Incidence of Barley Yellow Dwarf Viruses in California Cereals

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ABSTRACT

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An ELISA survey of barley yellow dwarf virus (BYDV) in four major cereal-producing areas showed regional and year-to-year differences in the frequency of BYDV types detected. PAV types were most commonly detected in the 1,115 oat, wheat, and barley samples assayed, followed by MAV and RPV types. Replicated field trials also were conducted in 1986 and 1987 to assess and compare the incidence of BYDV types in randomly collected and symptomatic samples. Chi-square analysis showed that symptomatic sampling overestimated the amount of PAV and RPV types relative to their incidence in random samples.

Barley yellow dwarf (BYD) is caused by any of a group of luteoviruses called the barley yellow dwarf viruses (BYDVs). These viruses continue to be major pathogens of small grains in California (1,3,8,12). Losses continue on an annual basis even though plant breeding efforts have produced virus-resistant or virus-tolerant cultivars of barley, oat, and wheat and crop management minimizes the exposure of seedlings to aphid flights (8). One explanation for these losses lies with the causative agents themselves. In general, each BYDV has specific aphid vectors and antigenic characteristics (13). The various BYDVs (BYDV types) have been named using acronyms derived from their aphid vector specificities; the three most studied BYDV types are RPV, MAV, and PAV (13). Research has shown that, besides their aphid vector and antigenic differences, the various BYDVs also have different effects on commercial cereal cultivars. For example, barley accessions with the yd2 gene were resistant to a PAV isolate, but not to an RPV isolate (2). Similar results have been obtained using California isolates of PAV and RPV BYDV types (9).

To manage disease and field-screen cereal cultivars for BYD reactions, it is necessary to know the incidence of the various BYDVs to correctly evaluate cultivar response to viral infection. An early survey in California showed that BYDVs (varying in virulence) were found in all cereal-producing counties (1). No attempt was made to identify the serotype (BYDV type) of these isolates. A more recent study incorporated Rochow's virus designations (13) and identification of BYDV types. Serological and aphid transmission assays of 128 symptomatic samples collected from eight California locations showed that all were infected by BYDVs of the PAV, MAV, and RPV types, either singly or in combination (6).

Because of the large acreage of cereals grown annually in California, a 2-yr survey of the state's four major grain-producing regions was carried out during 1987–1988 to assess and compare the incidence of the various BYDVs in commercial cereal cultivars. Also, field trials of oat, wheat, and barley were planted in 1986 and 1987 at the plant pathology farm at the University of California, Davis. These trials were sampled both randomly and by collecting symptomatic plants to assess the actual percentage of BYDV-infected plants and to determine whether a potential bias for a given BYDV type occurs as a result of symptomatic sampling. A preliminary report including a portion of these data has been published (8).

MATERIALS AND METHODS

Regional BYDV survey. Survey samples of symptomatic small grains were collected statewide from commercial cereal plantings made between late March and early April of 1987 and 1988. Plants generally were mature and beginning to head. Because typical BYD symptoms are not always easy to observe in mature commercial fields, plants were considered symptomatic only if entire leaves were discolored, in addition to the typical primary distal discoloration. Commercial wheat, barley, and oat samples were collected from California's four major cereal-producing areas—the Sacramento, San Joaquin, Salinas, and Imperial valleys—which historically have suffered economic losses caused by BYD. Five to seven sites in each major area were sampled; 12–16 samples were taken from each site in each of the study years. A total of 561 samples were collected in 1987; 554 were collected in 1988. Samples were frozen at −20 C for 2–3 mo after collection until enzyme-linked immunosorbent assays (ELISA) could be performed.

Field trials were conducted to assess the potential effects of symptom bias on selection of plants with a given BYDV type. Plantings were done in the autumns of 1986 and 1987 at the Davis site and included *Avena sativa* L. 'California Red', *Triticum aestivum* L. 'Tadinia', and *Hordeum vulgare* L. 'Prato' and 'California Mariout' (CM 72). Each cultivar was planted in 3 × 6 m randomized blocks, replicated four times. Twelve symptomatic, postflowering samples were collected from each replicate, for a total of 48 samples per cultivar per year ($n = 192$). Thirteen random samples were taken per replicate, for a total of 52 samples per cultivar per year ($n = 208$).

Serological analysis. ELISA tests were used to specifically assess plants for BYDV infection by the PAV, RPV, and MAV types. Slight modifications of previous procedures were followed (5,10,11) using polyclonal antisera to New York (NY) BYDVs (NY-PAV, NY-MAV, NY-RPV) and monoclonal antibodies of NY-PAV (MAV-3), NY-MAV (MAV-1), and NY-RPV (RPV-1, RPV-2, RPV-3) (10). All samples, including healthy and known BYDV-infected controls, were tested against antisera to all three of the above BYDV types.

Microtiter plates were coated with polyclonal immunoglobulin (IgG) (1987) or with monoclonal antibodies (1988) as described by Creamer and Falk (5). Samples were prepared by extracting approximately 0.5 g of leaf tissue using a leaf squeezer and collecting sap in a microfuge tube containing about 1.0 ml of 0.1 M sodium phosphate (pH 7.0). Each sample was centrifuged at 12,000 × g for 7 min, and then 200 µl per well was loaded into each of two wells

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on three ELISA plates. Each plate contained paired replicates of unknown samples, noninfected controls (two or three per plate), and positive and heterologous controls of NY or California isolates of PAV, MAV, and RPV BYDVs (two or three per plate). Each set of samples was analyzed by testing one of the three plates with PAV antibodies, one with MAV antibodies, and one with RPV antibodies. All control plants (including noninfected controls) were oat cultivar California Red.

Alkaline phosphatase-conjugated polyclonal IgG (2.0 μg/ml), followed by addition of *p*-nitrophenyl phosphate, was used to detect BYDV-positive samples in both 1987 and 1988 tests. Absorbance values were assessed by spectrophotometer at 405 nm (A_{405}). Field samples were considered positive if their A_{405} value was greater than the noninfected (control) mean plus four standard deviations (control + 4 SD), and also greater than the mean A_{405} of heterologous antisera (reactions for known BYDV types with heterologous BYDV antibodies).

Table 1. Mean barley yellow dwarf virus enzyme-linked immunosorbent assay (ELISA) absorbance (A_{405}) values for all samples tested in 1987 and 1988

Antibodies[a]	Healthy control[b]	Healthy + 4 SD[c]	Heterologous control[d]	Homologous control[e]	Field sample (range)[f]
1987 tests					
MAV	0.114	0.212	0.243	0.758	0.513 (0.246–1.98)
PAV	0.106	0.193	0.118	1.330	0.939 (0.201–1.98)
RPV	0.101	0.245	0.130	0.922	0.477 (0.246–1.83)
1988 tests					
MAV	0.073	0.107	0.082	1.280	0.608 (0.166–2.98)
PAV	0.078	0.126	0.074	1.140	1.104 (0.137–2.98)
RPV	0.109	0.162	0.117	1.060	0.621 (0.153–2.43)

[a] The 1987 samples were assayed using only rabbit polyclonal antibodies to the given barley yellow dwarf virus (BYDV) serotype. The 1988 samples were assayed by coating plates using monoclonal antibodies and using rabbit polyclonal antibodies conjugated with alkaline phosphatase to detect trapped antigens.
[b] Mean A_{405} value for reactions of all healthy control (*Avena sativa* 'California Red') samples from all tests with the respective antisera.
[c] Cumulative threshold value for differentiating healthy and BYDV-infected samples. Actual threshold values for each set of analyses were determined for each set of ELISA tests.
[d] Highest A_{405} value for reaction of the given BYDV antibody with heterologous BYDV serotype control antigens (e.g., MAV antibodies with either PAV or RPV control antigens, whichever was higher).
[e] Grand mean A_{405} values for reactions of all positive control samples with homologous antibodies.
[f] Cumulative mean and range of A_{405} values for all field samples that were scored positive with the respective antibody.

RESULTS

A summary of critical ELISA values for the 1,115 survey samples, healthy controls, and BYDV-infected controls for both polyclonal and monoclonal antibodies is shown in Table 1. Heterologous reactions did not exceed the threshold values (control + 4 SD), except for the PAV cross-reaction with NY-MAV polyclonal antisera (0.212 and

Table 2. Numbers of ELISA-positive samples from the symptomatic cultivated cereal survey of California's major cereal-producing areas in 1987 and 1988

Area	Year	Number of samples[a]	BYDV-positive samples[b]								
			MAV	PAV	RPV	MP	MR	PR	MPR	Total[c]	Percent[d]
San Joaquin Valley	1987	135	1	34	13	49	0	2	1	100	74
	1988	112	5	55	6	0	0	5	0	76	68
Sacramento Valley	1987	256	7	51	23	2	0	4	3	90	35
	1988	278	2	62	16	2	0	5	3	90	32
Salinas Valley	1987	101	17	25	9	0	0	0	0	51	51
	1988	94	42	26	4	1	4	1	0	78	83
Imperial Valley	1987	69	1	18	0	1	0	0	0	20	29
	1988	70	0	31	4	1	0	0	0	36	51

[a] Total number of samples collected and assayed from each area.
[b] MP, MR, and PR are samples that reacted positively for MAV and PAV, MAV and RPV, and PAV and RPV, respectively. MPR denotes samples that scored positive for all three BYDV types.
[c] Total number of BYDV-positive samples.
[d] Percentage of total samples positive for BYDV.

Table 3. Barley yellow dwarf virus ELISA-positive samples from replicated field plots of oats, barley, and wheat

	MAV[b]		PAV[b]		RPV[b]		MP[b]		MR[b]		PR[b]		MPR[b]		Total[c]		Percent[d]	
Sample[a]	86	87	86	87	86	87	86	87	86	87	86	87	86	87	86	87	86	87
Symptomatic																		
Cal Red	2	0	10	32	7	3	0	2	0	0	2	2	0	0	21	37	44	77
Tadinia	0	0	12	18	7	6	1	0	0	0	0	0	0	0	20	24	42	50
Prato	2	0	5	5	2	0	0	0	0	0	0	2	0	0	9	7	19	15
CM 72	1	2	7	4	7	3	1	0	0	0	2	1	0	3	18	13	38	27
Random																		
Cal Red	0	4	6	24	0	0	0	7	0	1	0	0	0	0	6	36	12	69
Tadinia	1	1	3	1	0	2	0	0	0	0	0	0	0	0	4	4	8	8
Prato	0	0	5	2	0	0	0	0	0	0	0	0	0	0	5	2	10	4
CM 72	0	3	4	2	1	0	0	0	0	1	1	0	0	0	6	6	12	12

[a] Forty-eight symptomatic samples of oat (*Avena sativa* 'California Red' [Cal Red]), wheat (*Triticum aestivum* 'Tadinia'), and barley (*Hordeum vulgare* 'Prato' and 'California Mariout' [CM 72]) were collected in 1986 and 1987. Fifty-two random samples of each were also collected from the 1986 and 1987 plots; these were assayed separately.
[b] Number of samples that were scored positive for the given BYDV type. MP, MR, and PR indicate samples positive for MAV and PAV, MAV and RPV, and PAV and RPV, respectively. MPR indicates samples positive for MAV, RPV, and PAV.
[c] Total number of BYDV-positive samples.
[d] Percentage of samples positive for BYDV.

0.243, respectively). In 1987, a field sample that showed low MAV-positive values (0.212–0.300) was considered MAV-positive only if it was also negative for PAV. Conversely, if a sample gave a low positive reaction for PAV, the low MAV-positive value was taken as a heterologous reaction and the sample was scored only as PAV-positive. Similar heterologous cross-reactions between MAV polyclonal antisera and PAV antigens have been documented (9).

In 1988, positive A_{405} values were generally higher for samples processed using monoclonal antibodies, and the healthy background was always lower than with polyclonal antibodies (Table 1). This allowed more confidence for interpreting mixed infections and low-A_{405}, ELISA-positive infections that were obtained from a few 1988 field samples.

Regional BYDV survey. ELISA analysis of survey samples showed that PAV was the BYDV type most commonly detected, followed by MAV and then RPV (Table 2). However, both year-to-year and geographical differences in BYDV type incidence were found.

In both years, PAV types were most commonly detected in San Joaquin Valley samples. Samples that reacted positively for both PAV and MAV were common in 1987 but not in 1988. Mixed infections of PAV and RPV were relatively rare in both years, and only one plant was found that reacted positively for PAV, MAV, and RPV. ELISA analysis of the Sacramento Valley samples showed similar results for each of the 2 yr. Positives for PAV were most common, followed by RPV and then MAV. Mixed infections were relatively uncommon. PAV-positive samples were also common among Imperial Valley samples in both years; MAV and RPV types were relatively rare (Table 2). In contrast to other California cereal-growing regions, samples reacting positively for MAV were found relatively frequently in the Salinas Valley (Table 2). MAV positives made up 33% of the total Salinas Valley BYDV positives in 1987 and 58% of the total in 1988.

Comparison of random and symptomatic sampling. BYDV-positive samples from 1986 and 1987 field plot studies were examined by year, cultivar, and BYDV type (Table 3). Overall incidence of BYDV types was generally similar in both years. (The only major exception was that PAV types were more common in samples from oat plots in 1987 than they were in 1986 samples.) The data from each treatment (year, cultivar, and BYDV type) were converted to percent incidence and analyzed by ANOVA using a model incorporating two sampling methods, four genotypes, and seven possible BYDV infections (MAV, PAV, RPV, MP, PR, MR, and MPR). Plot data for 1986 and 1987 were considered replicates. Our analyses showed significant differences ($P > 0.01$) among BYDV-positive samples for cultivars, BYDV type, and sampling method. Additionally, significant interactions ($P > 0.01$) were seen for cultivars and BYDV type and for sampling method and BYDV type.

Chi-square analysis of these data also showed significant interactions between sampling method and BYDV type. To determine which BYDV types contributed to this interaction, data from both years and all cultivars were pooled and BYDV type in symptomatic and random samples were compared. Data were analyzed as seven possible BYDV infections to determine which BYDVs contributed to the significant interaction. Both PAV and RPV types were significantly greater ($P > 0.01$) in the symptomatic samples than in the random samples. No other BYDV infections (MAV, MP, RP, MR, or MPR) were significantly different in random and symptomatic samples.

DISCUSSION

The San Joaquin, Sacramento, Salinas, and Imperial valleys are the four main cereal-producing regions in California. These areas account for approximately 92% (37, 27, 15, and 13%, respectively) of California's total cereal production, with over 1 million acres under cultivation (8). Specific estimates of losses caused by BYDV in each of these areas can be made only if we have information on the various types of BYDVs present, the severity of infection on the host cultivars, and the time of infection. Our survey and field plot data show that, in general, PAV types are the most common BYDVs in California cereals. This observation agrees with a previous California study (6) and with studies in other cereal-producing areas in the United States (4,7). Some of the samples in our study failed to react for MAV, RPV, or PAV BYDV types, probably as a result their collection late in the season when discolorations caused by other problems can be common, especially in wheats. We believe it is unlikely that those plants scored as BYDV-negative could have been infected by another BYDV type (such as SGV). An earlier study of 128 BYDV-symptomatic samples in California failed to detect any BYDV types other than MAV, RPV, and PAV (6).

When we assayed symptomatic plants in our field plots for BYDV type, PAV and RPV types were detected more frequently than would be expected from their natural occurrences (as determined by assaying randomly collected samples). This implies that PAV- and RPV-type BYDVs caused more of the BYD symptoms seen in our field plots. This probably also contributed to the incidence of these BYDV types seen in our survey. Consequently, PAV and RPV types may be overrepresented in our survey data, while MAV types and mixed infections are probably represented more accurately. However, our data show that the different regions of California have unique combinations of the various BYDVs (for example, MAV types were more common in the Salinas Valley samples than in others) and these may fluctuate from year to year.

The Imperial Valley is the only main cereal-producing area that showed both low amounts of RPV types and few mixed infections. Because of this, PAV-resistant cultivars, such as tolerant wheats, and barleys containing the yd2 gene, should perform well there. In the three other California cereal-growing regions, the presence of RPV types and mixed infections on a regular basis may affect the severity of BYD seen in the field. Previous greenhouse yield-loss trials conducted with California BYDV isolates showed that infections with mixed MAV and PAV types caused significant reduction of plant height and 1,000-kernel weight in the wheat variety Yecora Rojo, the most commonly planted wheat in the San Joaquin Valley (9). All yield parameters measured in the study were significantly affected by the RPV isolate used; for Yecora Rojo, total dry and head weights were 12% less than those of healthy plants. In the same study, the wheat variety Anza (commonly planted in the Sacramento Valley) showed tolerance to both PAV and MAV types, but not to RPV infections or to those containing both MAV and PAV.

Similarly, the PAV-resistant oat variety Ogle and barleys containing the yd2 gene are susceptible to RPV infections. The survey data from our study and yield loss studies using various BYDVs (9) show how these viruses continue to damage California's cereal crops. If data on time of infection and BYDV isolate-severity data can be obtained, then it may be possible to produce a functional model of BYDV-induced losses.

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