Status of the Barley Yellow Dwarf Problem of Winter Wheat in Eastern Washington

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ABSTRACT

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In the 14 eastern counties of Washington, barley yellow dwarf virus (BYDV) infected about 20% of winter wheat in the 3 yr 1982–1984. High disease incidence was associated with geographical location and planting date. The highest disease incidence was associated with irrigated areas that support the aphid vectors during the summer and with early planting dates. The predominant isolate of BYDV is vector-nonspecific and PAV-like.

Barley yellow dwarf (BYD) of winter wheat is of major concern in Washington. Infection usually occurs in the fall, resulting in the greatest damage. Plants are stunted, and both the number of tillers per plant and the amount of grain per head are reduced. The yellowing of leaves indicative of the disease usually does not occur until late April and early May. The yellowing so important to visual diagnosis is very dependent on climatic factors such as light intensity. Because cloudy weather is very common in late fall and early spring in the Pacific Northwest, disease incidence and lost yield potential are difficult to estimate. To provide these data, a 3-yr disease incidence survey has been conducted in eastern Washington with an enzyme-linked immunosorbent assay (ELISA) based on BYDV-specific antibodies developed in mice.

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MATERIALS AND METHODS

Virus and aphid culture. BYDV was propagated in barley (*Hordeum vulgare* L. 'Luther'). Nonviruliferous aphids were raised on healthy barley. For transmission studies, aphids were held on infected

Table 1. Number of barley seedlings infected after transmission of a vector-nonspecific barley yellow dwarf virus isolate (PAV-WA-78-20)

	No. aphids used for transmission per seedling[a]			
Aphid species	**1**	**5**	**10**	**15**
Rhopalosiphum padi	11/12[b]	12/12	12/12	12/12
Schizaphis graminum	3/12	9/12	12/12	12/12
Macrosiphum avenae	1/12	4/12	8/12	11/12
R. maidis	0/12	2/12	2/12	2/12

[a] Aphids given 3-day inoculation feeding on seedlings after 24-hr acquistion feeding on detached leaves.

[b] Numerator = number of plants infected as determined by symptoms; denominator = total number of plants in which transmission was attempted.

tissue pieces for 24 hr and transferred to healthy seedlings for 3 days. Routinely, five aphids were used per test plant. The aphids were killed with pirimicarb (Pirimor 50WP), and the plants were maintained under fluorescent lights (7,000 lux, 16 hr/day). Aphid species used included local collections of *Rhopalosiphum padi* L., *R. maidis* Fitch, *Sitobion (Macrosiphum) avenae* Fabricius, and *Schizaphis graminum* (Rond.). The nonviruliferous colonies were established by isolating nymphs from reproducing females maintained on moist filter paper.

Antigen preparation. BYDV was purified from 1-kg batches of Luther barley by extraction in 0.2 M citrate buffer, pH 6.4, chloroform clarification, differential centrifugation, and several cycles of sucrose density gradient centrifugation. Virus preparations free of detectable host material after density gradient centrifugation were used at concentrations of at least 20 μg/ml. A vector-nonspecific isolate (PAV-WA 78-20) prepared for antibody production is described with respect to vector specificity and serology in Tables 1 and 2.

Antibody production. Purified BYDV (20 μg/ml) was emulsified with Freund's complete adjuvant (1:1), and 0.2-ml aliquots of the emulsion were injected into the peritoneal cavities of 6- to 8-wk-old Swiss-Webster and Balb/C mice. Antigen in adjuvant emulsions was stored at −80 C between injections. Injections were repeated at 5- to 7-day intervals until ascitic fluid was produced, as indicated by swelling of the abdomen. The amount and rate of abdominal swelling varied considerably among mice, and not all mice became swollen. Therefore, injection series were routinely made with four to six mice and lasted for at least six injections.

Antibody-containing fluid was removed from the peritoneal cavities with a syringe and an 18-gauge needle. Typically, ascitic fluids were collected from 60% of both Swiss-Webster and inbred Balb/C mice. The volume of ascitic fluids collected varied between 1 and 20 ml per mouse per tapping; 2–3 ml was typical. Alternatively, the fluid was removed surgically from the peritoneal cavity with a Pasteur pipet. In these instances, spleens were subsequently removed and the contained lymphocytes were fused with myeloma cells for monoclonal antibody production. Details of the hybridoma work will be given elsewhere. Ascitic fluids were either diluted threefold to fivefold with phosphate-buffered saline (PBS), pH 7.4, centrifuged to remove debris and frozen for future fractionation or immediately processed by ion-exchange chromatography.

Antibody purification. For anion-exchange chromatography, ascitic fluids were percolated through a DE-22 cellulose column (1 × 15 cm) equilibrated with PBS. The antibodies, which did not adhere to the column in the presence of PBS, were collected as a single peak in the void volume. Alternatively, mouse antibodies were affinity-purified using a modification of the procedure described by Ey et al (3). Ascitic fluid was adjusted to pH 8.6 with NaOH and passed through the column. Antibodies were eluted from the column with 0.1 M citrate buffer, pH 3.0, and dialyzed against PBS, pH 7.4.

ELISA. All direct ELISA was performed as described by Clark and Adams (2), except all incubations were at 25 C for 3 hr and Type VII S alkaline phosphatase (Sigma) was conjugated to immunoglobulins (1 mg Ig/ml, 2.6:1 ratio) by glutaraldehyde (0.06%). For indirect ELISA, virus samples were absorbed to the plate in PBS, pH 7.4, for 3 hr at 25 C. Rabbit or goat antimouse conjugate (Sigma) was used at dilutions of 1:3,200 for the detection of virus-specific antibodies. Immunoglobulin concentration for direct ELISA was 1 μg/ml, and for indirect ELISA, 0.5 μg/ml. When monoclonal antibodies were used, tissue-culture media containing the antibodies were diluted and directly used in indirect ELISA without purification. All immunoglobulin and conjugate treatments were in PBS containing 1% ovalbumin and 2% polyvinylpyrrolidione. Tissue samples were extracted in PBS at 1 g/10 ml.

Field sampling. The number of fields sampled in each county studied in Washington State was proportional to the winter wheat acreage in each county; 10 was the maximum number of fields sampled in any county. Twenty seedlings or tillers were collected at random in each field within about 91 m perpendicular to the rows. Data concerning planting dates, cultivar, and nutrition were collected through county extension agents and directly from the farmers for 2 of the 3 yr of the survey. Samples were sealed in plastic bags and sent to the ELISA laboratory at the Irrigated Agriculture Research and Extension Center, Prosser, WA, to be tested for BYDV.

RESULTS AND DISCUSSION

BYDV strain identity. Aphid transmission studies conducted since 1977 with several hundred isolates from wheat, barley, corn, and many grasses revealed isolates that were transmitted relatively efficiently by *R. padi, S. avenae*, and *S. graminum*. Table 1 gives the transmission rates by four cereal

Table 2. Direct ELISA reactions of four Washington State isolates of barley yellow dwarf virus (BYDV) in comparison to BYDV-type isolates

Sample[a]	$A_{405\,nm}$ in reaction with BYDV-specific globulin shown[b]			
	RPV[c]	MAV	PAV	RMV
PAV-WA 78-20[d]	0.006	0.203	0.895	0.014
PAV-WA 78-13	0.018	0.162	0.845	0.011
Alate RP from field	0.101	0.255	1.010	0.086
Healthy oats	0.000	0.004	0.005	0.000
RPV-NY	*1.380*[e]	0.000	0.007	0.015
MAV-NY	0.016	*1.300*	0.156	0.004
PAV-NY	0.015	0.126	*0.905*	0.000
RMV-NY	0.041	0.000	0.000	*0.348*

[a] Antigens were prepared by grinding 3 g of frozen tissue first in liquid nitrogen and then in 1.5 ml of phosphate-buffered saline containing Tween 20. Samples were clarified with chloroform and centrifugation. The last four samples are type isolates.

[b] Tested by W. F. Rochow, USDA, ARS, at Cornell University, New York. ELISA employed direct coating of the plate with BYDV-specific antibodies in carbonate buffer, pH 9.6. Antigen was detected with isolate-specific alkaline phosphatase-conjugated antibodies.

[c] RPV, transmitted specifically by *Rhopalosiphum padi*; MAV, transmitted specifically by *Macrosiphum avenae*; PAV, transmitted nonspecifically; and RMV, transmitted specifically by *R. maidis*.

[d] Vector-nonspecific isolate.

[e] Homologous reactions are in italics.

Table 3. Results of indirect ELISA testing of isolates of barley yellow dwarf virus using mice polyvalent and monoclonal immunoglobulins prepared against PAV[a]

Sample[b]	$A_{405\,nm}$	
	Polyclonal antibodies (PL-82)	Monoclonal antibodies (32-41)
PAV-WA	1.22	0.64
SGV-ID	1.08	0.75
MAV-CA	0.28	0.47
RMV-MT	0.04	0.23
Healthy barley	0.05	0.19

[a] Indirect ELISA employed mouse polyclonal ascitic fluid at 0.5 μg/ml and monoclonal antibody contained in cell culture supernatant diluted 1:160. Goat-antimouse IgG alkaline phosphatase conjugate was diluted 1:3,200.

[b] Virus samples were prepared by grinding tissue in phosphate-buffered saline containing Tween 20, ovalbumin (0.2%), and PVP (2%), 1 g tissue per 10 ml. The SGV isolate was supplied by G. Bishop, University of Idaho, MAV by F. Gildow, Pennsylvania State University, and RMV by T. Carroll, Montana State University.

aphids of a typical BYDV isolate (78-20). *R. padi, S. graminum*, and *S. avenae* were good vectors but differed in their efficiencies. *R. padi* was the most efficient, and *R. maidis* only rarely transmitted Washington isolates of BYDV. Based on transmission characteristics of many isolates determined over an 8-yr period (1977–1985), the predominant isolate appears to be vector-nonspecific and PAV-like (5).

The serological identity predominating among the isolates was tested in 1978 and several times since by W. Rochow, USDA, ARS, at Cornell University. The serological identities of three isolates are shown in Table 2. Isolates 78-13 and 78-20 were positive for PAV in ELISA but also cross-reacted with MAV-specific antibodies (Table 2). However, in serial transmission tests (three or four aphid transfers) with 22 isolates, including 78-13 and 78-20 already cited, using four aphid species, MAV and other vector-specific isolates were not separated from suspected mixed infections (6).

Polyvalent antibodies produced in mice against our Washington PAV-like isolate (PAV-WA) readily detect BYDV (Table 3). Although our antibodies were prepared against a PAV-like isolate, they probably would detect MAV if present in our survey samples because the antibodies cross-react slightly with MAV-infected tissue samples supplied by W. Rochow, USDA, ARS, Cornell University, and F. Gildow, Pennsylvania State University.

It should be noted that in 1985, *R. maidis*-specific isolates (RMV) were found in the Columbia Basin on barley (K. Pike, *personal communication*). The isolates appear to be associated with barley, because *R. maidis* shows a host preference for barley over wheat. However, it is not yet clear how important an RMV-like isolate will be in Washington on winter wheat.

Table 4. Incidence of barley yellow dwarf virus in winter wheat in 14 counties in eastern Washington State, 1982–1984

County	Incidence (%)			
	1982	1983	1984	3 yr av.
Adams	19.1	13.7	35.7	22.8
Benton	33.1	28.7	30.0	30.6
Columbia	8.6	10.0	25.0	14.5
Douglas	4.3	···	0.0	4.3
Franklin	11.8	25.0	49.0	28.6
Garfield	0.0	11.6	20.6	10.7
Grant	31.5	23.7	26.0	27.1
Klickitat	0.0	0.0	···	0.0
Lincoln	13.5	11.25	7.0	10.6
Spokane	7.1	6.6	···	6.6
Stevens	10.2	···	···	10.2
Walla Walla	35.5	37.5	34.0	35.6
Whitman	21.3	26.3	8.7	18.8
Yakima	23.6	10.0	···	16.8
Eastern Washington[a]	17.9	21.1	22.4	20.5

[a]To calculate regional disease incidence, county data were adjusted to reflect the acreage of winter wheat in each county.

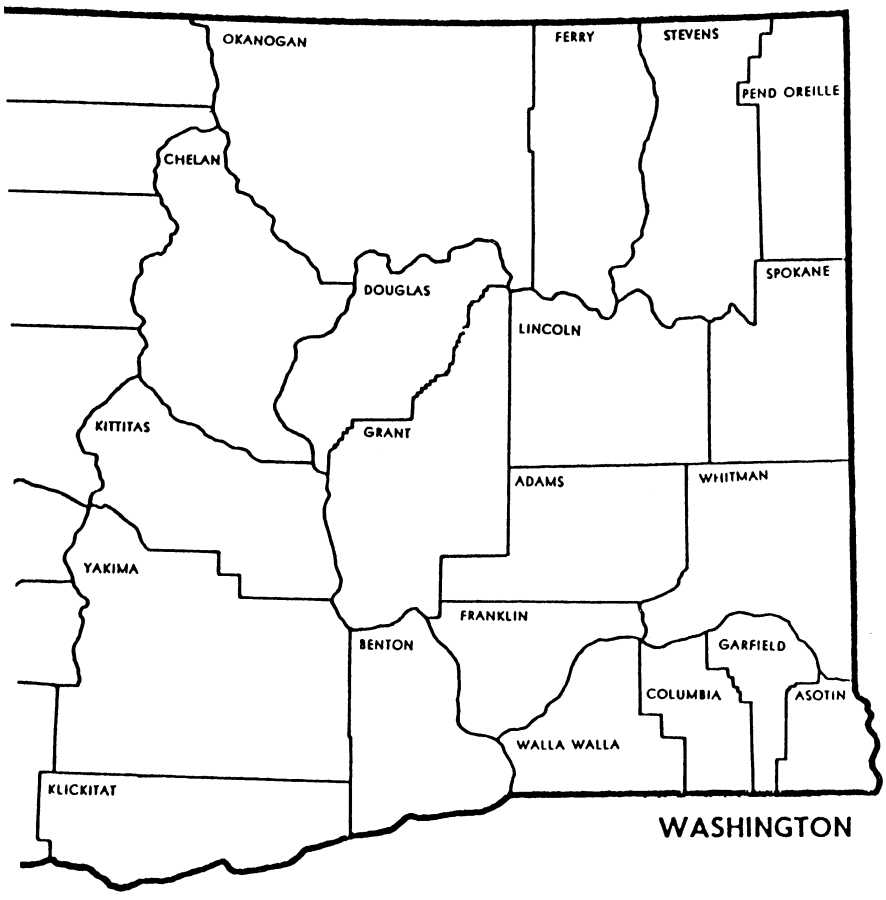

Fig. 1. Counties of eastern Washington.

Disease incidence. Winter wheat fields in eastern Washington were surveyed during February and March of 1982, 1983, and 1984 for BYDV. February and March were selected for sampling because symptoms were not yet evident and therefore would not bias the sampling, snow cover was not present, and all infections were the result of fall or winter aphid activity. The results of the survey are summarized in Table 4. Disease incidence in eastern Washington has been fairly constant, averaging about 20%. This value was derived by multiplying county incidence data by the relative amount of acreage in each county. The disease incidence was the greatest in Walla Walla, Benton, Franklin, and Grant counties (Table 4). These counties account for about 36% of the winter wheat acreage. They contain many irrigated acres of wheat, barley, and corn that harbor both virus and vectors during the summer, when few graminaceous aphids are found in the drylands to the east. The disease incidence in the above four counties varied little during the 3 yr of the survey. In contrast, disease incidences in the dryland counties to the north and east (Fig. 1) varied considerably from year to year. For example, the disease incidences in Adams and Whitman counties ranged from 14 to 36% and 9 to 26%, respectively, during the 3 yr of the survey. Factors that affect disease incidence in the primarily dryland counties include planting dates, wind patterns, and proximity to virus and vector reservoirs.

Planting date is the major determinant of BYD disease levels in eastern

Washington. In 1982, disease incidence was three times higher on wheat planted before 15 September (27 vs. 8.9%). In 1984, incidence was 2.5 times higher (38.6 vs. 15.5%) with early planting. Disease levels are lower with later planting dates in all counties. However, the optimum date for disease control and yield varies because of the effect of weather on aphid biology and plant yield. Planting dates and the proportion of early- to late-planted wheat are determined by moisture availability, type of cropping plan, erosion control methods, disease control practices, and other factors that vary geographically. For example, because eastern Whitman County receives slightly more than 50 cm of rain a year, cropping is annual with little fallow land. Most winter wheat is planted later than in western Whitman County, where rainfall is much lower. The disease incidence in 1982 was 2.5% (eastern Whitman) vs. 46% (western Whitman). Late August or early September experimental plantings in eastern Whitman County are often highly infected.

The major local sources of virus and vectors in the dryland counties during late summer and early fall that could account for the fall infection of the winter wheat have not been identified. A major source probably does not exist because of the dryness of the climate of eastern Washington during July and August. Large populations of viruliferous aphids are commonly found on corn and late-spring-planted wheat and barley grown under irrigation in Walla Walla, Benton, Franklin, and Grant counties, which are west of the dryland counties (1). High disease incidence is common when wheat is planted adjacent to these reservoirs. Long-distance migration of viruliferous aphids to the drylands is probable, assisted by the prevailing winds that are generally westerly and southwesterly. High disease incidence is common in Adams, Whitman, Columbia, and Garfield counties downwind from irrigated areas. In contrast, epidemics are sporadic or disease incidence is low in Douglas, Okanogan, Stevens, upper Lincoln, and Spokane counties north of the prevailing winds from the irrigated areas. Many of the northern county isolates do not infect corn (4). These non-corn-infective isolates have not been found in the Columbia Basin or to the east of the major irrigated area. They might be of local origin, because BYDV and aphid vectors can be found year-round in some seasons on grasses along waterways, lakes, ponds, and springs in Okanogan County.

In conclusion, there are areas in eastern Washington that repeatedly have high incidences of BYDV infection and there are areas with sporadic incidences. Disease incidences in the latter areas are affected by location in relation to virus and vector sources, meteorological conditions, and planting dates. However, all areas are potentially vulnerable to BYDV epidemics.

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