Identification of Barley Yellow Dwarf Viruses: Comparison of Biological and Serological Methods

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ABSTRACT

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During 1979 and 1980, parallel tests were made for variants of barley yellow dwarf virus in 216 field-collected samples of small grains by enzyme-linked immunosorbent assays (EIA) with four antisera and by aphid transmission tests with four vector species. In 174 of the comparisons, results of both methods agreed; in 39, the EIA procedure resulted in more complete information than the aphid tests; in 2, the methods gave different results; and in 1, the biological test was more informative than the serological one. Of 265 barley yellow dwarf virus isolates identified from 187 plants, 164 were similar to PAV (virus transmitted nonspecifically by *Rhopalosiphum padi* and *Sitobion avenae*); 69 resembled RMV (transmitted specifically by *R. maidis*); 20 were similar to RPV (transmitted specifically by *R. padi*); 9 were similar to MAV (transmitted specifically by *S.* [= *Macrosiphum*] *avenae*); and 3 resembled SGV (transmitted specifically by *Schizaphis graminum*). The EIA procedure was especially useful in detecting mixed infections, which were found in 60 of the 187 infected plants. Although the EIA procedure had many advantages, some limitations were encountered, especially in identification of isolates similar to RMV.

Additional key words: aphid vectors, ELISA, luteovirus

Rochow and Duffus (10) recently discussed diagnosis of diseases caused by luteoviruses and pointed out the promise shown by enzyme-linked immunosorbent assays (EIA) in identification of luteoviruses that cause barley yellow dwarf (5,8). In an initial 1978 study, we made parallel tests of field-collected samples by EIA with four antisera and by aphid transmission tests with four vector species. Results of those tests illustrated some advantages of EIA procedures (7). During the 1979 and 1980 growing seasons, we made a series of additional comparative tests with both the biological and serological methods. This paper describes results of those parallel tests. It also compares various aspects of the two methods for identifying isolates of barley yellow dwarf virus (BYDV).

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MATERIALS AND METHODS

Many of the collections of oats, wheat, or barley were made from test plots maintained by M. E. Sorrells in Tompkins County near Ithaca, NY. Other samples were collected by me or T. A. Zitter in other counties of New York. Thirteen samples in 1979 and 33 in 1980 were sent by cooperators from nine different states. Each sample usually consisted of a separate plant. I removed two or three adjacent leaves for use in the aphid transmission tests. The remaining leaves, together with stem and sheath tissues, were used for the EIA test.

For the EIA test, I prepared 3.0 g of finely chopped tissue by grinding it first in a mortar and pestle in liquid nitrogen, then with 2.0 ml of phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20, and finally together with 4.0 ml of chloroform before low-speed centrifugation. The EIA tests were carried out on the clarified juice as described previously with four virus-specific globulins (7–9). The globulins used in 1979 were the same preparations made for the initial studies (7,9). Globulins used in 1980 were from a different batch, prepared and labeled as described previously (9). As before, globulins were not purified, but antiserum used to prepare them was previously absorbed with an equal volume of a concentrate of juice from healthy oats, a step that probably contributed to low background readings (7,9). In 1980, the healthy oat juice was concentrated 512-fold. An EIA reaction was usually considered positive only if the absorbance (405 nm, 1-mm path) was above 0.04, a value generally within the visible range. Because the standard deviation for controls in most tests was below 0.005, the detection threshold was much higher than the twice-background range often used in such tests.

The four aphid species used were *Rhopalosiphum padi* (Linnaeus), *R. maidis* (Fitch), *Sitobion* (= *Macrosiphum*) *avenae* (Fabricius), and *Schizaphis graminum* (Rondani). Transmission tests involved a 2-day acquisition feeding and a 5-day inoculation test feeding as described previously (3,6). The test plant was Coast Black oats (*Avena byzantina* C. Koch). In every test, aphids fed on healthy tissue were used as controls.

Viruses recovered from the field-collected samples were compared with five characterized BYDV isolates (3,6,9). These five are RPV, transmitted specifically by *R. padi*; RMV, transmitted specifically by *R. maidis*; MAV, transmitted specifically by *S.* (= *Macrosiphum*) *avenae*; SGV, transmitted specifically by *Schizaphis graminum*; and PAV, transmitted nonspecifically by *R. padi* and *S. avenae*. *Schizaphis graminum* also transmits RPV and PAV, but less consistently than does *R. padi*. On the basis of both serological properties and cytologic effects, the five viruses form two groups: RPV and RMV in one; PAV, MAV, and SGV in the other (10). Because the initial test of a field sample is usually not adequate for thorough identification of

virus isolates, many additional transmission tests were made of test plants.

RESULTS AND DISCUSSION

In both years, about 80% of the comparisons with four globulins in EIA tests and four aphid species in virus transmission tests were in agreement. Of 216 comparisons, 174 had identical results (Table 1, A–G). For 39 samples, results of the EIA procedure were more complete than those of the aphid tests (Table 1, H–O). In two tests, results of the methods conflicted (Table 1, P and Q), and in one case the aphid transmission test produced more information than did EIA (Table 1, R). As in recent years, isolates similar to PAV were the most common. Of the 265 isolates identified from 187 plants, 164 were similar to PAV, 69 resembled RMV, 20 were similar to RPV, 9 were similar to MAV, and 3 resembled SGV (Table 1). The SGV-like isolates came from wheat collected in Idaho by Robert L. Forster. For three consecutive years, these "rare" isolates have been found in Idaho. Some of them appear less vector-specific than others and may be more similar to isolates described by Gill (1,2) than to SGV (3).

Isolates similar to all five characterized ones were found in 1979; virus isolates similar to four of the five were encountered in 1980. The EIA procedure was especially useful in detecting mixed infections, which occurred in 60 of the 187 plants (Table 1). Comparative features of the two techniques can be examined in detail by a closer look at the data that permitted the virus identifications summarized in Table 1.

A clear advantage of the EIA procedure over aphid transmission tests was the time needed to identify viruses. The EIA procedure took 2 days; each aphid test took at least 4 wk. Thorough identification of viruses by means of the aphid procedure requires several months because it is necessary to make subsequent transmission tests of plants that become infected initially. For example, in the 94 identifications of PAV by both methods (Table 2, A), seven of 282 test plants became infected in the original tests with *R. maidis*. In most of these plants, severity of symptoms paralleled those of plants infected by means of the other vectors; this indicated that the viruses were similar to PAV and not RMV, which causes milder symptoms. The only way to be sure that they were not RMV-like viruses, however, was to make subsequent transmission tests of the seven infected plants. When this was done, none of 24 plants became infected in tests using *R. maidis*, but the other vectors transmitted virus to most plants. Thus, the isolates in question were identified as similar to PAV.

It is especially necessary to make subsequent tests of plants infected by viruses that are similar to one of the vector-specific isolates. Thus, the MAV-like isolate identified in 1979 by both methods (Table 2, F) was identified in the aphid tests only because subsequent tests showed the virus to be transmissible by *S. avenae* but not by any of the other vectors. In several similar cases, PAV-like isolates had been transmitted from the field-collected plant by *S. avenae*, but not by *R. padi*; subsequent tests revealed their true identity. In 79 other cases, virus transmitted from a plant was studied to determine whether both *R. padi* and *S. avenae* had transmitted the same kind of isolate; in all instances the viruses were found to be similar to PAV. Further tests are needed to identify RPV-like isolates and differentiate them from those similar to PAV. The four isolates in group G (Table 2) were identified in the biological tests as RPV because they were transmitted in subsequent tests to 18 of 21 plants by *R. padi*, but to 0 of 21 plants by *S. avenae*.

In some cases, the EIA procedure gave more complete results than the biological one. In the absence of EIA tests, the 12 samples in groups H and K (Table 2) would have been considered negative. But parallel serological tests clearly showed the presence of PAV or RMV. Similarly, the 13 plants in group J (Table 2) were found to be doubly infected only in EIA tests, because *R. maidis* did not recover any RMV-like virus from those samples. The single transmission by *R. maidis* in the original test (Table 2, J) was found to involve a PAV-like isolate because in additional tests the virus was transmitted to all six plants by both *R. padi* and *S. avenae*, but to none of six plants by *R. maidis*.

In other tests, the EIA procedure was more accurate than the biological one. In one example (Table 2, P), results of the EIA test were very clear for an RPV-like isolate; the initial aphid transmission data produced a similar result. But when subsequent tests were made of virus recovered initially by *R. padi*, virus was transmitted to four of 15 plants by *S. avenae* and to all 15 plants by *R. padi*. The relatively severe symptoms of the infected plants, efficient transmission by *R. padi*, and less efficient transmission by *S. avenae* all fit the usual pattern for isolates similar to PAV, the identification recorded for the biological test (Table 2, P). The EIA data suggest a more valid interpretation: this isolate was similar to RPV, and the four transmissions by *S. avenae* merely illustrate the relative nature of vector specificity and the variation among isolates in any one luteovirus group (6).

The serological procedure was sometimes more sensitive than the biological one. During hot summer months, when many of these tests were made, it was sometimes a problem to determine whether a negative finding represented failure of aphids to transmit virus or failure of symptoms to develop in test plants infected by a "mild" isolate. We often test symptomless plants to evaluate this problem. We made such tests on the symptomless plants that had been infested with *R. maidis* for group K (Table 2) because EIA tests showed RMV to be present. That these test plants were not infected was clearly confirmed both in additional EIA tests and by failure to obtain infection in any of 48 additional test plants. Results of such tests with both methods usually agree. However, in one of the samples in group J, for example, the original EIA test clearly showed a mixture of viruses similar to PAV and

Table 1. Summary of 216 tests for barley yellow dwarf virus (BYDV) by enzyme-linked immunosorbent assay (EIA) and aphid transmission tests of samples collected in the field during 2 yr

	Samples in group (no.)		BYDV isolate similar to that shown[a]	
Group	1979	1980	EIA	Aphids
A	55	39	PAV	PAV
B	22	10	PAV + RMV	PAV + RMV
C	15	14	None	None
D	8	3	RMV	RMV
E	1	2	SGV	SGV
F	1	0	MAV	MAV
G	0	4	RPV	RPV
H	6	3	PAV	None
I	4	2	PAV + RPV	PAV
J	4	9	PAV + RMV	PAV
K	1	2	RMV	None
L	5	0	PAV + RPV + MAV + RMV	PAV
M	1	0	PAV + RPV + MAV + RMV	None
N	1	0	PAV + RPV + MAV + RMV	MAV
O	1	0	PAV + RPV + MAV + RMV	RMV
P	1	0	RPV	PAV
Q	0	1	RPV + PAV	PAV + RMV
R	0	1	None?	RMV

[a] PAV = virus transmitted nonspecifically by *Rhopalosiphum padi* and *Sitobion avenae*; RMV = transmitted specifically by *R. maidis*; RPV = transmitted specifically by *R. padi*; MAV = transmitted specifically by *S.* (= *Macrosiphum*) *avenae*; and SGV = transmitted specifically by *Schizaphis graminum*.

RMV, but only *R. padi* and *S. avenae* recovered virus. When a symptomless test plant that had been infested with *R. maidis* was tested, no virus was transmitted by *R. maidis*, but EIA results showed the plant to be infected by an RMV-like isolate.

One more advantage of the EIA procedure was its differentiation of viruses not readily separated in aphid transmission tests. Seven samples (Table 2, I and Q) were found to be doubly infected by viruses similar to PAV and RPV. Because both of these viruses are efficiently transmitted by *R. padi*, it is not feasible to differentiate them in routine aphid transmission tests. Thus, such mixtures go undetected in biological tests. We have confirmed the validity of the original EIA test in several such cases by serological tests of the infected test plants. In some cases, *R. padi* recovered both viruses from the field plant; in other cases, the vector transmitted only the PAV-like one.

Another feature of the EIA procedure is that it can be used for samples in such poor condition that lack of aphid feeding would preclude biological tests. Data from such tests are not shown here, but during 1979 and 1980 we were able to identify viruses in 50 other samples sent by mail from nine states, only because the EIA procedure was available.

Although the EIA procedure had many advantages, there were some significant limitations. They result from the same general property that makes EIA so useful: its marked specificity. Heterologous reactions generally are weak in EIA tests compared with other kinds of serological procedures (4,9). But they do occur, and they are especially useful in tests of BYDV isolates. Heterologous reactions between isolates similar to MAV and PAV were consistent and confirmed the homologous one (Table 2). Because I do not yet have an antiserum for SGV, the weak but consistent heterologous reactions with MAV and PAV globulins are the basis for identification of SGV-like isolates in EIA tests (9). Heterologous reactions between isolates similar to RPV and RMV were generally weak, except for some RMV-like variants described below.

Although this pattern of homologous and heterologous reaction is consistent for most samples, variations that occur sometimes cloud interpretation of results. In many of the 94 identifications of PAV-like isolates of group A (Table 2), reactions with RPV globulin were below 0.010, as is the case in most reactions with healthy controls. In about 15 of the tests, however, reactions with RPV globulin were around 0.040. That this was a feature of the particular sample was suggested by 13 additional EIA tests of such isolates in which the mean RPV globulin reaction was 0.009. With four isolates, however, the mean reaction with RPV globulin in initial tests was 0.051; in six additional tests of these isolates, the mean of reactions with RPV globulins was 0.30, a value that is probably insignificant compared with the 1.02 mean of homologous reactions. But these variations represent a range within PAV-like isolates that could make it difficult to evaluate mixtures of isolates similar to PAV and RPV.

Limitations in use of EIA procedures for BYDV identification were illustrated especially by variations in homologous and heterologous reactions for RMV-like isolates. Many of the 69 RMV-like

Table 2. Results of 216 parallel tests of field-collected samples for barley yellow dwarf virus (BYDV) by enzyme-linked immunosorbent assay (EIA) and virus transmission tests with four aphid species

	Enzyme-linked immunosorbent assay					Virus transmission				
		Absorbance at 405 nm[b]					Transmission with aphid species shown[c]			
Group	Plants infected (no.)[a]	RPV	MAV	PAV	RMV	Plants infected (no.)	*Rhopalosiphum padi*	*Sitobion avenae*	*R. maidis*	*Schizaphis graminum*
A	94 PAV	0.015	0.120	0.884	0.008	94 PAV	241/282	184/282	7/282	164/282
B	32 PAV + RMV	0.072	0.174	0.874	0.126	32 PAV + RMV	96/96	70/96	67/96	55/96
C	29 None	0.005	0.006	0.004	0.005	29 None	0/87	0/87	0/87	0/86
D	11 RMV	0.054	0.014	0.013	0.219	11 RMV	2/33	0/33	29/33	15/33
E	3 SGV	0.002	0.037	0.029	0.004	3 SGV	2/9	4/9	0/9	8/9
F	1 MAV	0.037	1.10	0.088	0.013	1 MAV	0/3	3/3	0/3	0/3
G	4 RPV	0.886	0.003	0.003	0.019	4 RPV	12/12	0/12	0/12	3/12
H	9 PAV	0.011	0.096	0.866	0.011	9 None	0/27	0/27	0/27	0/27
I	6 PAV + RPV	0.342	0.107	0.805	0.028	6 PAV	18/18	15/18	0/18	11/18
J	13 PAV + RMV	0.042	0.115	0.776	0.087	13 PAV	30/39	24/39	1/39	22/39
K	3 RMV	0.019	0.001	0.002	0.057	3 None	0/9	0/9	0/9	0/9
L	5 PAV + RPV + MAV + RMV	1.10	0.712	1.36	0.414	5 PAV	15/15	15/15	1/15	11/15
M	1 PAV + RPV + MAV + RMV	0.595	0.440	1.10	0.331	1 None	0/3	0/3	0/3	0/3
N	1 PAV + RPV + MAV + RMV	0.850	1.15	0.740	0.440	1 MAV	0/3	3/3	0/3	0/3
O	1 PAV + RPV + MAV + RMV	1.35	0.990	0.975	0.640	1 RMV	0/3	0/3	1/3	1/3
P	1 RPV	1.10	0.008	0.006	0.007	1 PAV	3/3	0/3	0/3	1/3
Q	1 PAV + RPV	0.470	0.057	0.290	0.015	1 PAV + RMV	3/3	3/3	1/3	0/3
R	1 None?	0.031	0.004	0.025	0.015	1 RMV	0/3	0/3	2/3	0/3
Healthy controls	27 None	0.005	0.006	0.006	0.006	38 Aphid controls	0/114	0/114	0/114	0/114
Virus controls	RPV	0.866	0.010	0.006	0.004	RPV	30/30	0/30	0/30	9/30
	MAV	0.016	0.825	0.061	0.006	MAV	3/30	30/30	0/30	0/30
	PAV	0.006	0.062	0.566	0.004	PAV	30/30	26/30	0/30	9/30
	RMV	0.022	0.006	0.005	0.190	RMV	2/30	0/30	29/30	2/30
	SGV	0.002	0.037	0.044	0.006	SGV	0/30	0/30	0/30	28/30

[a] With isolates similar to those shown. PAV = virus transmitted nonspecifically by *Rhopalosiphum padi* and *Sitobion avenae*; RMV = transmitted specifically by *R. maidis*; RPV = transmitted specifically by *R. padi*; MAV = transmitted specifically by *S.* (= *Macrosiphum*) *avenae*; and SGV = transmitted specifically by *Schizaphis graminum*.

[b] Using antiserum for isolate shown. Values are means of absorbance for number of individual wells (plants) shown at left in each case. All readings were made of undiluted reactants in a microcell with a 1-mm light path.

[c] Number of plants infected/number of plants infested with about 10 aphids of species indicated. Tests were made in parallel with four aphid species shown. Data are only for original test of field-collected sample; subsequent tests necessary for virus identification are described in text.

Table 3. Original and subsequent enzyme-linked immunosorbent assays of four RMV-like isolates from field-collected samples in 1980

Tests of plants in serial transmission[a]	A_{405} (1-mm light path) in tests of isolate (and source) shown with RMV- and RPV-specific globulins[b]							
	Isolate 1 (NY wheat)		Isolate 2 (NY barley)		Isolate 3 (NY wheat)		Isolate 4 (Idaho wheat)	
	RMV	RPV	RMV	RPV	RMV	RPV	RMV	RPV
Original	0.141	0.068	0.035	0.030	0.034	0.010	0.015	0.031
First	0.061	0.010	0.155	0.023	0.049	0.021	0.006	0.004
Second	0.155	0.026	0.056	0.006	0.039	0.026	0.000	0.009
Third	0.081	0.006	0.051	0.004	0.035	0.020	0.000	0.015
Fourth	0.154	0.011	0.100	0.008	0.075	0.035	0.010	0.015

[a] In parallel aphid transmission tests, all isolates were transmitted regularly by *Rhopalosiphum maidis* (to 79 of 90 plants), but not consistently by any of the other three aphid vectors (to 10 of 255 plants). None of 64 controls became infected.

[b] The mean reading of eight healthy controls was 0.002 for RMV (virus transmitted specifically by *R. maidis*) globulin and 0.006 for RPV (virus transmitted specifically by *R. padi*) globulin. Mean readings for RMV controls were 0.156 for RMV globulin and 0.011 for RPV globulin.

isolates reacted with the four globulins as does RMV (Table 2, controls). These isolates had a relatively strong homologous reaction with RMV globulin and a weak heterologous one with RPV globulin; reactions with MAV and PAV globulins were essentially negative (Table 3, isolate 1). About 12 of these RMV-like isolates reacted relatively weakly with RMV-specific globulin, but relatively strongly with RPV-globulin. (Hence the relatively high RPV-globulin readings of means for groups B and D in Table 2.)

Such isolates seemed to cover a range of variation. At one extreme were those that gave a weak but nearly equal homologous and heterologous reaction in the initial test. When subsequent tests were made of such isolates (Table 3, isolate 2), however, the pattern was more similar to that of RMV and isolate 1 in Table 3. Another group of isolates, illustrated by isolate 3 in Table 3, had a relatively weak homologous reaction with RMV-globulin and a relatively strong one with RPV globulin, a pattern that continued in tests of plants infected during subsequent serial transfers of the isolate. At the end of this spectrum of variation was one isolate (Table 2, R) for which the initial EIA result was too weak to be clear, and subsequent assays of plants from serial transmissions also produced ambiguous results (Table 3, isolate 4). In all cases, however, these isolates were readily transmitted by *R. maidis* but not by other aphid species in subsequent transmission tests. The variation occurred in the serological test, not in the biological one. These data suggest a limitation of the EIA procedure that could be significant in situations where isolates similar to these "atypical" ones predominate.

Perhaps an RMV-like isolate was not detected in EIA of sample Q because it was similar to the one just described for sample R (Table 2). Both of these samples were wheat from Idaho. The presence of an RMV-like isolate in sample Q was confirmed in transmission tests of the one plant originally infected by means of *R. maidis* (Table 2, Q). Subsequent tests by both methods confirmed a PAV-like isolate, but not the RPV-like one. Thus, *R. padi* apparently transmitted only the PAV-like isolate, not the RPV-like one from the wheat sample. This explanation is the most likely because there is no reason to question the high (0.470) RPV-globulin reading (Table 2, Q). But the anomaly described next suggests that even high readings could be misleading at times.

An unusual result in 1979 was the strong reaction of eight samples with all four virus-specific globulins (Table 2, L–O). In none of these eight cases was more than one virus isolate recovered by aphids; in one case, aphids did not recover any virus. The samples were all spring oats, results came from tests done during two different weeks, and the samples were from two different locations. In each case, the EIA plate contained other samples that gave more normal reactions. Controls on both plates were negative. Perhaps these eight plants really were infected by all four viruses, but this seems unlikely.

On the other hand, we have essentially never obtained false positives in hundreds of tests of many different types during several years. For example, results were clear-cut for all 29 field-collected samples found to be free of BYDV in these tests (Table 2, C). Perhaps these eight samples represent extremes in homologous and heterologous reactions. Perhaps the plants contained mixtures of other luteoviruses not transmissible by the aphids we used but that reacted with the antisera. Perhaps the results were anomalies of the procedure or an error in handling samples. Whatever the explanation, the data show that even a generally dependable test like EIA must be evaluated with care. We plan to continue to maintain our aphid colonies!

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