Field Variants of Barley Yellow Dwarf Virus: Detection and Fluctuation During Twenty Years

W. F. Rochow

Research plant pathologist, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, and professor of plant pathology, Cornell University, Ithaca, NY 14853.

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ABSTRACT

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All 181 isolates of barley yellow dwarf virus (BYDV) that were recovered from field-collected oats, wheat, or barley during 1975 and 1976 resembled one of five characterized isolates found previously. Most of them resembled PAV, an isolate of BYDV which is transmitted nonspecifically by *Rhopalosiphum padi, Macrosiphum avenae,* and *Schizaphis graminum.* More than one variant of BYDV was recovered from nine of 57 winter grain plants. Only one mixed infection was detected among 114 spring oat samples. Since these tests complete 20 consecutive years of evaluation of BYDV isolates in New York, a compilation was made of the yearly results. Identification of 1,055 BYDV isolates from spring oats showed that isolates similar to four of five characterized ones were present almost every year. A gradual change in predominating isolate type has occurred in the 20 yr, from isolates similar to the vector-specific MAV (specifically transmitted by *M. avenae*) to those similar to PAV. A similar pattern was detected in tests of winter grains during the same period. Mixed infections by more than one isolate of BYDV were common in winter grains but rare in oats during most years. Further evaluations based upon two kinds of serological tests confirmed the identifications of 39 isolates of BYDV. One test was a neutralization of infectivity based on serological blocking of aphid transmission from treated virus preparations. The other was the enzyme-linked immunosorbent assay procedure, which has many advantages and shows promise for future work.

Additional key words: aphid vectors, ELISA tests, *Rhopalosiphum maidis*, virus identification.

Barley yellow dwarf is a serious, widespread disease of small grains and grasses caused by a group of aphid-transmitted luteoviruses. Confirmation of disease in the field is especially difficult because isolates of barley yellow dwarf virus exhibit pronounced specificities for the different aphid species that serve as vectors (13,14). During each growing season, we use four aphid species to make comparative transmission tests from field-collected samples. These tests allow confirmation of field diagnosis of the disease, permit evaluation of variability among isolates of BYDV, and provide information about the relative distribution of different BYDV variants among growing seasons.

Although aphid transmission testing has been useful, the procedure has a number of disadvantages. The work is tedious, much time is required, relatively few samples can be tested during one growing season, and special facilities are needed. Many laboratories are not equipped to handle aphid vectors which, along with test seedlings, must be grown in advance. The process takes three to five weeks and uses much growth chamber and greenhouse space. A goal of many workers has been to simplify field testing procedures for BYDV and similar viruses. Serological tests have been of some use for this in the past, but BYDV serology usually required concentrated virus preparations (1,16), electron microscopy (12), or additional aphid transmission procedures (17,23). The new enzyme-linked immunosorbent assay (EIA) overcomes many of these disadvantages and offers much promise for use as a routine test for BYDV (2,10,11).

This paper has three purposes. First, it reports results of tests of field samples collected during 1975 and 1976. Second, it summarizes a 20-year change in predominating BYDV isolates in New York State. Third, it confirms the usefulness of the EIA technique for identification of BYDV isolates from the field.

MATERIALS AND METHODS

Most of the field collections were made from test plots maintained by N. F. Jensen, Department of Plant Breeding, Cornell University, Ithaca, NY. Locations for collections included the Cornell McGowan Farm, the Snyder Farm, the Tailby Farm, and the Caldwell Field, all near Ithaca, New York. Other samples included those collected by Otto Schultz from various areas in New York State, or samples sent to the Cornell Extension Service for diagnosis. Samples received from other workers in various states also were included in some tests. Each sample represented a different plant; usually two or three adjacent leaves were divided to provide the four pieces of plant tissue used in each comparative transmission test.

The four aphid species used in transmission tests were *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Macrosiphum avenae* (Fabr.), and *Schizaphis graminum* (Rondani). Each species represents the same clone of the aphid used in our previous tests (13). Comparative tests with the four aphid species were carried out as previously described (13,14,24) using a two-day acquisition feeding on detached leaves at 15 C, and a 5-day inoculation test feeding at 21 C in a growth chamber. The test plant for all experiments was oats (*Avena byzantina* K. Koch 'Coast Black'). For each test, each of three 6-day-old oat seedlings was infested with about 10 aphids. In every test, at least 30 aphids of each group were used as controls.

Identification of BYDV isolates transmitted from the field samples was based on the pattern of transmission by the four aphid species, on relative symptom severity, and on the results of subsequent comparative transmission tests from selected plants that became infected in the initial test. The latter were needed especially to reveal mixed infections by more than one variant of BYDV, and to identify vector-specific isolates. The field-collected isolates were compared with the five distinct BYDV isolates found in previous tests (8,14). These five characterized isolates are RPV, transmitted

specifically by *R. padi*; RMV, transmitted specifically by *R. maidis*; MAV, transmitted specifically by *M. avenae*; SGV, transmitted specifically by *S. graminum*, and PAV, transmitted nonspecifically by *R. padi* and *M. avenae*. *S. graminum* also transmits RPV and PAV, but less consistently than does *R. padi*.

Identification of BYDV isolates also was evaluated in two kinds of serological tests. One was an infectivity assay that utilizes serological blocking of aphid transmission, a type of infectivity neutralization test described previously (17). This method is based on allowing aphids to feed through membranes on virus inocula previously incubated with virus-specific antisera. This test was used specifically to differentiate isolates similar to RPV from those thought to be similar to PAV.

The second serological test was the enzyme-linked immunosorbent assay (EIA) carried out essentially as described by Clark and Adams (2). Paired wells in round-bottom microELISA® (Dynatech Laboratories, Inc., Alexandria, VA) substrate plates were charged at each of four steps with 200 μl of liquid. Between each step, wells were rinsed three times (3 min each) with phosphate-buffered saline (PBS), pH 7.4, containing 0.05% Tween-20 (2). First, wells were precoated with globulin (at 20 μg/ml) diluted in carbonate buffer, pH 9.6 (2), and incubated for ~5 hr at 37 C. The globulin had been prepared by two cycles of ammonium sulfate precipitation from antiserum specific for the RPV or MAV isolate of BYDV previously absorbed with an equal volume of a 540-fold concentrate of healthy oats. The globulin was not fractionated as described by Clark and Adams (2). Second, virus extracted from plant tissue was placed in the wells and incubated overnight at 4 C. Virus samples usually were prepared by grinding 3 g of tissue in 1.5 ml of PBS with a mortar and pestle. The tissue was then ground for an additional 1-2 min in 4 ml of chloroform. Parallel preparations of healthy oats and leaves from selected infected plants were used as controls. The extract was clarified by low-speed centrifugation and tested directly, and at a dilution of 1:5. Purified virus (16) was used in preliminary tests. Third, each well was charged with alkaline phosphatase-conjugated gamma globulin (1:200 dilution of stock) and incubated 4 hr at 37 C. Fourth, disodium *p*-nitrophenyl phosphate substrate, at a concentration of 1.0 mg/ml in 10% diethanolamine buffer, pH 9.8, was added (2). Reactions were stopped after 45 min by adding 50 μl of 3 M NaOH. Paired wells for each sample were evaluated separately by visual rating of the yellow color and by reading absorbance ($A_{405\ nm}$) of the undiluted contents of each well in a Beckman DB spectrophotometer using a microcell with a 1-mm light path.

TABLE 1. Identity of barley yellow dwarf virus (BYDV) isolates recovered from single plants collected near Ithaca, New York, and tested in comparative transmissions with *Rhopalosiphum padi*, *R. maidis*, *Macrosiphum avenae*, and *Schizaphis graminum*

Year and source host of samples	No. plants from which BYDV was recovered over no. tested	No. plants found to be infected with BYDV isolate (or isolates) similar to that specified[a]				
		RPV	MAV	RMV	PAV	Mixtures[b]
1975 Wheat	17/23	0	0	0	16	1 PAV + RPV
1975 Barley	11/11	0	0	2	9	0
1975 Oats	56/60	5	1	5	45	0
1976 Wheat	17/18	1	0	1	15	0
1976 Barley	12/12	0	0	1	3	8 PAV + RMV
1976 Oats	58/58	0	0	0	57	1 PAV + SGV

[a]Identity of the six isolates similar to RPV was established by subsequent comparative transmissions to 84 of 84 plants by *R. padi*, and to one of 84 plants by *M. avenae*. The isolate similar to MAV was identified in tests that involved transmission of virus by *M. avenae* to 11 of 12 plants, and by *R. padi* to 0 of 12. Identity of nine isolates similar to RMV was confirmed in tests in which *R. maidis* transmitted virus to 37 of 45 plants, *R. padi* to 0 of 45, and *M. avenae* to 0 of 45. Isolates similar to PAV were confirmed in 252 subsequent tests in which *R. padi* transmitted virus to 846 of 849 plants, *R. maidis* to 2 of 135, *M. avenae* to 620 of 849, and *S. graminum* to 18 of 33 plants. Data for controls are given in the text.

[b]Data for these mixed infections are given in Table 2.

RESULTS

Tests of field-collected samples from New York State. All virus isolates identified in 1975 and 1976 were similar to one or the other of the five BYDV isolates previously found (Table 1). The most common isolates were those similar to PAV. In tests of oats, for example, 80% of 56 isolates identified in 1975 were similar to PAV. Isolates similar to RPV accounted for 9% of the 1975 samples. Another 9% were similar to RMV, and 2% (one isolate) resembled MAV. All 58 oat plants tested in 1976 contained PAV-like isolates, one of them in a mixed infection with an isolate similar to SGV (Table 1). Isolates similar to PAV also predominated in samples of winter wheat and winter barley collected during both seasons (Table 1). From nine of the 57 infected winter wheat and barley plants, a PAV-like isolate was recovered in combination with an isolate similar to either RMV or RPV (Table 1 and 2). As had been observed in previous years (23,24), mixed infections were more common in winter grains than in spring oats. The single mixed infection in oats is unusual because the SGV-like isolate was transmitted from the field sample by *M. avenae* (Tables 1, 2). If *S. graminum* also transmitted the SGV-like isolate from the field sample, it did so together with a PAV-like isolate (Table 2).

In both years, symptoms of BYDV in oats were observed first in fields near Ithaca during the first week of June. In 1975, there was little secondary virus spread; the percentage of diseased plants remained low. In contrast, virus spread during June, 1976, was pronounced, and the disease epidemic became rather severe. This was especially true in many late-planted fields throughout New

TABLE 2. Summary of comparative transmission tests with *Rhopalosiphum padi* (RP), *R. maidis* (RM), *Macrosiphum avenae* (MA), and *Schizaphis graminum* (SG) that enabled identification of mixed infections by more than one variant of barley yellow dwarf virus (BYDV) in field-collected samples

BYDV isolates identified as similar to that shown	Cases tested from Table 1 (no.)	Aphid species that transmitted BYDV in original test of field sample	Transmission by aphid species shown in subsequent tests of plants infected by means of aphid species shown at left in original test of field samples[a]			
			RP	MA	RM	SG
PAV + RPV	1	RP	15/15	6/15	—	—
		SG	15/15	0/15	—	—
PAV + RMV	8	RP	29/29	24/30	—	—
		MA	30/30	22/30	—	—
		RM	7/42	6/42	22/42	5/15
		SG	6/6	4/6	0/6	—
PAV + SGV	1	MA	1/42	0/42	2/42	26/42
		SG	39/39	30/39	1/39	20/39

[a]Numerator is number of plants that became infected; denominator is the number infested with about 10 aphids for 5-day inoculation test feeding. Controls were among those for which data are given in the text.

York where wet weather had delayed oat planting; in many fields nearly 100% of the plants became infected, one of the worst outbreaks of BYDV in New York in many years.

Tests of field-collected samples from other states. In 1975, I received samples of wheat and oats from R. T. Gudauskas in Alabama, and samples of oats collected in Virginia and Pennsylvania by R. E. Hite. All 14 isolates of BYDV recovered from these samples were similar to PAV. Together with the original comparative tests, made by means of four aphid species, the isolates were identified in 29 additional tests with at least two aphid species.

Isolates similar to PAV also were the most common ones in tests of samples from six states in 1976. Only PAV-like isolates were identified in oats from South Carolina (G. C. Kingsland), in wheat from Kentucky (T. P. Pirone), in seven collections of wheat from Kansas (C. L. Niblett), in five collections of wheat from North Dakota (R. G. Timian), and in a collection from South Dakota (S. G. Jensen). The 28 PAV-like isolates identified from these collections were evaluated in 57 additional comparative tests in which BYDV was transmitted to 247 of 264 plants by *R. padi*, to 158 of 264 plants by *M. avenae*, to 0 of 66 plants by *R. maidis*, and to 23 of 51 by *S. graminum*.

Of seven samples of barley supplied in 1976 by R. E. Hite from Pennsylvania, three were found to be infected only by PAV-like isolates, two by RMV-like isolates, and two plants were doubly infected by isolates similar to PAV and RMV. From samples of wheat and oats also collected in Pennsylvania, the three isolates recovered from each set of samples included two similar to PAV and one similar to RPV. These nine PAV-like isolates were identified in additional tests in which *R. padi* transmitted virus to 33 of 33 plants; *M. avenae* transmitted virus to 19 of 33. The RMV-like isolates were identified in five tests in which *R. padi* and *M. avenae* failed to transmit virus, but *R. maidis* transmitted virus to 13 of 15 plants. The RPV-like isolates were transmitted by *R. padi* to all 33 plants, but *M. avenae* failed to transmit virus to any of 33 parallel plants.

In tests of ten random samples of tall fescue grass (*Festuca arundinacea* Schreb.) collected from borders around the small-grain test plot area in Columbia, Missouri, by Dale T. Sechler, four were infected with PAV-like isolates, which was in agreement with a previous test made locally (25).

Aphid controls were used in every experiment. In 1975, 14 experiments involved tests of field-collected material. Virus isolates were identified in 22 additional experiments done over a period of many months. None of 153 plants infested as controls in the initial tests of field material became infected, and none of 147 control plants in the subsequent transmissions became infected. In 1976, the tests involved 17 initial experiments and 13 additional ones. None of 363 control plants in these experiments became infected.

Fluctuation in predominating isolates during 20 years. Because tests in 1976 completed the twentieth consecutive year of this work, it seemed worthwhile to summarize all results. I reviewed all of my early notes from the period and modified a previous 10-yr summary (13) to identify each isolate recovered in tests of spring oats from New York. Although most of the 1,055 samples involved were collected from fields near Ithaca, NY, data from collections in other parts of New York State are included here to increase the sample base. Identifications of isolates, especially those similar to PAV and RPV, were less reliable in the early years than in recent years when we did many serial comparative transmission tests. This compilation is useful only in a general way owing to year-to-year variation in the number of samples studied, the thoroughness of tests, and whether serology was used to evaluate and confirm isolate identification. The compilation is strengthened by the fact that essentially all the samples were collected by one person, similar procedures were used to make the tests, and the same clone of each of the four aphid species was used throughout the period.

Perhaps the most significant observation is that isolates similar to four of the five that have been characterized were recovered from oats almost every year (Table 3). Isolates similar to PAV were found in each of the 20 years, those similar to MAV and to RPV were found in 18 of the 20, and those similar to RMV were detected in all but three of the 18 years in which tests were made. (In 1957 and 1958, *R. maidis* was not used.) Isolates similar to SGV, which have been rare in New York, were found in only three of the years, but some isolates like SGV may have been overlooked prior to 1971. Virus transmissions from field samples by *S. graminum* usually involved isolates similar to PAV or RPV. We did not always make additional tests, adequate to identify isolates that might have been similar to SGV, until Gill (5) described such isolates in Manitoba in 1969 and we reported them in New York in 1971 (8,22).

The relative prevalence of the four kinds of BYDV variants has fluctuated during the 20-yr period (Table 3). The gradual decline in dominance of isolates similar to MAV during the first portion of the period continued in recent years except for 1967 and 1968. At the same time, there has been a gradual increase in isolates similar to PAV, the variant of BYDV which has been most common in all recent years. Each year the percentage of isolates similar to RPV was under 20% except for 1966, 1970, and 1971. Similarly, RMV-like isolates usually were present in $<10\%$ of the collections except for 1964 and 1969.

Tests of winter grains during the 20-yr period were less thorough than those for oats. In general, however, the patterns of variation for isolates recovered from winter grains collected in New York were similar to those for oats. During the first 10 yr of the period,

TABLE 3. Distribution of variants of barley yellow dwarf virus recovered from oats collected in New York State over a period of 20 yr

Year	Isolates identified (no.)	Isolates (%) similar to:				
		MAV	RPV	RMV	PAV	SGV
1957	29	90	7	...	3	...
1958	42	88	0	...	12	...
1959	42	86	2	2	10	0
1960	72	89	1	1	8	0
1961	41	93	5	0	2	0
1962	34	85	9	0	6	0
1963	10	55	18	9	18	0
1964	28	29	11	35	25	0
1965	36	31	19	8	42	0
1966	67	19	27	4	49	0
1967	76	36	10	8	46	0
1968	54	61	6	6	28	0
1969	90	8	16	17	57	2
1970	47	6	26	9	59	0
1971	53	4	28	8	60	0
1972	81	3	17	3	76	1
1973	63	3	16	3	78	0
1974	75	0	7	9	84	0
1975	56	2	9	9	80	0
1976	59	0	0	0	98	2

isolates similar to MAV were encountered more often than others. Of the 52 isolates identified through 1966, 29 were similar to MAV. In 1961, for example, 14 of 19 isolates were similar to MAV. In 1966, only two of ten isolates were similar to MAV, a percentage amazingly similar to the 19% for oats (Table 3). During the second 10 yr of the period we tested more samples of winter grains than in the early years; 114 isolates from wheat and 221 isolates from barley were identified. In general, isolates transmitted by *R. padi* were recovered more often from wheat than barley, and isolates transmitted by *R. maidis* and *M. avenae* were more common in samples of barley than in those of wheat. For both winter grains and oats, isolates similar to PAV were the most common ones identified during the past 10 yr. For wheat, 63% of the isolates were similar to PAV; for barley, 46% were PAV-like ones. Of the 114 isolates from wheat, 11% were similar to RPV; only 2% of the 221 isolates from barley resembled RPV. Isolates similar to RMV represented 41% of those recovered from barley; 21% of those recovered from wheat. For MAV-like isolates, those from barley were 11% of the total, those from wheat, 4%.

The one consistent difference between results of tests of spring oats and winter grains was the likelihood of finding mixed infections. Mixtures were rare in oats, but common in winter grains. During the last 10 yr; for example, 11 of 103 wheat and 49 of 172 barley plants proved to be doubly infected. In all but one of these 60 mixed infections, isolates similar to RMV were present in combination with those similar to PAV, MAV, or RPV. One mixture contained isolates similar to PAV and RPV (Table 2). One triple infection of isolates similar to RMV, MAV, and PAV was detected (20).

Serological tests of field-collected isolates. Use of virus-specific antiserum provides an independent method to study reliability of virus isolate identifications made by comparative aphid transmission tests. In the past, serological tests always have been limited by the low titer of BYDV in infected plants, and by the availability of the concentrated virus inocula required in such tests. For example, there was no simple technique for serologically testing individual plants. In previous work, we used serological blocking of aphid transmission to make tests of that kind because it was the most sensitive technique available (23). In 1975, we used the latter procedure to study the identity of 11 isolates identified as similar to RPV or PAV in aphid transmission tests. Samples of these two isolates were selected because differentiation between them is the most difficult to identify by the aphid transmission tests. Three infected oat plants were picked for each of the 11 isolates. The tissue was frozen, ground in liquid nitrogen, clarified by treatment with chloroform, and a concentrated virus suspension was prepared by high-speed centrifugation (16). Each final virus preparation was divided into two portions, one for incubation with antiserum specific for the RPV and the other for the PAV isolate. After incubation of virus and antisera, the preparations were mixed with sucrose and placed in membrane-feeding tubes for acquisition feeding by *R. padi* (16,17). In every case the serological test confirmed the previous biological identification (Table 4). The five PAV-like isolates were neutralized by PAV antiserum, but not by the RPV serum. The six RPV-like isolates were neutralized only by the RPV serum. These tests permitted confirmation of the isolate identifications, but the procedures are tedious and time-consuming.

All tests carried out with the EIA procedure were done in parallel with gamma globulin from RPV-specific antiserum and from MAV-specific serum. Initial experiments with purified virus confirmed the usefulness of EIA as modified by Lister and Rochow (10,11). Nanogram amounts of RPV and MAV could be detected; the serological difference between the two isolates found in previous tests (1,16) also was detected by EIA (Fig. 1). No heterologous reactions have occurred for RPV and MAV. Tests with clarified preparations made from leaf tissue showed that strong reactions occurred in tests with only a few leaves from an infected plant. Other studies (11) showed no disadvantages due to freezing a leaf

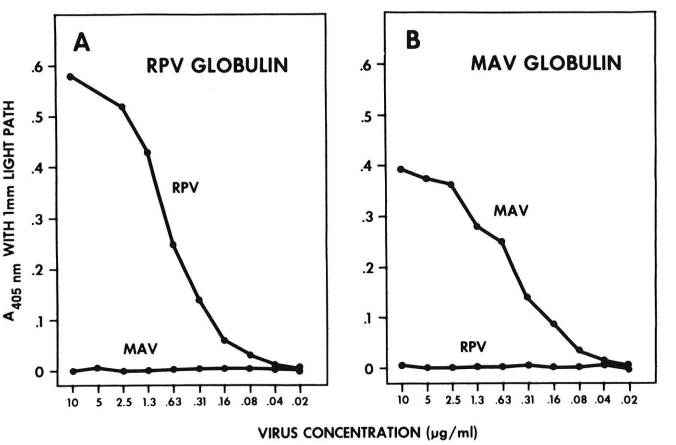

Fig. 1. Reaction of the RPV and MAV isolates of barley yellow dwarf virus in enzyme-linked immunosorbent assay tests with both homologous and heterologous γ-globulin. Antigen for each test was diluted from purified preparations of the RPV and MAV isolate and tested in parallel at the same virus concentration with RPV globulin (A) or MAV globulin (B). Each point is the mean of two readings which differed by 10% or less in all but two cases (11 and 19% differences) for all readings above 0.100.

TABLE 4. Identification of barley yellow dwarf virus isolates by aphid transmission by serological neutralization of infectivity of virus prepared from frozen oat tissue, and comparison with the results of aphid transmission tests

Host/plant and New York State field sources of isolates collected in 1975	Transmission[a] by aphid species:		Identification of isolate as similar to that shown	Transmission[b] by *R. padi* in tests with virus-specific antiserum shown	
	R. padi	*M. avenae*		RPV	PAV
Oats, McGowan	12/12	4/12	PAV	7	0
Oats, McGowan	12/12	4/12	PAV	12	0
Wheat, Caldwell	15/15	6/15	PAV	6	0
Oats, Tailby	12/12	1/12	PAV	3	0
Oats, McGowan	12/12	4/12	PAV	4	0
Oats, McGowan	12/12	0/12	RPV	0	11
Oats, Tailby	12/12	0/12	RPV	0	12
Oats, Caldwell	12/12	1/12	RPV	0	10
Oats, Caldwell	12/12	0/12	RPV	0	11
Wheat, Caldwell	15/15	0/15	RPV	0	2
Oats, Caldwell	15/15	0/15	RPV	0	9
RPV control	15/15	0/15	RPV	0	11

[a]Numerator represents the number of plants that became infected and the denominator the total number infested with about 10 aphids for a 5-day inoculation test feeding. None of 72 plants infested with control (not BYDV access-fed) aphids became infected in the tests that involved four to five serial transfers of each isolate.

[b]Number of 12 plants that became infected by means of *R. padi* that fed through membranes on a portion of a virus preparation previously incubated with virus-specific antiserum. None of 24 controls became infected.

sample to be tested, or using chloroform to aid clarification.

In three separate experiments, various field-collected isolates that had been stored in infected tissue in a freezer (at about -20 C), were thawed overnight at 4 C, and used to make a clarified preparation. Each preparation was tested in duplicate wells in plates with both RPV and MAV globulin. Each was tested both undiluted and at a dilution of 1:5, but data from the diluted samples are not detailed here. Controls in each test included virus from purified preparations, samples of leaves from plants infected with known BYDV isolates, and several samples of healthy oats. Tests included samples from the 1976 collections described here, as well as samples from collections made in 1968 with H. Jedlinski (20). The samples were selected to include a range of collection location and crop plant.

Thirteen isolates previously identified by aphid transmission as similar to RPV also were clearly identified by EIA (Table 5). Four isolates previously identified as similar to MAV had clearly visible, strong reactions with MAV globulin but were negative in parallel tests with the RPV globulin (Table 5). Even the 1:5 dilutions of these RPV- and MAV-like samples were positive with the homologous globulin in visual ratings. Identification of 11 isolates similar to PAV also was confirmed in these tests, although the data are less striking than those for the other two groups. In all cases the PAV-like isolates reacted with the MAV globulin, but the reactions were weak (Table 5). Previous work has shown that PAV and MAV are serologically related (1,16). The relative weakness of the heterologous reaction of PAV with MAV globulin in EIA tests is discussed by Lister and Rochow (11). The lack of any parallel PAV reaction with RPV globulin shows that the isolates in question are not related to RPV. The main reason for using these tests was the difficulty of distinguishing between RPV and PAV by biological assays.

DISCUSSION

Stability and consistency of the patterns of virus-vector specificity represent the most significant feature of tests that were conducted during a 20-yr period. The stable transmission pattern is not unique for isolates studied in New York. For example, isolates similar to PAV, RPV, and RMV were involved in an epidemic of barley yellow dwarf in Manitoba and Saskatchewan in 1974 (5). Vector-specific isolates similar to RPV and RMV appeared to be involved in a serious outbreak of the disease in Ontario in 1969 (7). The remarkable similarity of properties of BYDV collected in Manitoba and in New York was discussed in a recent study by Rochow and Gill (19).

One practical result of our understanding of vector specificity among isolates of BYDV is development of the procedure needed to confirm diagnosis of the disease by aphid transmission tests. Use of more than one aphid species is clearly necessary in most cases. Gill (5) uses five aphid species; I routinely use four. Another area of importance is the role of virus variants in breeding cultivars for tolerance to BYDV. The results of recent studies emphasize the differential reaction of barley lines to variants of BYDV (6,9). Most workers now consider barley yellow dwarf to be a disease

TABLE 5. Identification of barley yellow dwarf virus (BYDV) isolates by enzyme-linked immunosorbent assay (EIA) of extracts of frozen samples, and comparison with results of previous aphid transmission tests

Sample source and year frozen	Transmission[a] by		Isolate previously identified as similar to BYDV isolate:	$A_{405\ nm}$ in 1978 EIA test of sample with γ-globulin shown[b]	
	R. padi	*M. avenae*		RPV	MAV
NY barley '68	6/6	0/6	RPV	.495	.005
NY oat '68	12/12	0/12	RPV	.380	.006
IL oat '68	6/6	0/6	RPV	.490	.005
IL oat '68	12/12	0/12	RPV	.407	.006
IL oat '68	5/5	0/6	RPV	.285	.005
IL oat '68	12/12	0/12	RPV	.450	.005
NY wheat '76	15/15	0/15	RPV	.265	.003
NY oat '76	12/12	0/12	RPV	.235	.002
PA wheat '76	18/18	0/18	RPV	.433	.003
PA oat '76	17/17	0/18	RPV	.286	.003
IL oat '68	12/12	0/12	RPV	.465	.008
IL oat '68	6/6	0/6	RPV	.640	.005
IL oat '68	12/12	0/12	RPV	.560	.005
NY oat '68	1/18	18/18	MAV	.006	.360
NY oat '68	0/6	6/6	MAV	.008	.385
NY oat '68	0/6	6/6	MAV	.006	.350
NY oat '68	0/6	6/6	MAV	.002	.239
IL oat '68	12/12	7/12	PAV	.008	.023
IL oat '68	9/9	7/9	PAV	.006	.023
NY oat '68	3/3	1/3	PAV	.006	.015
IL oat '68	12/12	8/12	PAV	.003	.021
IL oat '68	30/30	18/30	PAV	.011	.025
IL oat '68	15/15	11/15	PAV	.008	.023
IL oat '68	20/21	13/21	PAV	.010	.023
IL oat '68	6/6	1/6	PAV	.002	.025
KN wheat '76	18/18	1/18	PAV	.009	.020
KN wheat '76	17/17	0/18	PAV	.008	.040
KN wheat '76	15/18	12/18	PAV	.009	.030
RPV controls			RPV	.480	.005
MAV controls			MAV	.009	.686
PAV controls			PAV	.010	.037
Healthy controls			None	.009	.006

[a]Numerator is number of plants that became infected; denominator is number infested with about 10 aphids for 5-day inoculation test feeding. None of about 84 plants infested as controls became infected in the tests that involved four to five serial transfers of each isolate.

[b]Values are means of absorbance for undiluted reacted substrate from each of two wells, measured in a microcell with 1-mm light path. Values for healthy controls are means of 14 wells where individual readings ranged from .002 to .013. For each virus control, values are means of six wells.

caused by a group of viruses with a spectrum of relationships among them.

The ability to differentiate BYDV variants also has led to an understanding of some aspects of the epidemiology of the disease. Differences between four major features of the disease in Illinois and New York suggested functional field relationships between specific virus isolates and different aphid species (21). Having reliable techniques for differentiation among BYDV variants makes it possible to answer specific questions about the source of virus in the field. For example, in one study (24) we found that some BYDV-infected orchardgrass plants were not the source of the BYDV widespread in nearby small grains; all isolates recovered from the orchardgrass were similar to RPV but those from the nearby grains were similar to PAV (24).

Despite the general consistency of BYDV-vector relationships, enough variations and occasional discrepancies arise to remind us how little we really know about the details of interactions among plant viruses, aphid vectors, and susceptible plants. For example, in most years the identities of virus isolates recovered by testing field-collected aphids have been in agreement with those of BYDV isolates found to predominate by testing plant samples (13). In 1971, however, three of the four field-collected viruliferous *M. avenae* transmitted virus isolates similar to RPV, an event that should occur only rarely according to our understanding of specificity between RPV and its aphid vectors. In most years, we usually fail to recover BYDV from at least a few of the field-collected plants believed to be infected by the virus. For example, four such failures occurred for the 60 oats tested in 1975 (Table 1). Possible explanations for such failures have been discussed previously (19). Occasional failures in these transmission tests are not surprising, but they suggest the possibility that still other aphid species might have transmitted additional variants of BYDV, a concept that led to the discovery in 1961 of the RMV isolate (14). Another illustration of our ignorance can be seen in the identification of the SGV-like isolate reported here (Tables 1 and 2). Why was the virus isolate recovered initially from the field-collected sample by *M. avenae,* but no further transmissions by *M. avenae* occurred in the subsequent tests using 42 plants? Dependent transmission of virus from mixed infections and similarities among luteoviruses are some of the intriguing possible explanations for these anomalies (4,15,18).

Another frustration is the problem of understanding the reasons for fluctuations in predominance of different BYDV isolates during the 20 years discussed. An obvious possibility is that the change from MAV-like isolates to PAV-like ones simply reflects a change in predominance of aphid species from *M. avenae* to *R. padi*, but our observations of vectors, pathogen types, and disease incidence in small grains during each of these years do not support this explanation. The aphid species most commonly observed on oats during our weekly field collecting trips in 1975 and 1976 was *M. avenae*, just as in previous years (13,20,22–24). Moreover, we consistently found *M. avenae* on oats earlier in the season than we found *R. padi*. For example, in 1975 *M. avenae* was detected on oats on 9 June; but *R. padi* not until 14 July. In 1976, *M. avenae* appeared on 2 June; *R. padi* on 15 June. Although these are only observations, and not thorough measurements of aphid populations, they agree with data from one season of aphid trapping. In 1960, *M. avenae* represented 13.5% of the aphids captured in a blacklight trap; *R. padi* collections were 8.9% of the total (3). Other important factors that probably affect fluctuations in predominating BYDV isolates include changes in cultivars and acreages of small grains, relationships between spring and winter grain crops, the role of perennial grasses, and the whole range of variation in development and distribution of the various aphid species that transmit BYDV. Data on fluctuation of BYDV isolates from year to year are an important base for understanding the epidemiology of barley yellow dwarf. However, these data are only one piece of a large puzzle.

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