Identification of Luteoviruses of Small Grains from 1981 Through 1984 by Two Methods

W. F. ROCHOW, Research Plant Pathologist, Agricultural Research Service, U.S. Department of Agriculture, and Professor, IRMGARD MULLER, Research Support Specialist, and LAURA A. TUFFORD and DAWN M. SMITH, Research Technicians, Department of Plant Pathology, Cornell University, Ithaca, NY 14853

ABSTRACT

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From 1981 through 1984, parallel tests for variants of barley yellow dwarf virus (BYDV) were made on 206 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 171 of the comparisons, results of both methods agreed; in 29, EIA resulted in more complete information than the transmission tests; and in six, transmission tests were more informative than EIA. Each BYDV isolate was identified by comparison with five previously characterized ones: PAV, MAV, SGV, RPV, and RMV. Of 211 BYDV isolates identified in 172 plants, 155 were similar to PAV, 29 were similar to RMV, 20 were similar to RPV, 4 were similar to MAV, and 3 were similar to SGV. The EIA procedure was especially useful in detecting mixed infections, which were found in 36 of the 172 infected plants.

Identification of the different luteoviruses that cause barley yellow dwarf in a wide range of grains and grasses has been improved in recent years with enzyme-linked immunosorbent assay (EIA) (4,5,9,11). We have been evaluating this useful serological method by comparing it with parallel biological assays using aphids to transmit the viruses. In three preceding years, EIA produced as much or more information than did aphid transmission tests in most cases (8,10).

This report summarizes work during the past 4 yr (1981–1984), in which we used four virus-specific immunoglobulins in EIA and four aphid species in virus transmission tests to study 206 field-collected samples. Purposes of the work included identification of virus variants from the field, comparison of serological and biological methods, and comparison of results with those obtained in previous years.

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MATERIALS AND METHODS

Most collections of oats, wheat, or barley were made from test plots maintained by M. E. Sorrells in Tompkins County near Ithaca, NY. Some samples were also collected each year in other counties of New York. Twenty-nine samples from cooperators in Virginia, Montana, or Idaho were also tested in 1981. Each sample, usually a single plant, was handled as described previously (10,13). Adjacent leaves were used in aphid transmission tests; remaining tissue was used to prepare 3 g of finely chopped sample for extraction with a PT-20 probe of a Brinkmann Polytron Homogenizer. All samples for EIA were clarified with chloroform (11).

The EIA was carried out as described previously (8,10,11). Each sample was tested by the direct (double-sandwich) procedure with immunoglobulins prepared from four virus-specific antisera (11). Alkaline phosphatase reactions were measured at 405 nm with a Dynatech microELISA Reader Model MR-580 after 45 min at room temperature. A reaction was usually considered positive only if the absorbance was at least 0.1, a value within the visible range. Because the standard deviation for "healthy" controls in most tests was below 0.01, this detection threshold was much higher than the twice-background range often used in such tests.

Biological identifications were made in transmission tests with four aphid species: *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Sitobion* (= *Macrosiphum*) *avenae* (Fabricius), and *Schizaphis graminum* (Rondani). Tests were based on a 2-day acquisition feeding and a 5-day inoculation test feeding as described previously (9,10). The test plant was Coast Black oats (*Avena byzantina* Koch). In every test, some aphids fed on healthy tissue for use as controls.

In both biological and serological tests, viruses recovered from the field-collected samples were compared with five characterized isolates of barley yellow dwarf virus (BYDV) (11,12): RPV, transmitted specifically by *R. padi*; RMV, transmitted specifically by *R. maidis*; MAV, transmitted specifically by *S. avenae*; SGV, transmitted specifically by *S. graminum*; and PAV, transmitted nonspecifically by *R. padi* and *S. avenae*. *S. graminum* also transmits RPV and PAV but less consistently than does *R. padi*. Based on a range of properties, these five viruses form two groups: RPV and RMV in one and PAV, MAV, and SGV in the other (6,12). Many additional aphid transmission tests were made of infected test plants because initial virus transmission results from a field sample are not adequate for thorough identification of these luteoviruses. In most of the additional tests, EIA was again done in parallel with aphid transmission tests.

RESULTS AND DISCUSSION

In each of the 4 yr, results of most comparisons with four immunoglobulins in EIA and four aphid species in virus transmission tests were in agreement. Of 206 tests, results were identical in 171 or 83% (Table 1, groups A–F). Results of the two methods did not agree in 35 tests; for 29 of these, the EIA produced more complete information (Table 1, groups G–N), and in six tests, we learned more from transmission tests than from EIA (Table 1, groups O–R). Mixed infections by more than one of the viruses were detected in 36 of the 172 infected plants; 34 of the 36 cases were detected by serological tests, but only in 14 plants were they detected by transmission tests (Table 1).

As in other recent years (9,10), isolates similar to PAV were the most common (Table 1). Of the 211 isolates identified from 172 plants, 155 were similar to PAV; 29, to RMV; 20, to RPV; four, to MAV; and three, to SGV. The predominance of PAV-like isolates in New York continues

a trend away from predominance of MAV-like ones 25 yr ago (9). In fact, no MAV-like isolates were detected in any New York samples in these tests; the MAV- and SGV-like isolates identified here (in 1981) came from the samples sent by cooperators in Idaho or Montana. Isolates similar to RPV and RMV were found each year (Table 1).

Relative advantages and disadvantages of the two procedures are illustrated by the data in Table 2, which provided the basis for the summary in Table 1. The main advantage of the serological procedure over the biological one is saving of time. The EIA procedure takes 2 days; aphid transmission tests take at least 4 wk. Because additional transmission tests are usually needed, a thorough biological identification can take many months. For example, in the 116 tests in which we identified viruses similar to PAV by both methods (Table 2, group A), 16 of 348 test plants became infected initially by means of *R. maidis*. These plants were then sampled in additional comparative aphid transmission tests to determine whether *R. maidis* had recovered PAV-like virus or some other variant from the original field-collected plants. The EIA procedure, also used to test these plants, determined within 2 days that only PAV-like virus was involved in all cases. Parallel tests with aphids, which required many weeks, confirmed the serological results because virus was subsequently transmitted to all 51 test plants by *R. padi*, to most of the test plants by *S. avenae* and *S. graminum*, but to only two of 48 plants by *R. maidis*.

Although *R. padi* is the most efficient vector of PAV-like isolates, a few times each year, it failed to transmit these

Table 1. Summary of 206 tests for barley yellow dwarf virus (BYDV) by enzyme-linked immunosorbent assay (EIA) and aphid transmission tests of samples collected in the field during 4 yr

	No. of samples				BYDV isolate similarity identified by	
Group	1981	1982	1983	1984	EIA	Aphids[a]
A	47	33	16	20	PAV	PAV
B	24	4	3	3	None	None
C	3	0	1	4	RPV	RPV
D	2	0	0	0	MAV	MAV
E	1	0	0	0	RMV	RMV
F	0	5	5	0	PAV + RMV	PAV + RMV
G	5	2	4	0	PAV + RMV	PAV
H	0	0	0	1	PAV + RMV	RMV
I	3	3	2	1	PAV + RPV	PAV
J	2	0	0	0	RMV	None
K	0	0	1	1	PAV	None
L	1	0	0	0	MAV	None
M	0	0	2	0	PAV + RPV + RMV	PAV + RMV
N	0	0	1	0	PAV + RPV + RMV	PAV
O	3	0	0	0	MAV	SGV
P	1	0	0	0	MAV	PAV
Q	1	0	0	0	MAV	MAV + PAV
R	0	0	1	0	PAV	PAV + RMV

[a] PAV 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*, which also transmits RPV and PAV.

Table 2. Results of 206 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

	EIA					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.)[a]	*Rhopalosiphum padi*	*Sitobion avenae*	*R. maidis*	*Schizaphis graminum*
A	116 PAV	0.013	0.159	0.848	0.022	116 PAV	315/348	291/348	16/348	233/348
B	34 None	0.013	0.013	0.012	0.024	34 None	0/102	0/102	0/102	0/102
C	8 RPV	1.028	0.012	0.006	0.021	8 RPV	24/24	4/24	0/24	6/24
D	2 MAV	0.013	1.043	0.051	0.076	2 MAV	0/6	6/6	0/6	0/6
E	1 RMV	0.296	0.008	0.007	0.575	1 RMV	1/3	0/3	3/3	2/3
F	10 PAV + RMV	0.032	0.172	0.827	0.229	10 PAV + RMV	30/30	14/15	14/15	13/15
G	11 PAV + RMV	0.037	0.138	0.655	0.247	11 PAV	25/33	25/33	0/33	21/33
H	1 PAV + RMV	0.018	0.141	1.305	0.143	1 RMV	0/3	0/3	1/3	0/3
I	9 PAV + RPV	0.714	0.109	0.573	0.011	9 PAV	27/27	19/27	0/27	15/27
J	2 RMV	0.256	0.037	0.041	0.322	2 None	0/6	0/6	0/6	0/6
K	2 PAV	0.005	0.156	1.156	0.000	2 None	0/6	0/6	0/6	0/6
L	1 MAV	0.049	0.202	0.017	0.035	1 None	0/3	0/3	0/3	0/3
M	2 PAV + RPV + RMV	0.678	0.067	0.215	0.090	2 PAV + RMV	6/6	5/6	4/6	3/6
N	1 PAV + RPV + RMV	0.899	0.123	0.577	0.089	1 PAV	3/3	3/3	0/3	2/3
O	3 MAV	0.013	0.285	0.157	0.001	3 SGV	2/9	6/9	1/9	9/9
P	1 MAV	0.049	0.152	0.000	0.036	1 PAV	3/3	0/3	0/3	0/3
Q	1 MAV	0.114	1.117	0.188	0.020	1 MAV + PAV	1/3	3/3	0/3	0/3
R	1 PAV	0.002	0.082	0.173	0.013	1 PAV + RMV	3/3	1/3	2/3	3/3
	33 Healthy controls	0.013	0.010	0.011	0.017	22 Aphid controls	0/66	0/66	0/66	0/66
Virus controls	RPV	0.671	0.014	0.017	0.021	RPV	69/69	1/69	0/69	18/69
	MAV	0.006	1.068	0.111	0.036	MAV	2/69	69/69	0/69	0/69
	PAV	0.014	0.189	0.922	0.025	PAV	69/69	61/69	2/69	33/69
	RMV	0.012	0.013	0.015	0.531	RMV	7/69	1/69	69/69	19/69
	SGV	0.007	0.086	0.061	0.014	SGV	2/69	1/69	0/69	69/69

[a] With isolates similar to those shown. PAV 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*, which also transmits RPV and PAV.

[b] Using antiserum for isolate shown. Values are means of absorbance for number of individual plants shown at left in each case.

[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.

isolates from the field sample but did so regularly in subsequent comparisons. For example, in 1981, three of the PAV-like isolates were transmitted from the field sample only by *S. graminum*. EIA of each sample had clearly shown the presence of virus similar to PAV; seven additional transmission tests confirmed the serological result. Such rare occurrences no doubt merely illustrate the inherent variation in virus transmission by vectors, but the pattern also suggests the intriguing possibility of a kind of dependent virus transmission from a field-collected plant infected with luteoviruses other than those we can identify (2,7).

A series of subsequent transmission tests was needed, especially in biological tests, to identify the vector-specific isolates similar to RPV, MAV, and RMV (Table 2, groups C–E). Before EIA was available, such tests required at least four serial transmissions and took many months. Parallel use of EIA now reduces this time dramatically. Parallel tests permitted demonstration, for example, that the four original transmissions by *S. avenae* in tests of the eight RPV-like viruses (Table 2, group C) involved only RPV-like virus. Similarly, identity of RMV-like virus (Table 2, group E) was confirmed in subsequent tests both by aphids and EIA, showing that virus transmitted originally by *R. padi* and *S. graminum* was RMV-like and not similar to one of the other isolates.

Another advantage of the serological procedure is its ability to differentiate viruses not readily separated in aphid transmission tests. Mixed infections of viruses similar to PAV and RPV can be identified in transmission tests only by tedious procedures involving use of many single aphids. Moreover, *R. padi* does not always recover both RPV- and PAV-like viruses from doubly infected, field-collected samples. For example, in seven of 12 tests that detected mixtures (Table 1, groups I, M, and N) we found, by making additional EIA of infected test plants, that *R. padi* had transmitted both viruses. From four plants, only PAV-like virus had been transmitted, and from one, only RPV-like virus.

The serological procedure was sometimes more accurate than the biological one. In 18 of the 29 cases in which results of serological tests were more complete than biological ones, the reason was that aphids failed to transmit virus from the field samples to indicator plants. This discrepancy between methods probably results in part from the fact that the condition of the tissue is more critical for aphid feeding than for virus extraction for EIA.

A special advantage of the EIA procedure is that identifications can be based on both heterologous and homologous data, especially for isolates similar to PAV and MAV. Sometimes we think that the ratio of these absorbance values for PAV-like isolates varies with relative severity of symptoms of test plants. In each of 2 yr, we made preliminary tests of this observation by first making aphid transmission tests in parallel with *R. padi* and *S. avenae*, then grouping infected test plants for EIA on the basis of symptoms. In 1982, the absorbance ratio for MAV/PAV immunoglobulins varied from 0.21 to 0.40 for 17 isolates. The lowest value was from a plant with mild symptoms; the highest was from a plant with severe symptoms. However, there was a wide range within each group. In 1983, corresponding ratios varied from 0.14 to 0.32 for 21 isolates. The lowest was from a plant with moderate symptoms; the highest was again from one with severe symptoms. We did not have enough samples or enough replicates to analyze these differences and evaluate the large variation among samples, but this illustrates the kind of additional data that can be obtained in EIA that might be useful for future applications.

Another advantage of the EIA procedure was that it permitted demonstration that a single aphid in nature can transmit two viruses. This result occurred when we collected 16 individual *R. padi* or *S. avenae* in the field, allowed each aphid to feed for 5 days on a single test plant, then tested the plants to determine which virus or viruses the aphids had transmitted. Four of the aphids transmitted virus. In one case, *S. avenae* transmitted virus similar to PAV. One *R. padi* transmitted virus similar to RPV, another transmitted virus similar to PAV. The third *R. padi* transmitted a mixture of both RPV- and PAV-like viruses, a mixture easily detected only by EIA.

The role of EIA in testing tissue not suitable for aphid feeding because of desiccation was also apparent each year. We assayed more than 700 samples from 35 locations only by EIA in various cooperative tests. In three of the four years, we identified viruses similar to all five of the isolates used for comparison. Of the 291 plants found infected, 42 contained more than one luteovirus. Even if these plants had been suitable for use in aphid transmission tests, we could have tested only a small percentage of them in this way.

The value of identifying luteoviruses in parallel by the two methods was illustrated by the six cases in which we learned more from transmission tests than from serological ones. In the absence of aphid transmission tests, three samples would have been erroneously identified as containing MAV-like viruses (Table 2, group O). In all three cases, the absorbance values in tests with MAV and PAV immunoglobulins were moderately low but were generally above the level of heterologous readings we usually obtain for SGV-like viruses. Many subsequent tests showed, however, that the viruses were similar to SGV, not MAV. In nine such tests of plants from the greenhouse, the mean absorbance values for assays with each of the virus-specific immunoglobulins were 0.009 (RPV), 0.081 (MAV), 0.056 (PAV), and 0.008 (RMV). These values for tests with MAV and PAV immunoglobulins are more in line with the relatively weak heterologous readings usually obtained for SGV (10,11). Moreover, in the parallel tests with aphids, the viruses were transmitted to all 42 test plants by *S. graminum* but to only two of the 42 plants by both *R. padi* and *S. avenae* and to none of the plants by *R. maidis*. These SGV-like isolates came from samples sent from Idaho by R. L. Forster, continuing a trend for SGV-like isolates to be relatively common in Idaho (3,5,10).

In another case, EIA produced a relatively weak identification of MAV-like virus, but aphid transmission tests clearly showed that the virus was similar to PAV (Table 2, group P). In five additional EIA, infected test plants consistently had severe reactions typical for PAV-like virus, and virus was transmitted to 18 of 18 test plants by *R. padi*. Mixed infections were detected in two plants by aphids, but not by EIA. In one case (Table 2, group Q), only MAV-like virus was detected by EIA; the 0.114 value for RPV immunoglobulin was not considered positive in this case because background values were higher than usual in the plate because of "edge effects" (1). Tests with aphids, however, showed a mixture of viruses similar to MAV and PAV (Table 2, group Q). These tests revealed that the single transmission from the field plant by *R. padi* involved PAV-like virus, but in the same test, *S. avenae* transmitted MAV-like virus. Seven additional EIA comparisons confirmed this.

In the final case, EIA failed to detect RMV-like virus, but tests with aphids showed it to be present in addition to a PAV-like isolate (Table 2, group R). Thus virus transmitted originally by *R. maidis* was subsequently transmitted only by *R. maidis*, and parallel EIA showed a consistent pattern similar to the atypical RMV-like virus encountered previously (10). In these tests, infected plants produced a relatively weak RMV reaction (mean absorbance of 0.072) and a relatively strong RPV heterologous reaction (mean absorbance of 0.042). This kind of virus variant is more likely to be detected by aphids than by our immunoglobulin, a good illustration of why we admire our aphids so much!

These data complete work during seven consecutive years in which we have compared the two methods of identifying luteoviruses from a total of 503 samples of small grains (8,10). Results of tests in each year were remarkably similar. The

EIA procedure generally was as good as (403 samples) or better than (90 samples) the biological one. But in five of the seven years, a few examples showed that the aphids can sometimes have the last word and help remind us how much we yet have to learn about plant luteoviruses.

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