Barley Yellow Dwarf in California: Vector Competence and Luteovirus Identification

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ABSTRACT

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Samples of small grains collected in 1981 from eight counties in California were tested by aphid transmission and by enzyme-immunosorbent assays for luteoviruses that cause barley yellow dwarf. California isolates were compared with three previously characterized in New York: RPV, MAV, and PAV. Of 128 plants sampled, 75% were infected by luteoviruses similar to PAV, 19% by viruses similar to MAV, and 6% by RPV-like luteoviruses. When clones of aphids collected in California were compared with those from New York for transmission of five virus isolates, no difference in vector competence occurred among clones of *Rhopalosiphum padi*. Two California clones of *Metopolophium dirhodum* efficiently transmitted MAV and PAV but not a California luteovirus similar to PAV (CA-PAV). Both California and New York clones of *Sitobion avenae* transmitted MAV and PAV, but California *S. avenae* transmitted CA-PAV less efficiently than PAV. Differences in tolerance to infection by three luteoviruses from California occurred in some varieties of barley and oats.

Luteoviruses are small, isometric, RNA-containing plant viruses that replicate in phloem tissue of infected plants, are transmitted in a persistent-circulative manner by aphids, and cause a wide range of yellows diseases in many crop plants (13). Luteoviruses that cause barley yellow dwarf exhibit a range of biological, serological, and chemical properties. Variants of barley yellow dwarf virus (BYDV) include five characterized isolates that have been studied in New York (10,12,13). These isolates were first differentiated on the basis of virus-vector specificity. The PAV isolate is transmitted nonspecifically by *Rhopalosiphum padi* (L.), *Sitobion* (= *Macrosiphum*) *avenae* (F.), and *Schizaphis graminum* (Rond.). The RPV isolate is transmitted by *R. padi*. RMV is transmitted specifically by *R. maidis* (Fitch), MAV is transmitted specifically by *S. avenae*, and SGV is transmitted specifically by *S. graminum*. These five luteoviruses can be divided into two groups on the basis of ultrastructural changes in plant cells (5) and by serological properties (12). The RPV and RMV isolates are in one group; MAV, PAV, and SGV are in the other.

Little is known about identity of luteoviruses that cause barley yellow dwarf in California and other western states. The first description of barley yellow dwarf by Oswald and Houston (7) and other early studies in California by Allen (1) apparently involved luteoviruses similar to PAV. Similar isolates appear to be the most common ones in Washington (15) although a few vector-specific viruses were encountered in early work there (16). In Montana, Yount and Carroll (17) recently identified isolates similar to PAV, as well as some that resemble RMV and MAV. Recent serious outbreaks of barley yellow dwarf in western states, new luteovirus research programs in the region, and the importance of disease control through development of tolerant cultivars all underscore the need for a better understanding of luteoviruses present in the field.

The purpose of this study was to determine whether or not species of aphids that infest small grains in California were able to transmit a range of luteoviruses that cause the disease and to identify luteoviruses present in small grains and grasses in the state. Preliminary studies were also made of the reactions of some oat and barley varieties to three California luteovirus isolates.

MATERIALS AND METHODS

Aphids of three species, *R. padi*, *S. avenae*, and *Metopolophium dirhodum* (Walk.), were collected from barley plots at Berkeley (B), Davis (D), or Shafter (S), CA. A single apterous adult of each species was allowed to produce nymphs overnight on a detached leaf of healthy barley. The nymphs were then used to start colonies of each aphid clone on caged barley, *Hordeum vulgare* (L.), maintained in growth chambers at 15 C with constant light. New colonies were initiated every 2 wk by transferring apterous adults. For comparison of vectoring abilities, previously studied clones of *R. padi*, *S. avenae* and *R. maidis* from Ithaca (I), NY (10), were used. Each aphid clone was tested for its ability to transmit four characterized New York isolates of BYDV described above and one PAV-like isolate collected at Davis, CA (CA-PAV).

To begin a transmission test, virus-free aphids were placed on detached leaves of oats that were either healthy or infected with one of the BYDV isolates for a 2-day acquisition feeding at 15 C in the dark. Individual second and third instar nymphs were then caged singly on 7-day-old seedlings of California Red oats, *Avena sativa* L., for a 5-day inoculation test feeding. Seedlings were then sprayed with systemic insecticides and maintained in the greenhouse under aphid-free conditions. Plants were scored as infected or not infected during a 4- to 6-wk period.

Isolates of BYDV were collected from fields of barley, oats, or wheat (*Triticum aestivum* L.) or on occasion from wild oats (*Avena fatua* L.). Collections were made near Arbuckle (Yolo County), Berkeley (Alameda County), Dixon (Solano County), Gilroy (Santa Clara County), Kernville (Kern County), Placerville (El Dorado County), Salinas (Monterey County), and Valley Spring (Calaveras County), CA. Several leaves were taken from individual plants showing typical BYDV symptoms (13), wrapped in moist paper towels, and stored on ice until they were returned to the laboratory. Leaf pieces from each plant were washed, blotted dry, and placed into three dishes with tight lids. Approximately 50 aphids of *R. padi*, *S. avenae*, or *M. dirhodum* were allowed a 48-hr acquisition feeding on leaves in one of each of the three dishes for each sample. Ten aphids of each species were placed on each of three 7-day-old seedlings of California Red oats for a 5-day inoculation test feeding. The seedlings were sprayed and observed as

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described previously. The original field plant was presumed to be infected with PAV-like isolates if all three aphid species transmitted BYDV to seedlings. If only *R. padi* transmitted virus, a virus similar to RPV was suspected; if only *S. avenae* and *M. dirhodum* transmitted, only a MAV-like isolate was suspected. In all cases where vector-specific transmission of RPV- or MAV-like isolates was suspected or where unusual transmission patterns resulted, test plants were used for one or more additional tests. Plants apparently infected with PAV-like isolates were also occasionally retested, especially when mixed virus infections were suspected.

When vector tests were completed, samples of index-test plants were sent to Ithaca for use in enzyme immunoassay (EIA) with antisera to each of four characterized isolates of BYDV as previously described (10–12).

To assess the effect of different BYDV isolates on growth of some small grain varieties and to identify good indicator hosts for future studies, 7-day-old seedlings of California Red, Kanota, and Sierra oats, and Briggs and Prato barley were inoculated with California BYDV isolates similar to RPV, PAV, and MAV. Seedlings were grown in 10-cm clay pots and inoculated by means of aphids (*R. padi* for RPV and PAV and *S. avenae* for MAV) as described above. Each plant was given a visual symptom rating from 0 to 4, with 4 representing maximum stunting, reduced tillering, and yellowing of foliage, relative to control plants, which were rated 0. Plant growth was measured by removing the three plants from each pot, rinsing away the soil, and determining fresh and dry weights of roots and shoots.

RESULTS

No difference in ability to transmit the luteoviruses was observed among one New York and two California clones of *R. padi* (Table 1). Combined results of two tests showed that all three clones of *R. padi* transmitted RPV and PAV but not RMV or MAV. Both New York and California clones of *S. avenae* transmitted MAV and PAV but not RPV or RMV. The two California clones of *M. dirhodum* also transmitted only MAV and PAV. Aphids of *S. avenae*-B transmitted CA-PAV less efficiently than PAV, and CA-PAV was not transmitted by single aphids of either clone of *M. dirhodum* (Table 1). Additional tests were made to compare PAV and CA-PAV transmission by *S. avenae* and *M. dirhodum*.

When 20 seedlings were each infested with five aphids in each treatment, *S. avenae*-I transmitted PAV and CA-PAV to 20 and 18 plants, respectively, and *S. avenae*-B transmitted PAV and CA-PAV to 15 and 6 plants, respectively. These results verified less efficient transmission of CA-PAV by *S. avenae*-B and suggested an inherent difference between PAV and CA-PAV, as well as differences between clones of *S. avenae*. Aphids of *M. dirhodum*-D transmitted PAV and CA-PAV to 5 and 0 plants, and *M. dirhodum*-S transmitted PAV and CA-PAV to 3 and 1 plants, respectively. Thus, *M. dirhodum* is an inefficient vector of PAV, and especially of CA-PAV, with which only one of 40 plants infested with 300 aphids became infected. These results agree with similar observations for *M. dirhodum* by Gill (3). None of 16 plants infested with aphids fed on healthy plants as controls became infected.

To identify isolates of BYDV in California, a preliminary survey was made of small grain fields in eight counties. Data from four field samples and three controls are shown in Table 2. Data for the first three samples allowed identification of RPV-, MAV-, and PAV-like luteoviruses designated as CA-RPV, CA-MAV, and CA-PAV, respectively. Results from aphid transmission tests were confirmed by EIA tests, which indicated no serological differences between California and New York isolates. In 40 separate comparisons, results of EIA tests agreed with those of the aphid transmission tests. Sample 4 (Table 2) illustrates ambiguous transmission data occasionally obtained from a field sample. Virus from this sample was

Table 1. Transmission of barley yellow dwarf virus (BYDV) isolates by clones of *Rhopalosiphum padi, Sitobion avenae,* and *Metopolophium dirhodum* collected at Berkeley (B), Davis (D), or Shafter (S), CA, compared with those from Ithaca (I), NY

	No. of aphids (of 40) that transmitted the BYDV isolate indicated[b]				
Aphid clone[a]	RPV	MAV	RMV	PAV	CA-PAV
R. padi-I	36	0	0	30	28
R. padi-D	36	0	0	30	26
R. padi-S	29	0	0	27	27
M. dirhodum-D	0	25	0	8	0
M. dirhodum-S	0	21	0	16	0
S. avenae-I	0	35	0	26	26
S. avenae-B	0	30	0	26	13

[a] Colonies were started from one apterous adult aphid collected at each location and maintained on caged barley at 15 C under constant light.

[b] Data are combined results of two tests, each involving 20 plants, that used different virus sources and aphid colonies. No differences occurred in percentage transmission between tests. Aphids were given a 48-hr acquisition feeding on detached leaves from infected oats or healthy oats as controls. Single aphids were then allowed a 5-day inoculation feeding on 7-day-old California Red oats. None of 80 aphids of each clone, fed on healthy oats, transmitted virus to any of 16 plants. *Rhopalosiphum maidis* transmitted RMV to 20 of 40 plants when fed on the RMV source but to zero of four plants when fed on healthy oats.

Table 2. Comparison of virus recovery tests, transmission index tests, and enzyme-immunosorbent assay (EIA) of representative isolates of barley yellow dwarf luteoviruses collected in California (samples 1–4) compared with RPV, MAV, and PAV isolates from New York

	No. of infected plants in aphid transmission tests[a]						A_{405} in EIA test with Antiserum indicated[b]				Similar to isolate shown
	Recovery test			Index test							
Sample	Rp	Sa	Md	Rp	Sa	Md	RPV	MAV	PAV	RMV	
1	3	0	0	3	0	0	0.44	0.01	0.01	0.01	RPV
2	0	3	3	0	3	3	0.01	1.43	0.14	0.01	MAV
3	3	3	1	3	3	2	0.00	0.13	0.50	0.02	PAV
4	0	1	0	0	0	1	0.01	0.07	0.29	0.00	PAV
RPV	3	0	0	3	0	0	0.52	0.00	0.01	0.03	RPV
MAV	0	3	3	0	3	3	0.00	1.38	0.10	0.01	MAV
PAV	3	3	1	3	3	2	0.01	0.12	0.50	0.01	PAV

[a] Recovery and index tests were initiated by allowing *Rhopalosiphum padi* (Rp), *Sitobion avenae* (Sa), and *Metopolophium dirhodum* (Md) a 48-hr acquisition feeding on detached leaves from infected oats, followed by a 5-day inoculation feeding on 7-day-old California Red oat seedlings. Each seedling was infested with five to 10 aphids. Data are number of plants (of three) that developed symptoms. None of 12 plants infested with each aphid species, fed only on healthy oats, became infected.

[b] Mean readings of four controls for the RPV, MAV, PAV, and RMV globulins were 0.010, 0.010, 0.009, and 0.007, respectively.

identified in further index tests with *R. padi* and *S. avenae*, but this required a total of three transmission tests over a 6-mo period, using 54 plants. The unambiguous EIA data illustrate the value of the serological test (11).

Results of the survey (Table 3) show that luteoviruses similar to RPV, MAV, or PAV occur in several areas of California. Of 128 plants tested, 75% were infected with isolates similar to PAV, 19% contained virus similar to MAV, and 6% were infected by isolates similar to RPV. Only PAV-like isolates were identified at Arbuckle, Kernville, and Valley Spring; 12 of 14 plants from fields near Dixon were infected by viruses similar to PAV. Although more of the samples were barley than wheat or oats, all three luteoviruses were recovered from each kind of small grain. In a similar survey of small grains from fields at Davis during the spring of 1980, all 15 plants tested were infected with PAV-like virus. In addition, one was also infected with a virus similar to RPV, a mixture detected only in EIA tests.

Table 3. Identification of barley yellow dwarf virus (BYDV) isolates collected from small grains in California

Location of collection	No. of samples	No. of plants infected with virus similar to isolate shown[a]		
		RPV	PAV	MAV
Dixon	14	2	12	0
Arbuckle	20	0	20	0
Salinas	28	3	20	5
Valley Spring	13	0	13	0
Kernville	7	0	7	0
Berkeley	16	3	5	8
Placerville	19	0	15	4
Gilroy	11	0	4	7
Total	128	8	96	24

[a] Isolates were identified by a combination of aphid transmission tests and enzyme-immunosorbent assays. Isolates similar to RPV were specifically transmitted by *Rhopalosiphum padi*. The MAV-like isolates were transmitted by *Sitobion avenae* and *Metopolophium dirhodum*. All three aphid species transmitted isolates similar to PAV; however, *M. dirhodum* did so inefficiently. None of 48 plants infested as controls became infected.

Differences in symptom severity induced by three California isolates of BYDV were apparent in the oat and barley varieties tested (Table 4). The CA-PAV isolate caused less severe yellowing and growth reductions than did CA-RPV or CA-MAV. The CA-RPV isolate induced the most severe symptoms; it killed some young seedlings of California Red oats. Unmistakable symptoms were induced by all three viruses in California Red oats and, for this reason, California Red was selected for use as indicator host. The CA-PAV isolate was almost symptomless in Kanota oats even though CA-RPV and CA-MAV induced obvious symptoms. No symptoms occurred in Sierra oats and only mild ones occurred in Prato barley. Virus recovery tests with California Red oats, however, showed that both Sierra and Prato were infected. Briggs barley developed uniform symptoms to all three viruses and could also be used as an indicator host. Briggs barley was selected for maintaining aphid colonies because aphids reproduced well on this variety and, if accidental BYDV contamination should occur, symptoms would be readily observed.

Severe reduction of root growth occurred in California Red oats; fresh weights of roots from healthy plants and those from plants infected with CA-RPV, CA-PAV, and CA-MAV were 3.2, 1.1, 2.2, and 1.8 g/pot, respectively (mean of three replicates; infected significantly different from healthy at $P = 0.05$). Root growth of infected California Red oats was reduced to 34–68% of that of healthy plants. Root growth of infected Kanota oats and Briggs barley was reduced to approximately 70% of the healthy controls. No significant reduction in root weight occurred in Sierra oats or Prato barley. Dry weights of roots and shoots paralleled fresh weights.

DISCUSSION

When luteovirus diseases have been studied in detail, they usually have been found to involve viruses with a range of differences (13). This study shows that barley yellow dwarf in California is no exception. At least three luteoviruses can cause the disease, and aphids capable of transmitting all three viruses are present in the field. The results underscore the concept that barley yellow dwarf is caused by a group of luteoviruses (10). Because this study was not designed to identify all possible luteoviruses, other variants may also be present. We did not use *R. maidis* in recovery tests, for example, so isolates similar to RMV would not have been detected in aphid transmission tests or in serological tests made of infected test plants.

General similarities in transmitting abilities of aphid clones from New York and California agree with results of an early comparison of vectors from Washington and New York (2,9). Such results agree with the fact that the virus is often the major variable in virus-vector interactions. Differences among clones of these aphid species, however, can be important (14). In this study, for example, the two clones of *S. avenae* differed consistently in efficiency of transmission of PAV-like isolates. Both *S. avenae* and *M. dirhodum* transmitted PAV somewhat more efficiently than CA-PAV, although both CA-PAV and PAV reacted in the same way in EIA tests with four virus-specific globulins.

Information about the identity of luteoviruses that cause barley yellow dwarf in California is needed for several reasons. One is the apparent differential susceptibility of cereal grain cultivars to different California isolates of BYDV. The relevance of the greenhouse tests discussed here to the differential reaction of varieties infected in the field is unknown and cannot be predicted from these preliminary studies. The data suggest, however, that differences among small grain cultivars in response to infection by the various California luteoviruses could be important. In addition, studies at other locations have illustrated the need to understand differential reactions of barley lines to different isolates of BYDV (4,6).

The presence of BYDV isolates similar to RPV could have special significance in California. The RPV isolate of BYDV is closely related to beet western yellows

Table 4. Symptom severity and fresh weight of shoots from oat and barley varieties infected with California isolates of barley yellow dwarf virus[a]

BYDV isolate	California Red oats		Kanota oats		Sierra oats		Briggs barley		Prato barley	
	Disease[b] rating	Shoot[c] fr wt (g)	Disease rating	Shoot fr wt (g)	Disease rating	Shoot fr wt (g)	Disease rating	Shoot fr wt (g)	Disease rating	Shoot fr wt (g)
Healthy	0	13.7	0	33.9	0	26.0	0	45.5	0	40.3
CA-RPV	3.5	6.5*	2.0	18.6*	0	20.5	2.0	30.5*	1.4	31.2
CA-PAV	2.0	10.2**	0.8	30.9	0	22.1	1.0	34.2**	0.9	47.1
CA-MAV	3.0	7.9*	1.5	19.3*	0	25.1	1.6	30.4*	0.6	41.7

[a] Aphids (*Rhopalosiphum padi* for CA-RPV and CA-PAV and *Sitobion avenae* for CA-MAV) were given a 2-day acquisition feeding on infected plants or on healthy plants as controls. They were then allowed a 5-day inoculation feeding on 7-day-old seedlings of each variety, which were sprayed with systemic insecticides and maintained in a greenhouse for 6 wk.

[b] Symptom severity was rated on a scale of 0–4, with 4 representing maximum stunting, yellowing, and reduced tillering, relative to controls. Rating is the mean of nine replicates.

[c] Data are means of three replicate pots of three plants each. * And ** indicate that the value is significantly different from healthy controls at 0.01 and 0.05 levels of probability, respectively.

virus and is also similar to the luteovirus that causes a serious disease of rice in Italy (13). Moreover, RPV is the luteovirus that is particularly effective in promoting dependent virus transmission of other viruses from mixed infections. Thus, the concept of luteoviruses that form one large, interacting system in nature may have special significance in California where so many different crops are grown (10,13).

Distribution of the three variants of BYDV among the various locations sampled illustrated another important variable in understanding barley yellow dwarf. Virus isolates similar to PAV predominated in samples from all but two locations; only PAV-like viruses were identified from three areas. If only these three areas had been sampled or if fewer samples had been collected, we might not have identified the other two luteoviruses present. Distribution of viruses in any one year may be quite different from another. Experience in other locations (3,8,10) suggests that a major challenge is the need to understand and eventually to predict the fluctuation in viruses and vectors that affect the seriousness of disease outbreaks.

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