Vector Relations

Anomalies in Serological and Vector Relationships of MAV-like Isolates of Barley Yellow Dwarf Virus from Australia and the U.S.A.

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ABSTRACT

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Some barley yellow dwarf virus isolates obtained from cereal plants in Victoria, Australia, during 1985–1986 were serologically similar to the MAV isolate of W. F. Rochow, but distinct from it in being readily transmissible by the aphid *Rhopalosiphum padi*. Most serological comparisons used the Purdue culture of Rochow's MAV, initially obtained from Cornell, but maintained by transfer by *Sitobion avenae* for 6 yr. However, detailed examination of the Purdue culture with monoclonal antibodies revealed that a change in its serological behavior had occurred during culture, although efficient transmission by *S. avenae* and not by *R. padi* had been maintained.

Additional keyword: luteovirus.

Luteoviruses (17,19), grouped under the name barley yellow dwarf virus (BYDV), share the characteristic of infecting gramineous plants, but differ in various other properties, especially their transmissibility by specific aphid vectors (12). Five isolates of BYDV have been distinguished by the following acronyms, based on their principal vector species: RPV, transmitted principally by *Rhopalosiphum padi* L.; RMV, by *R. maidis* Fitch.; MAV, by *Macrosiphum (Sitobion) avenae* Fabr.; SGV, by *Schizaphis graminum* Rond.; and PAV, by both *R. padi* and *S. avenae* (4,11). These isolates appear to exemplify five types of BYDV into which many isolates seem to fit and that are also distinguishable serologically (12). This is especially important in relation to screening survey samples by enzyme-linked immunosorbent assay

(ELISA), currently the predominant diagnostic method (8). It has been shown that even with polyclonal antisera (those most readily available), ELISA gives specific test results (16) largely consistent with those given by vector studies (13,15).

However, each of these BYDV types probably contains variants differing in characteristics (12,17), such as virulence, host range, serological behavior, and detailed vector relationships. Such differences could have important implications regarding the diagnosis, epidemiology, and economic effects of BYDV in a particular area. In this report, we describe isolates, collected in Australia, that illustrate this potential in resembling the MAV isolate of Rochow in ELISA tests (i.e., serotypically), but not in vector relationships.

MATERIALS AND METHODS

Virus isolates. The isolates used as standards were: MAV, obtained from dried oat (*Avena sativa* L.) leaf tissue, from subcultures of Rochow's isolate maintained by mass transfer of *S. avenae* between batches of plants during 6 yr at Purdue (9); P-PAV (6), similarly obtained from dried leaf tissue from subcultures maintained at Purdue; and V1-PAV, obtained from a subculture of a PAV-like isolate, separated from a mixture of PAV- and RPV-like isolates (V1) collected in Victoria, Australia, and maintained at Burnley Gardens (20,22). Oat cultivars Algeribee and Clintland 64 were used for isolate culture and for noninfected (healthy) control tissue at Burnley Gardens and Purdue, respectively. Test isolates were from selected plants in collections made in a series of surveys of cereal crops and grasses in Victoria, Australia, during 1985–1986 (21).

ELISA. Table 1 lists the polyclonal (pc) and monoclonal (mc) antisera used as sources of immunoglobulins (Ig's) for ELISA tests. Some of the serological specificities of these Ig's have been described in the references cited. In relation to this work, the reported behavior of Ig's from antisera to the MAV isolate is of special interest. Thus, pc MAV-Ig has been shown to react heterologously with PAV-like isolates in ELISA tests (3), although less so than homologously. The Ig's from monoclonal antisera to MAV (mc MAV-Ig's-1, -2, and -3) are reported to react strongly with MAV, although some degree of cross-reactivity with PAV was also indicated for mc MAV-Ig's-2 and -3 (7).

Use of mc MAV-Ig-1 was therefore indicated for definitive diagnosis of MAV-like isolates by ELISA, especially in mixed infections with PAV-like isolates. However, because only small supplies of the mc MAV-Ig's were available, the procedure followed was first to screen isolates with pc MAV-Ig, then to subject those reacting positively to retesting with mc MAV-Ig-1. ELISA was done with 200-μl samples in Immulon M-129 B microtiter plates (Dynatech Inc.) by standard direct, double-antibody sandwich (DAS) or by indirect (ID) procedures for the pc Ig's and mc Ig's, respectively (1). Ig's were prepared from the antisera by ammonium sulfate precipitation. To remove activity to normal host proteins, polyclonal (rabbit) antisera were cross-absorbed with preparations from healthy oats before Ig preparation, or they were so treated during ELISA by diluting conjugate in healthy oat extracts (8). Stock Ig preparations were stored at 1 mg/ml (A_{280nm} = 1.4) and used at a dilution of 1/1,000. Coating Ig's were diluted in sodium carbonate coating buffer, pH 9.6, and conjugates or monoclonal antibodies were diluted in sap extracts as described above, or in phosphate-buffered saline containing 0.05% Tween 20 and 0.02% ovalbumin (conjugate buffer).

Conjugates were made by labeling the stock Ig's with alkaline phosphatase by a one-step treatment with 0.06% glutaraldehyde for 4 hr at room temperature (1). The substrate was *p*-nitrophenyl phosphate at 1 mg/ml in diethanolamine/HCl buffer at pH 9.8. Reactions were stopped by adding 50 μl of 3 M NaOH. Some sample extracts for tests were obtained by squeezing fresh leaf in a roller press (Erich Pollahne, West Germany) and diluting the expressed sap with 0.1 M phosphate buffer, pH 7.0. Most extracts, however, were made by grinding fresh or dry leaf in liquid nitrogen, then regrinding in 0.1 M phosphate buffer, pH 7.0, at 1:10, w/v (9). Timing of the steps in ELISA varied, but for convenience was usually as follows: DAS-coating, 2–3 hr (37 C); sample application, 4–6 hr (37 C); conjugate, overnight (4 C); substrate, 30–60 min (20 C); ID-coating, 2–3 hr (37 C); sample application, 4–6 hr (37 C); second (monoclonal) antibody, overnight (4 C); anti-mouse conjugate (Sigma), 2 hr (37 C); and substrate, 30–60 min (20 C). Test results (A_{405nm}) were read in a Dynatech microElisa minireader. Duplicate wells were used for each sample, and results were averaged. Extracts from noninfected oats were used as controls. Readings equal to or exceeding twice the "background" values for extracts from noninfested control plants or from check plants infected with heterologous isolates were regarded as positive (21).

Vector studies. Six BYDV vector-aphid species from local sources were used in attempts to transmit the Australian MAV-like and PAV-like isolates from cereals and grasses to oats: *R. padi* L., *R. maidis* Fitch., *R. rufiabdominalis* Sasaki, *Metopolophium dirhodum* Walk., *Sitobion miscanthi* Takahashi, and *S. fragariae* Walk. (*S. fragariae* Walk, from Tasmania, was supplied by P. L. Guy and was used in the study of BYDV in Tasmanian pasture grasses; 5.) BYDV-free cultures of the aphids were maintained in a simple, secure rearing system (Ridland et al, *unpublished*). The aphids were reared on individual barley (*Hordeum vulgare* L. cv. Lara) seedlings grown on nutrient-soaked cotton pads in closed polystyrene cups. First-instar apterae from these cultures were placed on either test-leaf material (field samples or known positives) or on BYDV-free healthy controls and allowed to feed for 48 hr at 18 C. They were then transferred, three aphids per plant, to oat indicator seedlings growing individually in polystyrene cups, which were held for an additional 96 hr at 18 C for the transmission feed. Aphids were then removed with a fine artists' brush, and the oat seedlings potted into soil mix and grown in an insect-screened greenhouse for observation of symptoms at 4 and 8 wk.

A series of transmission tests were done. In each case the first tests were with material from the field, and subsequent tests were with laboratory-infected oats resulting from the previous experiment. Leaf samples taken at 8 wk from the fourth series of tests (experiment 4) were tested by ELISA to confirm the identity of the isolate that was transmitted.

TABLE 1. Sources of immunoglobulins (Ig's) for enzyme-linked immunosorbent assay

Ig type	Designation	Eliciting antigen	Source or reference
Polyclonal	pc V1-PAV	Mixture of RPV-like and PAV-like isolates from Victoria, Australia	20, 21, 22
	pc IL-PAV	PAV-like isolate from Illinois	2
	pc P-PAV	PAV-like isolate from Indiana	6
	pc TAS-PAV	PAV-like isolate from Tasmania, Australia	5
	pc NZ-PAV	PAV-like isolate from New Zealand	J. W. Ashby and M. F. Clark
	pc UK1-PAV	PAV-like ("B severe") isolate from U.K.	M. F. Clark
	pc UK2-PAV	PAV-like isolate from U.K.	D. G. Rose
	pc MAV	Type MAV of Rochow	11
Monoclonal	mc MAV-1	Type MAV of Rochow	7
	mc MAV-2	Type MAV of Rochow	7
	mc MAV-3	Type MAV of Rochow	7

RESULTS

ELISA tests. Table 2 summarizes ELISA test results for samples from representative cereal plants extracted when collected during surveys in the Balliang area of Victoria and also at intervals of 2–3 wk during subsequent maintenance in the greenhouse. These results illustrate how positive values obtained with Ig's from polyclonal antisera to MAV or PAV were refined by the use of the available monoclonal antisera. Thus, although most of the samples initially reacted with both the pc IL-PAV-Ig's and pc MAV-Ig's, they were later differentiated by the mc Ig's into two groups (Table 2, experiment 3), one comprising isolates reacting like the PAV-like standard (PAV serotypes), and the other comprising isolates reacting like the MAV standard (MAV serotypes). This behavior remained consistent in subsequent tests. Of special interest, however, was that no isolates of the MAV serotype, including the MAV standard itself, reacted with mc MAV-Ig's-2 or -3.

Because the occurrence of isolates serotypically like MAV was novel for Australia, we compared their reactions with that of the MAV standard, using the range of Ig's available, in tests that would be appropriate in diagnostic screening. ELISA values for DAS tests with selected antigens and pc Ig's are given in Table 3.

The following comparisons are noteworthy. Values for the V1-PAV isolate were typical for isolates of the PAV serotype. Coating and conjugated pc Ig's from antisera to PAV-like isolates reacted efficiently with V1-PAV, whereas pc Ig from the antiserum to MAV reacted efficiently with it only when used as coating Ig. We assume that heterologous reactions of this Ig used as conjugate are impaired by the glutaraldehyde treatment used for enzyme labeling. By contrast, in the reciprocal tests of MAV antigen with various Ig's as coat and conjugate, the results indicated that pc Ig's from antisera to the isolates V1-PAV and IL-PAV did not bind efficiently to MAV, whether used as coat or conjugate. However, pc Ig from antiserum to the TAS-PAV isolate apparently bound to MAV strongly, when used either as coat or conjugate. Indeed, the reciprocal binding abilities of the MAV and TAS-PAV pc Ig's seemed quite similar. This was also true with 4a/1 and 2a/1 isolates, which had been classified as MAV serotype in the initial screenings (Table 2), and the results with these isolates were overall very similar to those for MAV.

Similar experiments (Table 4), involving indirect ELISA with mc Ig's, confirmed that pc Ig's to TAS-PAV and the MAV standard bound MAV and the MAV serotype isolates from Victoria far more efficiently than isolates of the PAV serotype. This was confirmed in further experiments (Table 5) in which an extensive range of pc Ig's to PAV-like isolates was used to capture MAV, P-PAV, or Victorian MAV serotype isolates, and capture efficiency was monitored by DAS or by indirect ELISA with mc MAV-Ig-1. As expected, the MAV isolate was bound most efficiently by its homologous pc Ig, but that from the antiserum to the TAS-PAV isolate also bound it very efficiently. Interestingly, the Victorian MAV serotype isolates were bound equally efficiently by pc Ig's to either the MAV or TAS-PAV isolates, although the latter, like the pc Ig's from the other antisera to PAV-like isolates tested, reacted more efficiently with the PAV isolate than did pc MAV-Ig.

Aphid transmission experiments. The known aphid vectors of BYDV available to us at the Burnley laboratory did not include *Sitobion avenae*, which has not been reported in Australia. Therefore, transmissibility with this species, a definitive criterion for MAV (11), was not testable because of quarantine restrictions. Similarly, it was not possible to do parallel tests of the transmissibility of MAV with Australian aphids at Purdue. However, the vector studies done at Burnley (Table 6) indicated clearly that isolate 2a/1, a representative Victorian isolate of the MAV serotype, was far more efficiently transmitted by *R. padi* than by the *Sitobion* species tested. Trancapsidation (the encapsidation of an MAV-like genome with PAV-like coat protein) (14) was ruled out as an explanation of the efficient transmission of isolate 2a/1 by the ELISA test results for plants infected in one set of experiments (Table 6, experiment 4). All of them reacted positively with mc MAV-Ig-1, but negatively with pc Ig from antiserum to the IL-PAV isolate (Table 6).

Further comparisons among isolates of the MAV serotype. The anomalous serological behavior of the MAV serotype isolates tested in Australia, together with the anomalous vector relationships of the 2a/1 isolate (selected as typical of the Australian MAV serotype isolates) prompted further examination at Purdue of the MAV used as a standard (the Purdue culture of Rochow's MAV). In these experiments, the serological behavior of the Purdue culture was compared with that of newly acquired MAV from Rochow's stock culture maintained at Cornell (Table 7). Results for the newly acquired MAV confirmed the results of Hsu et al (7), showing that this antigen reacted with all three mc MAV-Ig's. By contrast, those from the Purdue MAV culture, used as a standard in the Australian tests, confirmed that, as in Table 1, this isolate reacted with mc MAV-Ig-1 but not with mc MAV-Ig's-2 and -3. However, frozen stored samples from earlier Purdue cultures of this isolate were available, and tests of these indicated that its reactivity with mc MAV-Ig's-2 and -3 had been lost during

TABLE 2. Enzyme-linked immunosorbent assay of samples from Balliang collection 1, of September 1985

Sample designation (source)	Reactions with indicated Ig's[a]							Serotype diagnosis
	Experiment 1		Experiment 2	Experiment 3				
	IL-PAV	MAV	MAV-1	MAV-1	MAV-2	MAV-3	IL-PAV	
Site 1								
1i/1 (barley)	1.71	0.42	0.01	0.02	0.30	0.19	0.54	PAV
1i/2 (barley)	1.82	0.44	0.01	0.03	0.38	0.28	0.76	PAV
1j/1 (oats)	0.55	0.12	···	0.02	0.35	0.25	0.84	PAV
1k/1 (barley)	1.36	0.38	···	0.02	0.21	0.10	0.46	PAV
1l/1 (oats)	0.10	0.11	···	0.71	0.06	0.02	0.02	MAV
Site 2								
2a/1 (oats)	0.16	0.72	1.14	0.70	0.04	0.02	0.03	MAV
Site 3								
3a/2 (barley)	0.13	0.44	0.84	0.37	0.18	0.09	0.34	Mixed
3b/1 (oats)	0.19	0.06	···	0.00	0.25	0.12	0.66	PAV
Site 4								
4a/1 (oats)	0.12	0.55	0.85	0.58	0.04	0.01	0.01	MAV
4a/2 (oats)	0.13	0.35	···	0.68	0.04	0.01	0.00	MAV
4a/3 (oats)	1.81	0.43	0.01	0.00	0.53	0.48	0.95	PAV
4B/2 (barley)	0.10	0.44	···	0.69	0.06	0.02	0.03	MAV
V1-PAV standard	1.12	0.11	0.02	0.02	0.65	0.48	1.70	···
MAV standard	0.14	1.40	0.69	0.54	0.07	0.06	0.08	···
Healthy control	0.02	0.02	0.02	0.01	0.05	0.02	0.04	···

[a] Ig's indicated are conjugates or monoclonal antibodies, all used at 1/1,000 dilution. Polyclonal IL-PAV-Ig was used as coating Ig with IL-PAV conjugate. Polyclonal MAV-Ig was used as coating Ig with MAV conjugate or monoclonal MAV-1, MAV-2, or MAV-3 antibodies (7). Results for experiment 1 are for the plants as collected; those for experiments 2 and 3 were obtained after maintaining the plants in the greenhouse for about 3 and 5 wk, respectively (20).

subculture (Table 7).

Checks of the transmissibility of the current Purdue culture of MAV were also carried out at Purdue. This culture showed behavior typical for MAV, but essentially reciprocal to that of 2a/1 in the Burnley tests. That is, it was readily transmissible by *S. avenae* but not by *R. padi*; transmission of this MAV by single aphids raised on an infected oat plant was successful in 0 of 30 attempts with *R. padi* and 17 of 30 attempts with *S. avenae*. Again, the plants infected in these experiments reacted positively with pc MAV-Ig and with mc MAV-Ig-1, but negatively with pc P-PAV-Ig.

TABLE 3. Comparative activity of selected antigens in enzyme-linked immunosorbent assays with homologous and heterologous coating and conjugate immunoglobulins (Ig's)

Antigen and conjugate Ig	Coating Ig			
	V1-PAV	IL-PAV	TAS-PAV	MAV
V1-PAV				
V1-PAV	0.42*[a]	0.53	0.51	0.45
IL-PAV	0.61	1.09*	1.02	0.78
TAS-PAV	1.16	1.56	1.65*	1.35
MAV	0.20	0.28	0.27	0.29*
MAV				
V1-PAV	0.02*	0.03	0.10	0.12
IL-PAV	0.02	0.03*	0.11	0.15
TAS-PAV	0.05	0.13	0.70*	0.88
MAV	0.06	0.13	0.91	1.23*
2a/1[b]				
V1-PAV	0.05*	0.13	0.46	0.49
IL-PAV	0.04	0.08*	0.29	0.34
TAS-PAV	0.08	0.23	0.99*	1.08
MAV	0.06	0.17	0.75	0.85*
Healthy				
V1-PAV	0.02*	0.01	0.02	0.02
IL-PAV	0.02	0.01*	0.01	0.02
TAS-PAV	0.01	0.01	0.01*	0.02
MAV	0.02	0.03	0.02	0.04*

[a] Reactions involving homologous coat and conjugate Ig's have asterisks. For sources of Ig's, see Table 1.
[b] Similar results were obtained in this experiment with isolate 4a/1, Table 2, and an additional MAV serotype isolate from Victoria.

DISCUSSION

This work establishes that isolates of BYDV that are serologically similar to Rochow's MAV (MAV serotypes) occur in Australia. However, the evidence from vector transmission studies indicates that these isolates are distinct from the MAV of Rochow in being efficiently transmissible by *R. padi*.

For practical reasons, vector transmissibility was used in early studies of BYDV as a fundamental characteristic differentiating isolates. Currently, serological differentiation has also come to be regarded as a convenient means of discriminating between isolates. Both characteristics are thought to reflect properties of the viral capsid (18) and have proved to be consistent to a remarkable

TABLE 4. Comparative activity of selected antigens in indirect enzyme-linked immunosorbent assays with monoclonal antibodies to Rochow's MAV isolate[a]

Antigen and second antibody	Coating pc immunuoglobulins[b]			
	V1-PAV	IL-PAV	TAS-PAV	MAV
V1-PAV				
MAV-1	0.01	0.01	0.01	0.02
MAV-2	0.72	0.84	0.82	0.81
MAV-3	0.68	0.76	0.81	0.71
MAV				
MAV-1	0.02	0.07	0.42	0.57
MAV-2	0.01	0.01	0.02	0.00
MAV-3	0.01	0.01	0.01	0.01
2a/1[c]				
MAV-1	0.16	0.31	0.89	0.99
MAV-2	0.01	0.01	0.01	0.02
MAV-3	0.01	0.00	0.02	0.02
Healthy				
MAV-1	0.00	0.00	0.00	0.01
MAV-2	0.01	0.00	0.00	0.02
MAV-3	0.00	0.01	0.00	0.01

[a] Monoclonal antibodies (7) were used as second antibodies after antigens were bound with the coating antibodies indicated.
[b] For sources of immunoglobulins, see Table 1.
[c] Similar results were obtained in this experiment with isolate 4a/1, Table 2, and an additional MAV serotype isolate from Victoria.

TABLE 5. Relative antigen binding by immunoglobulins (Ig's) from antisera to various PAV-like isolates and MAV, when used as coating Ig's to capture selected antigens in indirect (ID) or double-antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA)[a]

Antigen	Coating antibody						
	UK1-PAV	IL-PAV	NZ-PAV	UK2-PAV	P-PAV	TAS-PAV	MAV
MAV[b]	0.13 (0.15)	0.19 (0.22)	0.27 (0.30)	0.40 (0.45)	0.45 (0.51)	0.84 (0.94)	1.00 (1.12)
P-PAV[b]	··· (0.03)	··· (0.02)	··· (0.03)	··· (0.04)	··· (0.02)	··· (0.03)	··· (0.05)
A-MAV[c]	0.27 (0.15)	0.20 (0.11)	0.57 (0.32)	0.46 (0.26)	0.63 (0.35)	1.00 (0.56)	1.00 (0.56)
P-PAV[d]	0.71 (0.17)	2.58 (0.62)	1.29 (0.31)	1.29 (0.31)	1.75 (0.42)	2.33 (0.56)	1.00 (0.24)

[a] All antibody preparations were at 1/1,000 dilution. All antigen extracts were made by grinding leaf in 0.1 M, pH 7, potassium phosphate buffer/Tween 20 at 1:10 w/v. All values given are averages from duplicate tests of the antigens tested in each category. Binding ratios are in relation to MAV = 1.00. Mean ELISA values are given in parentheses. ELISA values for healthy control extracts were 0.01–0.05.
[b] Average values for two sets of extracts from dry leaf in ID ELISA, with mc MAV-Ig-1 as second antibody.
[c] Average values for four Victoria MAV serotype isolates, 2a/1, 4a/1, 4a/2, and 4b/2 (Table 2), in ID ELISA with mc MAV-Ig-1 as second antibody.
[d] Data obtained in DAS ELISA with pc IL-PAV conjugate.

TABLE 6. Transmissibility of a Victorian (MAV) serotype isolate of barley yellow dwarf virus (2a/1) by various aphids

Vector species	No. of infections[a]/no. of tests				Positive enzyme-linked immunosorbent assay (ELISA) reactions with immunoglobulins indicated[b]	
	Experiment 1	Experiment 2	Experiment 3	Experiment 4	pc IL-PAV	mc MAV-1
Rhopalosiphum padi	17/22	8/18	34/40	16/16	0	16
Sitobion miscanthi	2/13	0/19	···	1/20	0	1
Metopolophium dirhodum	1/18	···	1/21	9/14	0	9
S. fragariae	···	···	···	0/22	0	0
R. maidis	···	···	···	0/17	0	0
R. rufiabdominalis	···	···	32/40	···	···	···

[a] Infections indexed by typical symptoms on Algeribee oat plants.
[b] ELISA reaction results refer to test plants used in experiment 4.

TABLE 7. Enzyme-linked immunosorbent assay (ELISA) comparisons of MAV- and PAV-like isolates of barley yellow dwarf virus, using various immunoglobulins (Ig's)

Isolate and source	ELISA with Ig's indicated[a]				
	Plate 1—monoclonal Ig's			Plate 2—polyclonal Ig's	
	MAV-1	MAV-2	MAV-3	MAV	P-PAV
MAV (newly acquired from W. F. Rochow)	0.47	1.00	0.74	0.52	0.14
MAV (cultured 6 yr at Purdue)[b]	0.54	0.21	0.08	0.83	0.11
PAV (newly acquired from W. F. Rochow)	0.06	0.40	0.28	0.09	0.32
P-PAV (cultured at Purdue)	0.06	1.11	0.89	0.23	1.02
Healthy	0.06	0.16	0.08	0.07	0.09

[a] All ELISAs were done at the same time with the same extracts. Tests with monoclonal Ig's were done in a different plate from those with polyclonal Ig's. Procedures used were double-antibody sandwich and indirect ELISAs, as described in the text.

[b] Tests of frozen samples dated 7/15/83 and 8/11/83 were positive with all three monoclonal Ig's. Those for all eight subsequent samples available, collected at intervals between 6/7/85–3/5/86, were positive only with MAV-1.

degree. However, our results illustrate that this need not be the case, indicating that serological properties and vector specificity do not necessarily result from identical features of capsid structure.

An apparent change occurred in serological behavior, although not in vector specificity, of the type of Rochow's MAV as maintained by vector transmission over several years at Purdue. This change would not have been noted but for the availability of the monoclonal antibodies of Hsu et al (7), for both MAV's reacted similarly with the polyclonal antibodies available to us. One possible reason for this change is selection from a mixture of MAV-like viruses having generally similar vector relationships. Indeed, recent results in this laboratory are consistent with this hypothesis (10). The fact that aphid transmission is obligatory for the transfer of BYDV makes it impossible to obtain "pure" cultures, for all BYDV isolates are likely to comprise mixtures of viruses that happen to be similarly transmissible by the vector(s) used for passage. This must also be true of other luteoviruses. Chance selection by the vector, or selection pressures due to host or environmental conditions, may therefore affect the specific type of virus that predominates in a culture. In this way, it is conceivable also that the Australian MAV serotype isolates described here, which differ in vector relationships from Rochow's MAV, and thus probably also from other MAV-like viruses in North America and elsewhere, have been selected from introduced MAV-like infections by the aphid vectors that predominate in Australia.

Differences were observed in the ability of the Ig's from antisera to various PAV-like isolates to bind MAV serotype isolates. The results suggest, for example, that the TAS-PAV has closer serological affinities with MAV than do the other PAV-like isolates that were used for antiserum production. In fact, use of the pc TAS-PAV-Ig alone in screening samples would probably fail to distinguish between the PAV and MAV serotype isolates described here. Whether this implies that the TAS-PAV isolate is serologically closer akin to MAV than other PAVs, or that the TAS-PAV isolate is a mixture of PAV- and MAV-like isolates, is not known, as this isolate was not available to us during the work described.

LITERATURE CITED

1. Clark, M. F., Lister, R. M., and Bar-Joseph, M. 1986. ELISA techniques. Methods Enzymol. 118:742-766.
2. D'Arcy, C. J., and Hewings, A. D. 1986. Enzyme-linked immunosorbent assays for study of serological relationships and detection of three luteoviruses. Plant Pathol. 35:288-293.
3. Fargette, D., Lister, R. M., and Hood, E. L. 1982. Grasses as a reservoir of barley yellow dwarf virus in Indiana. Plant Dis. 66:1041-1045.
4. Gill, C. C. 1969. Annual variation in strains of barley yellow dwarf virus in Manitoba, and the occurrence of greenbug-specific strain isolates. Can. J. Bot. 47:1277-1283.
5. Guy, P. L., Johnstone, G. R., and Duffus, J. E. 1986. Occurrence and identity of barley yellow dwarf viruses in Tasmanian pasture grasses. Aust. J. Agric. Res. 37:43-53.
6. Hammond, J., Lister, R. M., and Foster, J. E. 1983. Purification, identity and some properties of an isolate of barley yellow dwarf virus in Indiana. J. Gen. Virol. 64:667-676.
7. Hsu, H. T., Aebig, J., and Rochow, W. F. 1984. Differences among monoclonal antibodies to barley yellow dwarf virus. Phytopathology 74:600-605.
8. Lister, R. M., and Rochow, W. F. 1979. Detection of barley yellow dwarf virus by enzyme-linked immunosorbent assay (ELISA). Phytopathology 69:649-654.
9. Lister, R. M., Clement, D., and Skaria, M. 1985. Stability of ELISA activity of barley yellow dwarf virus in leaf samples and extracts. Plant Dis. 69:854-857.
10. Lister, R. M., and Lei, C. H. 1987. Apparent selection of serologically distinct types of barley yellow dwarf virus from a single isolate. (Abstr.) Phytopathology 77:1706.
11. Rochow, W. F. 1969. Biological properties of four isolates of barley yellow dwarf virus. Phytopathology 59:1580-1589.
12. Rochow, W. F. 1970. Barley yellow dwarf virus. No. 32 in: Descriptions of Plant Viruses. Comm. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England.
13. Rochow, W. F. 1979. Comparative diagnosis of barley yellow dwarf virus by serological and aphid transmission tests. Plant Dis. Rep. 63:426-430.
14. Rochow, W. F. 1982. Dependent transmission by aphids of barley yellow dwarf luteoviruses from mixed infections. Phytopathology 72:302-305.
15. Rochow, W. F. 1982. Identification of barley yellow dwarf virus: Comparison of biological and serological methods. Plant Dis. 66:381-384.
16. Rochow, W. F., and Carmichael, L. E. 1979. Specificity among barley yellow dwarf viruses in enzyme immunosorbent assays. Virology 95:415-420.
17. Rochow, W. F., and Duffus, J. E. 1981. Luteoviruses and yellows diseases. Pages 147-170 in: Handbook of Plant Virus Infections and Comparative Diagnosis. E. Kurstak, ed. Elsevier-North Holland, Amsterdam.
18. Rochow, W. F., Foxe, M. J., and Muller, I. 1975. A mechanism of vector specificity for circulative aphid-transmitted plant viruses. Ann. NY Acad. Sci. 266:293-301.
19. Shepherd, R. J., Francki, R., Hirth, L., Hollings, M., Inouye, T., MacLeod, R., Purcifull, D., Sinha, R., Tremaine, J., Valenta, V., and Wetter, C. 1976. New groups of plant viruses approved by the International Committee on Taxonomy of Viruses, September 1975. Intervirology 6:181-184.
20. Sward, R. J., and Lister, R. M. 1987. The incidence of barley yellow dwarf viruses in wheat in Victoria. Aust. J. Agric. Res. 38:821-828.
21. Sward, R. J., and Lister, R. M. 1988. The identity of barley yellow dwarf virus isolates in cereals and grasses from Mainland Australia. Aust. J. Agric. Res. 39:375-384.
22. Waterhouse, P. M., Gerlach, W. L., and Miller, W. A. 1986. Serotype-specific and general luteovirus probes from cloned cDNA sequences of barley yellow dwarf virus. J. Gen. Virol. 67:1273-1281.