference in virus concentration ranged from 143 to 51 μg for young leaves, and from 19 to 3 μg for old leaves. The average yields represent almost a ten-fold difference in virus titer between young and old leaves (Table 2). Tests with PAV-infected tissue also revealed a differential in virus titer. In seven experiments, the difference in PAV concentration ranged from 33 to 7 μg for young leaves and 13 to 1 μg for old ones. The average yield of PAV for all experiments represented more than a three-fold difference in virus concentration for young leaves over old ones (Table 2). In contrast to results with the MAV and PAV isolates, the amount of RPV extracted from young or old leaves did not differ consistently. In each of eight experiments, virus titers ranged from 41 to 9 μg for

young leaves, and from 47 to 3 μg for old ones. The average RPV yield per 100 g of tissue was 29 for young leaves and 23 μg for old ones (Table 2).

Although few virus transmissions occurred, *R. padi* transmitted MAV more often after feeding on concentrates of young leaves than of old ones (Table 2). *R. padi* transmitted MAV from young leaf concentrates in two of four experiments, and from concentrates of old leaves in only one experiment. *M. avenae* transmitted MAV in the parallel tests to all plants from both kinds of preparations. Results of assays of the concentrates from PAV-infected leaves agreed with the differential transmission patterns of tests in which aphids acquired virus directly from leaves. In each of seven experiments, *M. avenae* transmitted PAV to most of the test plants from preparations of young leaves, but to few plants from concentrates of old leaves (Table 2). *R. padi* transmitted PAV to most plants from both kinds of concentrates. Results of the parallel tests with RPV again differed from those obtained with the other two virus isolates. The "nonvector" *M. avenae* was no more likely to transmit virus from preparations made from young leaves than from old ones; *R. padi* transmitted RPV consistently from both kinds (Table 2).

The parallel between the considerable difference in both virus titer and aphid transmission of virus from young and old MAV-infected leaves suggested that virus titer might be a major factor in overcoming the restrictive mechanism and allowing occasional transmissions of MAV by *R. padi*. To study this possibility, we made highly concentrated preparations of MAV from young leaves and then tested them by letting *R. padi* feed through membranes on the preparations. In a typical experiment, virus was concentrated by three cycles of differential centrifugation from about 200 g of MAV-infected leaves. This final preparation, usually 1.0 ml, was divided into two samples of 0.5 ml which were layered on linear-log sucrose gradients and centrifuged for 2 hours at 39,000 rpm at 4 C in the SW 41 rotor of the Beckman centrifuge. Virus titer was estimated by scanning the gradients, and the virus zone was collected for bioassay in membrane feeding tests with both aphid species. At concentrations of 60-164 μg of MAV per ml *R. padi* transmitted virus only occasionally. But transmission increased when concentrations were at least 180 μg/ml (Table 3). Although comparisons among the preparations are limited by the fact that each was made from a different source of tissue, the data suggest that virus titer is an important factor in the occasional transmission of MAV by the "nonvector" *R. padi*.

In similar experiments with the PAV and RPV isolates, virus titers of the preparations were always lower than those obtained for MAV, an observation also noted in other work (13). *M. avenae* transmitted PAV to all plants in each of four experiments, regardless of the virus titer (Table 3). Similarly, *M. avenae* transmitted RPV from four of six preparations; these data do not suggest any relationship between virus titer and the likelihood of transmission by the "nonvector".

Additional attempts were made to study the role of virus titer more directly by assaying a series of dilutions of a single virus preparation. These tests were inconclusive, however, because either virus titers were too low or the total amount of virus obtained in a preparation was insufficient to permit use in a series of dilutions. In six experiments with MAV, for example, initial virus concentrations of the preparations ranged from 60-120 μg per ml. Transmissions of MAV by the "nonvector" *R. padi* were few in tests of these preparations. This result agrees with the above observation that *R. padi* transmits MAV very rarely from preparations when virus titer is below 180 μg per ml.

TABLE 3. Transmission of isolates of barley yellow dwarf virus (BYDV) by *Macrosiphum avenae* and *Rhopalosiphum padi* fed through membranes on virus concentrates

BYDV isolate[a]	Virus concentration[b] (μg/ml)	Transmission[c] by aphid species shown	
		M. avenae	*R. padi*
MAV	60	12/12	2/24
	80	24/24	1/24
	116	24/24	2/24
	148	12/12	0/24
	164	12/12	1/24
	180	12/12	24/48
	198	48/48	13/48
	220	12/12	7/24
PAV	7	36/36	24/24
	14	24/24	12/12
	16	24/24	12/12
	23	24/24	12/12
RPV	23	16/36	21/22
	28	0/24	12/12
	44	1/36	24/24
	48	1/24	12/12
	50	5/24	10/10
	56	0/24	12/12

[a]BYDV isolates MAV and RPV are transmitted specifically by *Macrosiphum avenae* and *Rhopalosiphum padi*. Isolate PAV is transmitted efficiently by *R. padi* and inefficiently by *M. avenae*.

[b]All of the MAV concentrates and two of the RPV concentrates (23 and 44 μg/ml) were prepared exclusively from young leaves.

[c]Numerator is number of plants that became infected; denominator is number infested each with 10 aphids for a 5-day inoculation test feeding period at 21 C. None of 240 plants infested as controls became infected.

Because recent results suggested that grinding tissue in the presence of liquid nitrogen released more BYDV than did other extraction procedures (3), most preparations used here were made by use of liquid nitrogen. When we made some direct comparisons, we were surprised to find that virus titers in preparations made by use of liquid nitrogen were no greater, and sometimes less, than titers of preparations made by previously used procedures.

In each of seven experiments with the MAV isolate, more virus was obtained in preparations made without liquid nitrogen than in the parallel preparations made with it (Table 4). The average yield of MAV was about 47% higher for preparations made without liquid nitrogen than for those made with liquid nitrogen. In six experiments with the RPV isolate, the two methods did not consistently differ, but somewhat less virus was usually obtained from the preparations made with liquid nitrogen (Table 4). In two experiments with the PAV

TABLE 4. Virus yields from tissue extracted with or without liquid nitrogen, and virus transmission by *Macrosiphum avenae* and *Rhopalosiphum padi* from these concentrates of two isolates of barley yellow dwarf virus

Virus isolate[a]	Virus concentration (μg/ml) in preparations made with or without liquid nitrogen[b]		Transmission[c] by aphid species shown following acquisition feeding through membranes on concentrates prepared by the methods indicated			
			Without liquid nitrogen		With liquid nitrogen	
	Without	With	*M. avenae*	*R. padi*	*M. avenae*	*R. padi*
MAV	85	58	104/106	7/358	96/96	11/348
RPV	52	40	39/168	95/96	23/168	91/92

[a]BYDV isolates MAV and RPV are transmitted specifically by *Macrosiphum avenae* and *Rhopalosiphum padi*.
[b]Data are the averages from seven experiments with MAV and six experiments with RPV.
[c]Numerator is the total number of plants that became infected; denominator is the number infested each with 10 aphids for a 5-day inoculation test feeding period at 21 C following about 18-hour acquisition feedings through membranes at 15 C. None of 192 control plants became infected.

isolate of BYDV, virus titers were essentially identical in each type of preparation.

We also made tests to determine whether or not the "nonvector" aphid species was more likely to transmit from one kind of preparation or the other. No differences occurred in the parallel tests. MAV was transmitted to almost all plants by *M. avenae; R. padi* was no more likely to effect an occasional transmission from one kind of preparation than from the other. RPV was transmitted to almost all plants by *R. padi,* but *M. avenae* transmitted only occasionally from either kind of preparation (Table 4).

DISCUSSION.—Because the age of source tissue is so important in many aspects of plant virology, it is not surprising that vectors should transmit viruses better from some leaves than from others. Aphids often are more likely to transmit viruses from young leaves than from old ones, but this difference is not always found when comparisons are made (17). In the present work, for example, the vectors of each virus isolate transmitted virus equally well from old and young leaves. But virus transmission by "nonvectors" or inefficient transmitters was greatly influenced by the age of leaf used for acquisition feeding. Moreover, the differences were pronounced for certain virus-vector combinations, but not for others. As in study of other factors that affect vector specificity of BYDV isolates, the importance of any one variation depends on the specific virus isolate and aphid species being considered (10).

Differences between source leaves may have affected some previous studies of factors that influence vector specificity of BYDV. For example, in early work on importance of the acquisition feeding period (10), long acquisitions were provided by rearing "nonvector" aphids on virus-infected plants. The increased transmission by such aphids, compared with those given 2-day acquisition feedings on detached leaves, may have been influenced by the age of the leaves on the intact plant from which aphids acquired virus. That this effect was slight is indicated by the fact that long acquisition feedings were not more successful for "nonvectors" in tests with MAV, where leaf age is important, than in tests with RPV, where the effect of leaf age is not so important.

The general response of "nonvectors" or the inefficient transmitters to virus concentration suggests that virus titer in young and old leaves affects vector specificity for two of the three virus isolates. For MAV and PAV, young leaves not only were a better source of virus for aphids feeding on them, but provided substantially higher virus titers when preparations were made from them. In contrast, the "nonvector" *M. avenae* was no more likely to transmit RPV from young leaves than from old ones. This similarity was reflected in the generally equal virus titer in preparations made from the two kinds of leaves. If the salivary glands provide the restrictive barrier within the aphids, as suggested by Rochow (10) and Behncken (2), then high virus titers may simply increase the probability that a virus particle can overcome the restrictive properties of the glands and thus result in virus transmission by "nonvectors". But the present results, together with results of previous attempts to overcome specificity with concentrated inocula (9), suggest that more specific virus-aphid interactions are involved.

M. avenae transmitted RPV or PAV when exposed to relatively low virus concentrations in membrane-feeding experiments (10-50 μg per ml). But *R. padi* transmitted MAV consistently only when fed on concentrates containing at least 180 μg of virus per ml. Perhaps the restrictive barrier within *M. avenae* is susceptible to lower virus concentrations than that of *R. padi*. A difference between the two aphid species is suggested also by other (*unpublished*) experiments with varying concentrations of the virus isolate transmitted efficiently by each aphid species. *M. avenae* consistently transmits MAV in membrane feeding experiments from inocula containing about 0.01 μg per ml of virus; but *R. padi* effects comparable transmission of RPV only when inocula contain about 1.0 μg per ml of RPV. The importance of the virus isolate in the interaction with the aphid is shown by the relative amounts of the virus needed for transmission by *M. avenae* fed through membranes. Approximately 20-50 μg per ml of RPV, about 10-20 μg per ml of PAV, and about 0.01-0.1 μg per ml of MAV are needed for efficient transmission. Thus, although virus titer is an important factor, its importance varies with the virus isolate.

Variation among separate virus preparations limits interpretation of these results, as well as those of other studies we have made on bioassay of BYDV. That transmission is not related solely to the virus content of preparations is suggested by results of this study. For example, the highest rate of transmission by the "nonvector" in tests of six preparations of RPV was from the preparation with the least amount of virus (Table 3).

Unlike some preparations made from whole plants, this most active one was made only from young leaves. But another preparation made from young leaves in this series of experiments provided a poor source of virus (Table 3). These results emphasize the extent of variation among specific virus-aphid combinations, and also identify an important variable in the vector specificity of three isolates of BYDV.

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