Detection of Barley Yellow Dwarf Virus by Enzyme-Linked Immunosorbent Assay

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ABSTRACT

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Parallel tests with aphid-transfer techniques and enzyme-linked immunosorbent assay (ELISA) indicated that ELISA was sensitive enough to detect barley yellow dwarf virus in oat leaf extracts. The reaction was specific enough to distinguish easily RPV and MAV, two distinctive vector-specific isolates of the virus. A third type of isolate, PAV, sometimes reacted, usually weakly, in heterologous tests with MAV antiserum. ELISA appears to have potential for simplifying survey and assay work with this phloem-restricted luteovirus.

Barley yellow dwarf virus (BYDV) is an important aphid-borne and phloem-restricted virus that occurs world-wide in wheat, oats, and barley (15). It also is distributed widely in several perennial grasses, including bluegrass, red fescue, perennial ryegrass, and bromegrass. Because BYDV cannot be sap-transmitted, testing for its presence in plants until now has required manipulations of aphid vectors. Vector specificities of BYDV isolates add to the difficulties and require tests of virus acquisition and transmissibility by several specific aphid vectors for each sample tested (14). Moreover, although antisera to some strains of BYDV are available (17), the extremely low concentration of virus in extracts of infected leaves precludes using them in simple microprecipitin or gel diffusion tests for diagnosis and assay. Instead, such antisera have been most useful in the more complex infectivity-neutralization tests with aphids fed on virus preparations through membranes, and in serologically-specific centrifugal density gradient analysis or electron microscopy (12,15,18).

We now present evidence that antisera to two isolates of BYDV can be used in enzyme-linked immunosorbent assay (ELISA) for the detection and assay of BYDV in cereal leaf extracts. A preliminary note has been published (11). We also describe some factors that affect the use of such tests for these purposes. Potentially the technique can significantly simplify testing for BYDV, as illustrated in a companion study (16).

MATERIALS AND METHODS

Virus isolates. The isolates of BYDV involved in the various tests have been described (14,17). One isolate (PAV) is transmitted nonspecifically by both *Rhopalosiphum padi* L. and *Macrosiphum avenae* Fabr.; another isolate (RPV) is transmitted specifically by *R. padi*; and a third isolate (MAV) is transmitted specifically by *M. avenae*. Isolates RPV and MAV are serologically distinct and MAV and PAV are serologically related (1,16). The RMV isolate is transmitted specifically by *Rhopalosiphum maidis* (Fitch); its serological relationship to other BYDV isolates is unknown. Isolates were maintained in greenhouses at Cornell in oat plants (*Avena byzantina* C. Koch 'Coast Black').

Antisera. The antisera were similar to those described by Rochow et al (17). They were made at Cornell in 1975 by injecting rabbits with totals of 280 μg (MAV) or 260 μg (RPV). These totals were divided approximately evenly among two successive weekly intramuscular injections of virus emulsified with Freund's adjuvant, and an intravenous injection 3 wk later. Terminal bleedings

carried out 12 days after the final injection yielded antisera comparable to previous sera for MAV and RPV, which had homologous dilution end points in micro agar double-diffusion tests of 1:4,096 (1).

Preparation of immuno-globulins and EIA test procedures. At Purdue, EIA procedures followed the protocols of Voller et al (21), and Clark and Adams (6), as modified by Lister (10). Partially purified immunoglobulins (Ig's) were prepared by precipitation (half-saturation with ammonium sulfate), equilibration in half-strength phosphate-buffered saline (PBS) pH 7.4, and washing through DEAE cellulose in that buffer (8). The Ig's at 1 μg of protein per milliliter ($A_{280\,nm}$ = 1.4) were conjugated with alkaline phosphatase (Type VII, Sigma Chemical Co., St. Louis, MO 63178) at an enzyme: Ig ratio of 2:1 (w/w) with 0.06% glutaraldehyde (2), and were stored at 4 C with bovine serum albumin at 5 μg/ml, added after thorough dialysis.

Wells in polystyrene MicroELISA™ plates (No. 1-223-29; Dynatech Laboratories, Inc., Alexandria, VA 22314) were coated by incubating 250 μl of unlabeled Ig diluted in 0.05 M sodium carbonate, pH 9.6, at 36 C in each well for 4 hr. Peripheral wells were not used, but were kept filled with water to help stabilize the temperature. Plates were stacked or covered during incubation. After incubation they were rinsed three times with PBS containing 0.05% Tween-20 (PBS-Tween). Antigen preparations or cereal leaf extracts (see below) in PBS-Tween containing 2% polyvinyl pyrrolidone (PVP, M.W. 40,000; Sigma) then were incubated overnight at 6 C in the coated, rinsed wells to react with the bound Ig. After further rinsing, diluted enzyme-conjugated Ig was added to react with bound antigen during a further 4-hr incubation at 36 C. The conjugate was diluted in a buffer mixture containing PBS-Tween-PVP and 0.02% ovalbumin, or in an extract from healthy or infected Coast Black oat leaf tissue diluted 1:5 (w/v) in this buffer, or in the buffer extracts of healthy oat leaf tissue concentrated by ultracentrifugation (28,000 rpm for 4 hr, Spinco R30 rotor) and resuspension.

Finally, unreacted conjugate was rinsed away, and specific antibody-antigen reactions were assessed by adding *p*-nitrophenyl phosphate at 1 mg/ml in 10% diethanolamine buffer at pH 9.8. Reactions were stopped after 20–30 min by adding 50 μl/well of 3 M NaOH. Assay was by visual observation of the yellow nitrophenolate product or by reading absorbances ($A_{405\,nm}$) of well contents diluted fivefold with water. Those reactions giving obvious yellow color or absorbances equal to or greater than 2× the average for healthy control samples in the same experiment were regarded as positive. All tests were duplicated, and the results were highly reproducible.

Test samples used at Purdue were intact plants or leaves mailed from Cornell in chilled containers, and stored frozen at −20 C until used. For extraction, samples were cut into sections (2–3 cm), pulverized in liquid nitrogen with a mortar and pestle, ground in 0.1 M potassium phosphate buffer at pH 7.5 at a tissue:buffer ratio of 1:2 (w/v), followed by further grinding after adding a further two volumes of the PBS-Tween-PVP buffer. Purified virus preparations made at Cornell also were mailed to Purdue in chilled containers and stored at −20 C, diluted in phosphate buffer, until used.

For tests done at Cornell, the same general procedures were used (16). Minor differences included the use of plates with round-bottomed wells, 200 μl of liquid per well, and incubation of all plates in humidified plastic boxes. The major difference between the procedure used at Cornell and that described above was the use of immunoglobulin preparations not fractionated by chromatographic separation. Instead, virus-specific antisera were first absorbed with an equal volume of a 540-fold concentrate of an extract of healthy Coast Black oat. The total protein fraction (= "globulin preparation") was prepared by two cycles of precipitation with 50% saturated ammonium sulfate from this absorbed antiserum. Another difference between methods at the two locations was the use at Cornell of a microcell with a 1-mm light path, which permitting direct reading of reacted samples at 405 nm (A^{1mm}_{405nm}) without dilution of the well contents. Finally, as discussed below, sample preparation differed significantly in that liquid nitrogen was not used to prepare leaf samples at Cornell; instead, fresh leaf tissue was ground with a pestle and mortar with a buffer/chloroform mixture (3 g tissue with 1.5 ml PBS, then 4 ml of chloroform) and the aqueous phase was separated by low-speed centrifugation (10 min at 8,000 *g*), then used in ELISA.

Aphid transmission tests. Characterization of BYDV isolates by their specific vector relationships was as described elsewhere (14).

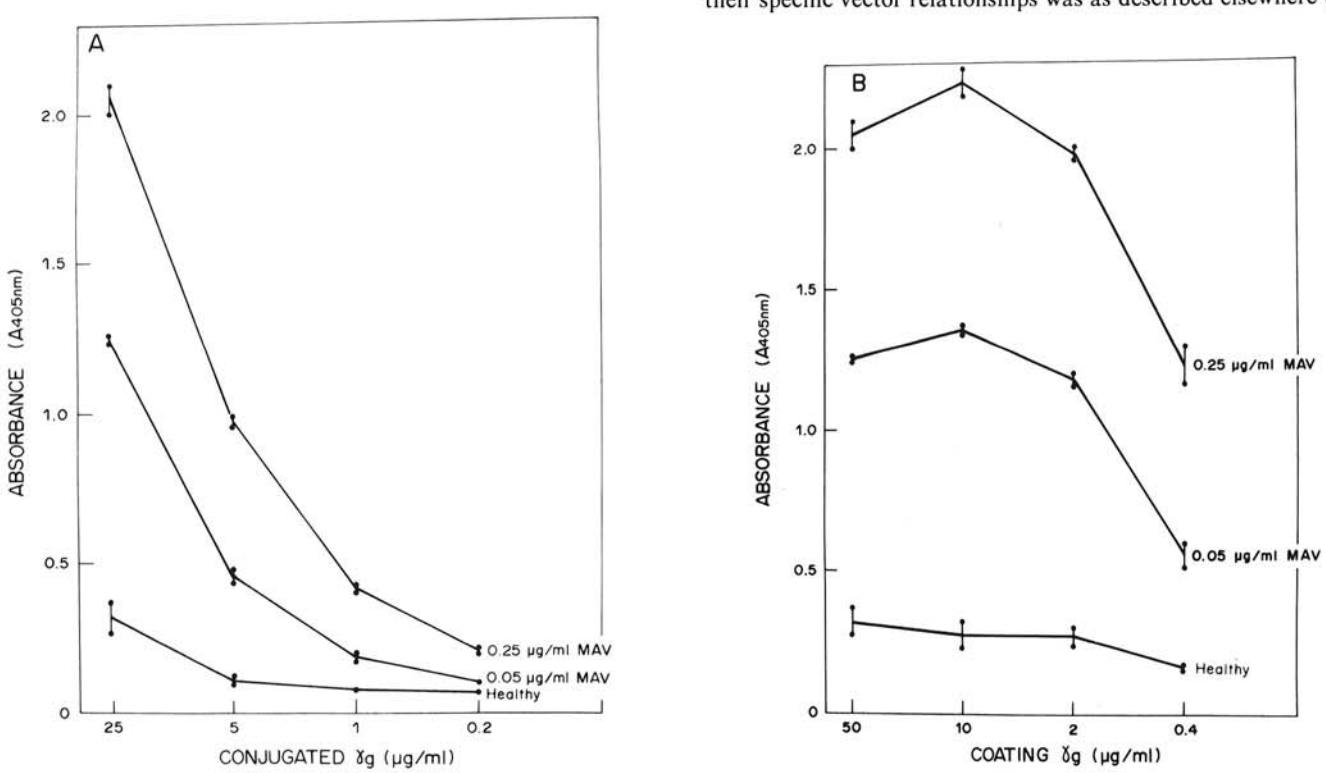

Fig. 1. ELISA absorbances (A_{405nm}) for five-fold dilutions of reacted substrate showing: **A**, Variation of ELISA absorbance with concentration of enzyme-conjugated anti-MAV Ig G (γg) used (with coating Ig G at 50 μg/ml); **B**, Variation of ELISA absorbance with concentrations of anti-MAV Ig G (γg) used for plate coating (with conjugate at 25 μg/ml).

RESULTS

Characteristics of Ig's. The sensitivity characteristics of the coating and enzyme-labeled Ig's used in experiments at Purdue are illustrated in Fig. 1A, B. These show plots of the ELISA absorbances obtained in tests between anti-MAV Ig's and the purified homologous antigen diluted in extracts of healthy tissue. Similar results were obtained in homologous tests with the anti-RPV Ig's. Over a range of Ig concentrations from 50 to 0.2 μg/ml, ELISA absorbances were more sensitive to variations in the concentration of enzyme-labeled conjugate than to variations in the concentration of coating Ig. But, although sensitivity increased with Ig conjugate concentration (especially above 5 μg/ml), the nonspecific background absorbance obtained with control extracts from healthy tissue also tended to increase when Ig conjugate was used at greater than 5 μg/ml (Fig. 1-A). This effect was even more marked with the RPV Ig's. For both MAV and RPV, similar sensitivities were obtained whether coating Ig was used at 2, 10, or 50 μg/ml. For simplicity and economy therefore, all coating and enzyme-labeled Ig's routinely were used at 5 μg/ml for screening tests.

Under these conditions, purified MAV or RPV diluted in healthy leaf extracts was detectable in typical experiments to levels of about 30-60 ng/ml, and ELISA absorbances had an essentially linear relationship to virus concentration over a range of values (Fig. 2). Clearly however, as is indicated by Fig. 1, these levels are not necessarily the limiting sensitivities of ELISA for these isolates of BYDV. Results with the labeled globulin preparations used at Cornell were similar (16), but the sensitivities were lower (see for example, Fig. 3). This would be expected as a result of reduced efficiency in labeling Ig's contaminated with other constituents of antisera and of leaf extract.

It was of special interest that the reactions were highly specific. Thus, in the Cornell tests with MAV and RPV, no cross-reactivity was detectable between virus preparations and heterologous Ig's (16). This result was consistent with previous findings of nonrelationship between these two isolates by other serological tests (1,16). In contrast with results of the earlier work which indicated that MAV antiserum detects PAV about as readily as MAV in gel diffusion tests (1) the Cornell experiments showed cross-reactivity between anti-MAV Ig and PAV preparations to be unexpectedly low (Fig. 3). Such improved discrimination between virus isolates has recently been shown for ELISA tests with other plant virus systems (9).

In the tests at Purdue, when enzyme-labeled Ig's were diluted 1:100 (v/v) with extracts of healthy leaves before use, significant cross-reactivity was noted between the anti-MAV Ig and RPV preparations although not in the reciprocal combination (Fig. 2A, B). This cross-reactivity was abolished making the dilutions of the labeled anti-MAV Ig in extracts from RPV-infected plants, but it also was abolished by using healthy leaf extract concentrated 20-fold for diluting the enzyme conjugate (Fig. 2B). We conclude that the MAV antiserum contained antibodies to normal host proteins that were present in RPV-infected plants at higher concentrations than in healthy plants, and that these proteins can remain as a constituent in some purified preparations, as noted previously in agar diffusion tests (1).

Diagnosis of "unknowns." In an initial set of tests (Table 1), leaf samples from infected oat plants in the greenhouse collection at Cornell were identified only by number, and sent to Purdue for ELISA testing using MAV Ig. Of 13 such samples sent in July, 1977, four were from healthy plants, four contained only MAV, two contained only RPV, one contained a mixture of these, and two contained other individual isolates of BYDV. All five samples that contained MAV were clearly identified in the Purdue ELISA (A_{405nm} = 0.562–0.800), while a weak positive reaction occurred with one sample from a plant infected with the PAV isolate (A_{405nm} = 0.190). The other samples were negative, giving ELISA absorbances in the range of those for the healthy samples (A_{405nm} = 0.106–0.115). At the same time, six field-collected samples were tested by transmission tests with four aphid species at Cornell and by ELISA at Purdue. Four of these samples were ELISA-positive. The aphid transmission tests showed that these samples were infected with virus isolates similar to PAV—the isolate serologically related to MAV—and that the two remaining (ELISA-negative) samples were infected with isolates of the RPV type (Table 1).

Comparisons with ELISA absorbances for tests of dilutions of purified virus indicated that MAV typically was present at about 200 ng/ml in extracts from infected plants. In a separate experiment however, re-extraction of the fibrous leaf residue by grinding with additional buffer was done for one MAV sample. This procedure yielded an extract containing about one-half of the virus concentration of the first extract.

For a second series of ELISA, both anti-MAV and anti-RPV Ig's were used to test samples from the Cornell collection. Many of these tests were of oat leaves from test plants used to identify field-collected isolates obtained during the summer of 1977. After identifications by ELISA were completed at Purdue, the results were

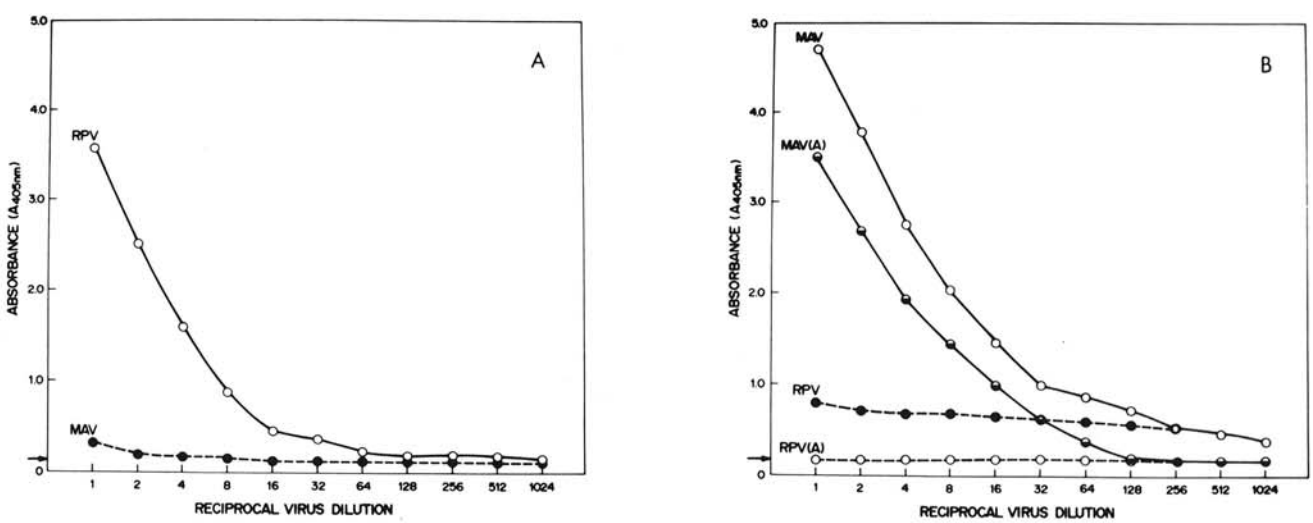

Fig. 2. ELISA absorbances (A_{405nm}) for five-fold dilutions of reacted substrate, obtained in homologous and heterologous titrations of 2 μg/ml preparations of: **A**, RPV and MAV against anti-RPV Ig and **B**, RPV and MAV against anti-MAV Ig. For all values, coating Ig's were at 5 μg/ml in buffer. Curves indicated as "MAV" and "RPV" are plots of values obtained with enzyme conjugates diluted to 5 μg/ml (1:100) with Coast Black oat extract. Curves indicated as "MAV (A)" and "RPV (A)" are plots of values obtained with enzyme conjugate diluted to 5 μg/ml with a 20-fold concentrate of oat extract. The results obtained when unconcentrated RPV-infected oat extract was used similarly to dilute the anti-MAV enzyme-labeled Ig fell along the RPV-A line. The arrow indicates the mean value obtained with healthy control extracts.

compared with those previously obtained in aphid transfer tests. Table 2 summarizes the results for all 40 such tests. Isolates of the MAV and RPV types were clearly identified by their homologous Ig's. Isolates similar to PAV usually yielded extracts giving weak positive reactions. Reactions of isolates similar to RMV were negative. Comparisons with ELISA absorbances for tests of standard virus preparations indicated that MAV concentrations in leaf extracts from these samples averaged about 500 μg/ml, but the concentration of RPV in extracts was lower (about 100 ng/ml). This result was confirmed by two further repetitions of tissue extraction from the positive samples. Test sensitivity was not improved and ELISA absorbances remained essentially unchanged when the ELISA plate wells were refilled with the leaf extracts twice at intervals of 2 hr at room temperature before a final (third) filling was incubated overnight in the cold.

Finally, a direct comparison was made of ELISA and aphid transmission tests run in parallel at Purdue and Cornell, respectively. In the ELISA tests both anti-MAV and anti-RPV Ig's were used, and the aphid transmission tests employed the appropriate two aphid species to distinguish these isolates. Sixteen oat test plants were selected from the Cornell collection. From each plant, one leaf was removed for the aphid tests and two additional leaves were mailed to Purdue for the ELISA tests. The results (Table 3) showed that, as expected, the aphid transmission tests clearly differentiated healthy plants, those infected with RPV, those infected with MAV, and those infected with both isolates. ELISA clearly identified MAV, but were less clear and consistent in identifying RPV. For three of the eight samples containing this isolate, certain diagnosis would not have been possible by ELISA alone, using the "2× healthy background" criterion for a positive $A_{405\ nm}$ value. Significantly, however, by the same criterion no false positive results were obtained in any tests.

Effect of extraction procedure on virus yield. The results of the ELISA screening done at Purdue indicated that the concentrations of RPV-type isolates obtained in leaf extracts were lower than those of MAV-type isolates, and could be marginal or inadequate for unequivocal diagnosis. However, when ELISA was incorporated into a study of field isolates at Cornell (16), 13 of 13 RPV-like isolates were readily diagnosed, with ELISA absorbances ranging from 0.265–0.640 in comparison with absorbances for extracts from healthy control tissue averaging 0.009. In the Cornell tests extracts from fresh tissue were used, made by grinding in a chloroform-buffer mixture, whereas at Purdue we used frozen tissue

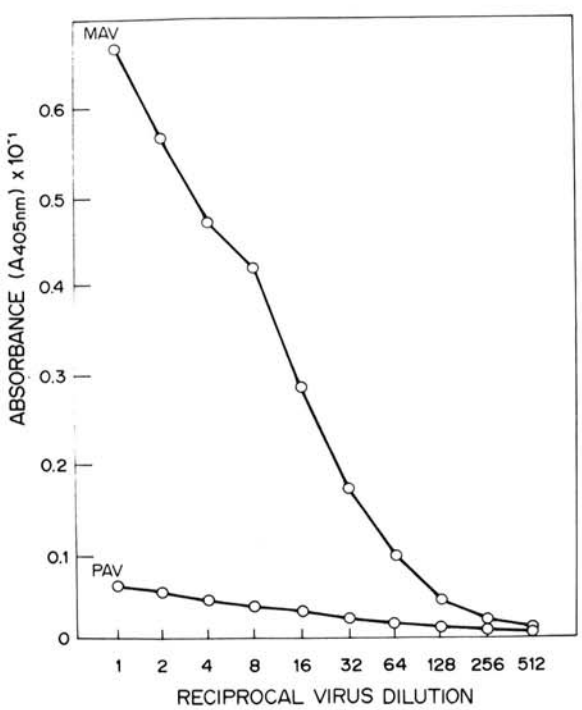

Fig. 3. ELISA absorbances (A_{405nm}^{1mm}) for undiluted reacted substrate, obtained in experiments at Cornell University in which dilutions of preparations of MAV and PAV, as indicated (each initially 10 μg/ml), were reacted with anti-MAV globulins, prepared as described in the text and used diluted to an estimated concentration of 20 μg/ml total protein. The mean value for reactions with healthy control extracts was 0.008. Readings above 0.040 were all associated with visible reactions.

TABLE 1. Results of initial enzyme-linked immunosorbent assay (ELISA) test series of Cornell barley yellow dwarf virus samples in oat leaves as received at Purdue as "unknowns," and tested with anti-MAV immunoglobulin (Ig) only

BYDV isolate in plant tested[a]	ELISA test result[b]		Transmission test result[c]
	visual	A_{405nm}	
MAV	+++	0.775	MAV
MAV	+++	0.800	MAV
MAV	++	0.562	MAV
MAV	+++	0.772	MAV
MAV + RPV	+++	0.750	MAV + RPV
RPV	—	0.114	RPV
RPV	—	0.108	RPV
RPV (FS)	—	0.111	(RPV)
RPV (FS)	—	0.100	(RPV)
PAV	?	0.190	PAV
PAV (FS)	++	0.596	(PAV)
PAV (FS)	+++	1.300	(PAV)
PAV (FS)	+++	0.920	(PAV)
PAV (FS)	++	0.460	(PAV)
RMV	—	0.114	RMV
None	—	0.115	0
None	—	0.110	0
None	—	0.106	0
None	—	0.110	0

[a](FS) indicates field sample concurrently undergoing aphid transmission tests at Cornell when received at Purdue. Status of other isolates was already known, as indicated.
[b]Visual readings were of undiluted reacted substrate in wells. Absorbance readings were means of duplicate readings for five-fold dilutions of reacted substrate.
[c]Isolate acronyms in parenthesis refer to results of aphid transmission tests run concurrently with ELISA tests.

TABLE 2. Results of enzyme-linked immunosorbent assay (ELISA) tests at Purdue of leaf samples of greenhouse-grown oat plants infected with barley yellow dwarf isolates collected at Cornell[a]

BYDV isolate similar to that shown	Plants tested (no.)	ELISA test with immunoglobulin (Ig)[b]	
		RPV Ig (A_{405nm}^{1mm})	MAV Ig (A_{405nm}^{1mm})
PAV	19	0.14 (0.05–0.22)	0.24 (0.08–0.76)
MAV	4	0.14 (0.07–0.24)	1.21 (1.00–1.42)
RPV	2	0.51 (0.47–0.54)	0.08 (0.05–0.10)
RMV	11	0.11 (0.08–0.15)	0.08 (0.05–0.10)
Healthy oats	4	0.09 (0.05–0.13)	0.07 (0.05–0.10)
Healthy control extract[c]		0.10	0.07
PBS control[c]		0.09	0.08

[a]All samples except standard controls were received at Purdue as "unknowns".
[b]Means of duplicate absorbance readings for five-fold dilutions of reacted substrate. For each test duplicate wells were used. Numbers in parentheses show range of readings for each kind of test.
[c]Standard controls.

extracted in buffers, after pulverization in liquid nitrogen (see below and "Materials and Methods"). Results of several comparative tests suggested that these differences in extraction procedure can significantly affect the yield of virus detectable by ELISA.

Thus, in experiments at Cornell, although freezing infected Coast Black oat tissue prior to extraction by the chloroform procedure had little or no effect on yields of MAV or RPV, comparisons of yields from fresh tissue extracted with or without chloroform indicated that yields of RPV were reduced when chloroform was not used, whereas yields of MAV seemed essentially unaffected. The effect of freezing tissue before virus extraction was tested by storing comparable 2-g tissue samples for both RPV and MAV overnight at either 4 C or −20 C. The samples were ground in 1 ml of PBS-Tween with a mortar and pestle, followed by additional grinding in 3 ml of chloroform. Each preparation was clarified by low-speed centrifugation and tested directly by ELISA. The absorbance (A_{405nm}^{1mm}) of a sample made from unfrozen RPV-infected tissue was 0.43; the corresponding reading for frozen tissue was 0.42. In another comparison, the A_{405nm}^{1mm} values for preparations from RPV-infected oat tissue were 0.45 and 0.37 for unfrozen and frozen tissue, respectively. Freezing also had no appreciable effect on results in similar tests done with MAV-infected tissue; in two pairs of comparisons with unfrozen and frozen MAV-infected tissue, A_{405nm}^{1mm} values were 0.43 and 0.37 respectively, and 0.40 and 0.37, respectively.

The effect of extracting fresh tissue with or without chloroform clarification also was tested at Cornell by extracting four comparable 2 g-samples of infected tissue for each of MAV and RPV. The A_{405nm}^{1mm} values for the two MAV samples made by grinding tissue with chloroform were 0.43 and 0.48; corresponding values of the two MAV samples made without chloroform were 0.46 and 0.37. In the tests with RPV, however, reactions of preparations made with chloroform gave A_{405nm}^{1mm} values of 0.34 and 0.36; corresponding values for parallel preparations made without chloroform were 0.22 and 0.12. The mean value for eight healthy control samples in these tests was 0.007.

Experiments at Purdue confirmed this effect of chloroform in extraction of RPV. Samples (3g) of Coast Black oat leaf infected with RPV or MAV, received from Cornell and stored frozen in the usual way, were extracted by one of the following procedures:

Treatment 1. Grind in 1.5 ml PBS/PVP/Tween with a mortar and pestle, add 4 ml chloroform and regrind, separate phases by low speed centrifugation, make aqueous phase to 12 ml with PBS/PVP/Tween, and test (= Cornell procedure product, diluted).

Treatment 2. Grind in 6 ml 0.1M phosphate, pH 7.0, add 12 ml chloroform and regrind, separate phases by low speed centrifugation, make aqueous phase to 12 ml with PSB/PVP/Tween, and test.

Treatment 3. Add liquid nitrogen and pulverize, grind in 6 ml 0.1M phosphate, pH 7.0, add 6 ml PBS/PVP/Tween and grind further, test supernatant after low-speed centrifugation (= Purdue procedure).

Treatment 4. As (3), but reverse the order of adding the phosphate and PBS buffers.

The ELISA absorbances (A_{405nm}) obtained for the products of these treatments from MAV-infected tissue did not differ significantly, but they did so with RPV-infected tissue (Table 4). Means of A_{405nm} values for the treatments of 1–4 were 0.688, 0.727, 0.568 and 0.482, respectively. Table 4 also illustrates the degree of reproducibility obtained. Treatments 1 and 2 (chloroform extractions) were not significantly different at $P = 0.01$ by Duncan's new multiple range test (20), but yields of treatment 3 were significantly lower than those of both of these treatments, and those of treatment 4 were significantly lower than any.

TABLE 3. Results of parallel tests of barley yellow dwarf virus (BYDV) isolates by enzyme-linked immunosorbent assay (ELISA) and aphid transmission done at Purdue and Cornell respectively[a]

BYDV isolate in plant tested	ELISA test with immunoglobulin (Ig) shown[b]		Transmission[c]	
	RPV Ig (A_{405nm})	MAV Ig (A_{405nm})	*R. padi*	*M. avenae*
MAV	0.06	1.24*	0	3
MAV	0.09	1.60*	0	3
MAV	0.06	1.44*	0	3
MAV	0.07	1.20*	0	3
RPV	0.83*	0.08	3	0
RPV	0.18	0.13	3	0
RPV	0.21*	0.07	3	0
RPV	0.19*	0.08	3	0
RPV	0.16	0.13	3	0
MAV + RPV	0.13	0.78*	3	3
MAV + RPV	0.28*	0.53*	3	3
MAV + RPV	0.20*	0.56*	3	3
None	0.07	0.07	0	0
None	0.07	0.09	0	0
None	0.06	0.07	0	0
None	0.06	0.12	0	0
Healthy sap control[d]	0.07	0.07	—	—
PBS control[d]	0.07	0.07	—	—

[a]The isolates were received at Purdue as "unknowns" in random sequence.
[b]Means of duplicate absorbance readings for five-fold dilutions of reacted substrates. Asterisked results regarded as definitely positive by visual readings.
[c]Number of three infested plants that became infected following 2-day acquisition and 5-day test feeding by about 10 aphids of species shown.
[d]Standard controls.

DISCUSSION

Our results show that ELISA is sufficiently sensitive to find important applications in detecting, diagnosing, and assaying some isolates of BYDV in cereal leaf extracts. Thus, even though concentrations of the RPV isolates sometimes were marginal for unequivocal detection when purified enzyme-conjugated Ig was used at 5 μg/ml, we think that, if necessary, increasing the Ig concentration or improving specific enzyme-labeling would overcome these difficulties. Moreover, factors as important as test sensitivity may be the efficiency and variability of virus extraction from leaf tissue. Rochow and Brakke (19,4) identified extraction to be a critical step in the production of virus for purification, and found that reextraction of fiber yielded additional amounts of virus, as it did in our initial ELISA. In our tests with fresh or frozen tissue, yields of detectable RPV (but not MAV) were improved by including chloroform in the extraction medium, which suggests that MAV and RPV isolates may be associated differently with cells or tend to bind differently to tissue fragments. Similarly, leaf age can be a critical factor in virus availability to vectors and for purification

TABLE 4. Comparative A_{405nm} readings for enzyme-linked immunosorbent assays (ELISA) of the RPV isolate of barley yellow dwarf virus as extracted by various procedures from frozen infected Coast Black oat leaves[a]

Treatments	Replicates 1	2	3	4	5	6	$\bar{x}$[b]	s
1	0.74	0.74	0.63	0.62	0.72	0.68	0.688 A	0.0538
2	0.77	0.78	0.68	0.65	0.74	0.75	0.727 A	0.0512
3	0.59	0.63	0.58	0.56	0.52	0.53	0.568 B	0.0407
4	0.53	0.53	0.49	0.51	0.43	0.40	0.482 C	0.055

[a]Extraction procedures are given in detail in the text. Treatments 1 and 2 used chloroform/buffer extraction, treatments 3 and 4 used grinding in liquid nitrogen followed by buffer extraction. Numbers are means of duplicate absorbance readings (A_{405nm}) for five-fold dilutions of reacted substrates.
[b]Means followed by different letters differ significantly at $P = 0.01$ by Duncan's new multiple range test (19), but means followed by a common letter do not.

(7). In the Purdue ELISA, leaf samples screened were usually unselected, being simply those available at the time of sampling. Preliminary tests to compare separated leaf, culm, stem, and root from greenhouse-grown Coast Black oats as sources for MAV and RPV for ELISA indicate that whereas young leaf is the best source for extracting MAV, the best source for RPV is culm tissue. Such differences could have implications regarding host/virus tissue relationships. Certainly, establishing methods for efficient and uniform extraction (monitoring the results by ELISA) is a prerequisite for such important potential applications as epidemiological studies, comparing plant breeders' selections for resistance to BYDV, and for optimizing yields in purification procedures.

Specific diagnosis is especially important in screening BYDV infections, because of vector and host specificity and differential host effects. In most cereals, and certainly in grasses, diagnosis cannot be achieved by symptom observation, so that specific identification of BYDV isolates in several locations is currently done year by year by laborious and time-consuming aphid-transfer methods. Further work is needed on the specific diagnosis of BYDV isolates with ELISA, but it appears to have vast potential for simplifying such surveys. If sufficient supplies of the appropriate specific antisera can be made available, specific diagnosis could become routine. For the present, however, both the supply of antisera and of purified virus preparations for further research into ELISA applications are limiting. As a start in overcoming this problem, we are attempting to develop stocks of high titer antisera to the MAV and RPV isolates. Initially we thought that use of these two antisera would permit field screening for three of the common variants of BYDV because of strong heterologous reactions between PAV and MAV antiserum in other kinds of serological tests (1). But the relatively weak heterologous reaction for PAV in ELISA (Fig. 3) now suggests that more than two antisera would be needed for adequate testing of field samples, an observation confirmed at Cornell in tests of field-collected samples during 1978.

While our work was in progress, we learned that ELISA has successfully been applied for detecting potato leaf roll virus (5), and citrus tristeza virus (3) (Bar-Joseph et al, *personal communication*) and the technique was also applied in detecting maize chlorotic dwarf virus (13). One of us (W.F.R.) also has used ELISA to detect BYDV in current field collections, and in frozen-stored leaf samples representing field collections made during 20 years of studies of the occurrence of the virus in New York State (16), with results that confirm and extend those reported here. We think that ELISA will find wide application in the detection and diagnosis of phloem-restricted viruses.

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