Seasonal Occurrence of Barley Yellow Dwarf Virus Serotypes in Small-Grain Cereals in the Valley of Mexico

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ABSTRACT

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We assessed the occurrence of serotypes of barley yellow dwarf virus (BYDV) in an area at the International Maize and Wheat Improvement Center (CIMMYT) used to screen germ plasm for field resistance to BYDV. Wheat and barley plants were labeled in the field, and leaves were sampled several times during plant development to monitor BYDV incidence. Samples were tested by enzyme-linked immunosorbent assay with a panel of monoclonal and polyclonal antibodies. Samples from plantings sowed on different dates were tested to investigate seasonal influences on BYDV occurrence. Up to 88% of the plants sowed in winter (November and January) became infected. Fewer infections were detected in summer sowings (May). MAV serotypes predominated in both winter and summer sowings.

Luteoviruses grouped under the name barley yellow dwarf virus (BYDV) cause the most important virus diseases of small-grain cereals and occur worldwide (6). The viruses, strains, variants, or serotypes involved are aphidborne (1), and the virus-vector relationships can be quite specific (18). Rochow and coworkers (21) described five isolates of BYDV representative of five serotypes: NY-RPV, transmitted specifically by *Rhopalosiphum padi* L.; NY-RMV, transmitted specifically by *R. maidis* (Fitch); NY-MAV, transmitted specifically by *Sitobion avenae* (Fabricius); NY-SGV, transmitted specifically by *Schizaphis graminum* Rondani; and NY-PAV, transmitted nonspecifically by *R. padi* and *S. avenae*. The prefix NY indicates that the isolates originated in New York State (19).

At the International Maize and Wheat Improvement Center (CIMMYT) in the Valley of Mexico, ongoing cereal breeding programs include screening wheat and barley germ plasm to select for tolerance or resistance (sensu Cooper and Jones [7]) to BYDV (3). The simplest approach to this process is to expose germ plasm to natural infection in the field. However, because different serotypes may affect cereals differently (2,20,22), it is important to obtain information on the occurrence and prevalence of specific BYDV serotypes. Although BYDV has been known in Mexico for at least 40 yr (17) and appears to be widespread in the Valley of Mexico (16), little is known about the seasonal prevalence of BYDV serotypes in this area. We report results of a series of surveys on the occurrence of BYDV serotypes in the Valley of Mexico during 1987–1991.

MATERIALS AND METHODS

Surveys. We surveyed wheats and barleys planted during the winters (sown in November or January) and summers (sown in May or early June) of 1987–1991 at CIMMYT's Atizapan station (19°16'N, 99°51'W, elevation 2,640 m). The valley is surrounded by mountains ranging in elevation from 2,200 to 5,300 m and has a temperate climate with dry winters and rainy summers.

Plant material and sampling. BYDV incidence was assessed over time in spring and winter bread wheat and barley nurseries. Plants were grown in two-row plots with rows either 1 m or 11 m long. Plots were hand-seeded with 10–14 seeds per meter (3). In all, 1,209 plants were selected for study (Table 1), and 3,410 samples from these plants were assessed by enzyme-linked immunosorbent assay (ELISA) (5) for the presence of specific serotypes of BYDV. The selected plants were marked to facilitate locating them during the course of the survey. Marked plants were on a grid of approximately 10 × 10 m. To eliminate edge effects, marked plants were in the center of the nurseries.

Leaf samples were collected from most labeled plants at four growth stages (25): the first true leaf (stage 21–23, early tillering); the third leaf (stage 25–29, late tillering); the leaf before the flag leaf (stage 61–69, anthesis); and the flag leaf (stage 80–89, dough development). For some plants, however, samples were collected at only two growth stages, late tillering and dough development (Table 1).

During the first 2 yr, leaf samples were air-dried at room temperature (12) and kept in envelopes until they were assessed by ELISA, which was done within 45 days; during the last 2 yr, leaf samples were assessed fresh. During the entire experiment, only a few plants (1.5%) that tested positive for infection subsequently yielded samples that tested negative for infection, and although this may have been caused by fluctuations in virus

Table 1. Surveys of the occurrence of barley yellow dwarf virus from 1987 to 1991 at Atizapan

Year	Species	Sowing date	Plants labeled (no.)	Samples tested (no.)
1987–1988	Winter bread wheat	November	176	704
1988	Spring bread wheat	January	93	372
1988	Spring bread wheat	May	147	294
1988	Spring barley	May	146	292
1989	Spring bread wheat	May	160	320
1989	Spring barley	May	98	196
1989–1990	Winter bread wheat	November	137	548
1990	Spring bread wheat	January	90	360
1990	Spring barley	January	79	158
1990–1991	Winter barley	November	83	166
Totals			1,209	3,410

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content, we decided not to consider these plants in the data analyses.

ELISA. Samples collected from 1987 to 1989 were assessed by indirect ELISA (5). Plates were coated with immunoglobulin from a rabbit polyclonal antiserum made to a mixture of BYDV serotypes from Mexico (27). Three monoclonal antibodies (MAbs) prepared in rats and specific for certain BYDV serotypes (24) were used as second antibodies: MAb 91, detecting PAV serotypes; MAb 92, detecting RPV serotypes; and MAFF2, detecting MAV serotypes. These second antibodies were detected with a commercial antirat immunoglobulin G conjugated with alkaline phosphatase.

Samples collected during 1990–1991 were evaluated by double-antibody sandwich ELISA (5) with a panel of polyclonal rabbit antibodies to representative isolates of five BYDV serotypes (8,12, 26): a subculture of NY-MAV, designated MAV-PS1 (13); the P-PAV isolate of Hammond et al (10); and the NY-RPV, NY-SGV, and NY-RMV isolates of Rochow (18), kindly supplied by W. F. Rochow and S. Gray from the collection maintained at Cornell University.

Air-dried leaf samples were ground in liquid nitrogen and then in extraction buffer (phosphate-buffered saline [PBS] containing polyvinylpyrrolidone, pH 7.0) at 1:25 (w/v). Fresh leaf samples were extracted in a roller press (E. Pollahne, Wennigsen, Germany), and extracts were diluted at 1:20 (w/v) in 0.1 M phosphate buffer, pH 7.0. To minimize background reactions, polyclonal antiserum conjugates were diluted in extracts from healthy plants made at 1:20 (w/v) in PBS containing 0.05% Tween 20.

All samples were tested in duplicate wells in Immunolon 2 microtiter plates (Dynatech Laboratories, Chantilly, VA). Negative control extracts from noninfected plants and positive control extracts from dried leaves of stock plants infected with the BYDV isolates named above were included in each plate.

For both kinds of ELISA, absorbances at 410 nm were measured in an MR 700 Microplate ELISA reader (Dynatech) after the substrate (*p*-nitrophenyl phosphate at 0.6 mg/ml) was incubated for 60 min at room temperature. ELISA values at least three times greater than those recorded for the healthy control extracts were regarded as indicating infections.

Cross (heterologous) reactions with respect to the positive controls did not occur with the monoclonal antibodies, but the MAV-PS1 polyclonal antiserum reacted slightly with P-PAV. On the basis of previous work, we interpreted samples that reacted with MAV-PS1 antiserum as MAV serotypes only if this was confirmed by monoclonal antibody reactions or if corresponding reactions with the P-PAV and NY-SGV antisera were negative or relatively low (13,14,23).

RESULTS AND DISCUSSION

Figure 1 and Table 2 summarize the cumulative percentages of infected plants detected over time, in relation to both individual serotypes and mixed infections, in all experiments. In most years, the incidence of BYDV was high by the soft-dough stage, ranging up to 88% for bread wheats and barleys sown in January or November. The generally lower incidence observed among bread wheats and barleys sown in May (Fig. 1 and Table 2) is consistent with reduced aphid activity associated with the regular occurrence of heavy rains during the summer months (4).

In general, MAV serotypes predominated regardless of planting date, year, or cereal type, except in samples from barleys sown in May 1989, in which MAV serotypes were absent and relatively little

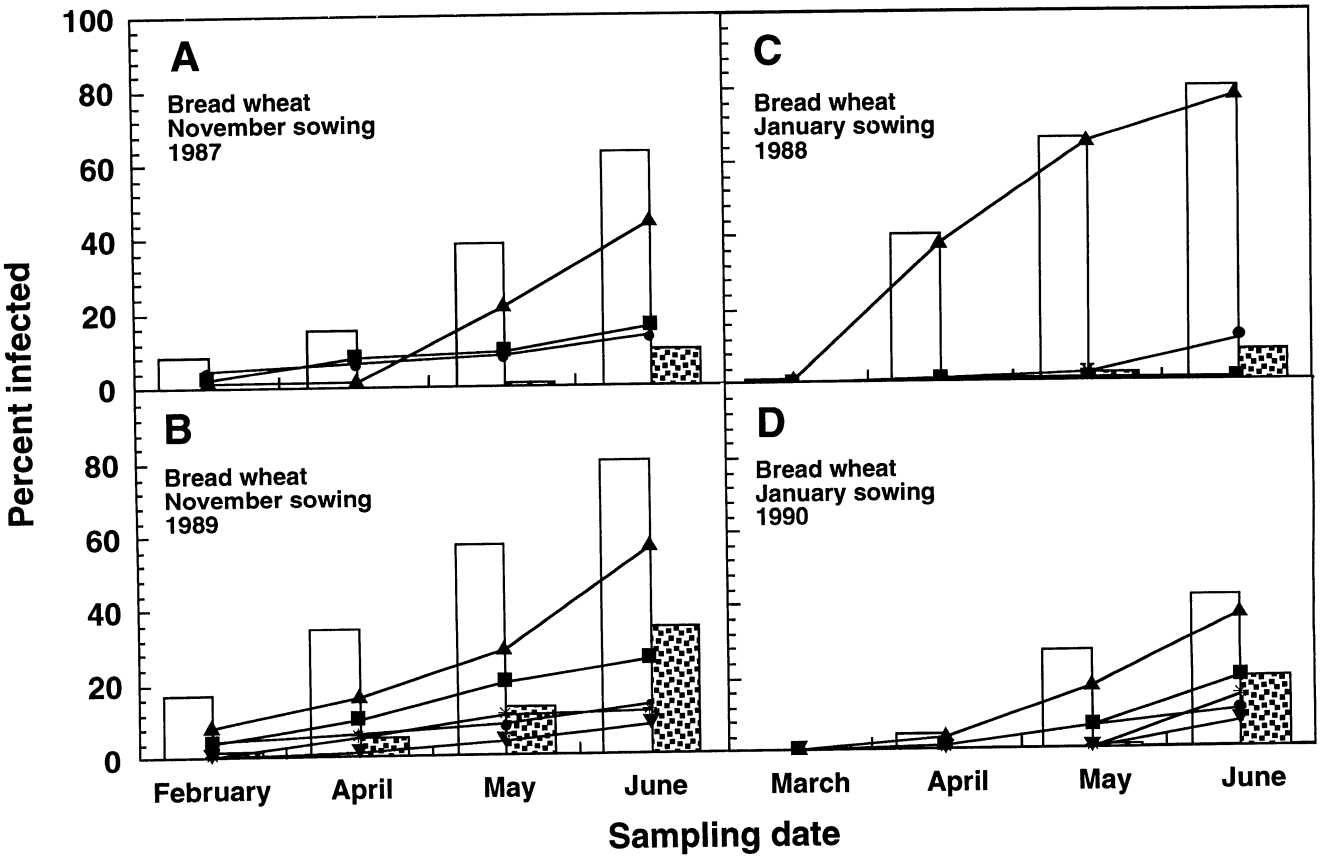

Fig. 1. Cumulative frequency of plants infected with barley yellow dwarf virus serotypes among field-grown wheats sown at Atizapan in **(A)** November 1987 (176 plants sampled on 9 February, 5 April, 4 May, and 6 June), **(B)** November 1989 (137 plants sampled on 21 February, 2 April, 2 May, and 11 June), **(C)** January 1988 (93 plants sampled on 8 March, 21 April, 23 May, and 16 June), and **(D)** January 1990 (90 plants sampled on 8 March, 25 April, 17 May, and 18 June). Open bars indicate the percentage of infected plants, and stippled bars indicate the percentage of plants with mixed infections. Symbols indicate the percentages of plants infected with serotypes PAV (■), MAV (▲), SGV (▼), RPV (●), and RMV (*). Plants were sampled at four stages of development: early tillering, late tillering, anthesis, and dough. Tests for SGV and RMV serotypes were not done during 1987–1988.

BYDV was detected (Table 2). The remaining serotypes were less common and occurred inconsistently. In contemporaneous investigations (15), *R. padi* and *R. maidis* were the predominant aphids captured in suction traps at this location. They are vectors of PAV and RMV serotypes, respectively, in Mexico (16; R. Ranieri, *unpublished*). The dominance of MAV serotypes thus may indicate that their vectors—primarily *Metopolophium dirhodum* and *S. avenae* in this region (16)—are more efficient, or that for some reason more individuals of these species are viruliferous than those of *R. padi* or *R. maidis*.

In barley, mixed infections eventually became somewhat more common in plants sown in November 1990 than in those sown in January (Table 2). The occurrence of mixed infections was reasonably consistent with the prevalence of individual serotypes. Mixtures of two, three, and even four serotypes were observed. For example, in the barley sown in January 1990, in which all the serotypes were represented in single infections, about 20, 35, and 45% of the mixed infections found in the June 1990 samples contained two, three, and four serotypes, respectively (Fig. 2A).

In bread wheat plants sown in November, infections were first detected in February samples, whereas in those sown in January, infections were first detected in April (Fig. 1). This result is consistent with the fact that aphids are absent or present only in very low numbers during the winter months (December–February) (15).

The epidemiology of BYDV is extremely complex, involving several vectors, several virus strains and variants, and a wide range of gramineous hosts (11). The relative frequency of BYDV serotypes and of mixed infections depends on the interactions in any particular season among virus serotypes, vectors, and hosts. More information on these interactions is needed for a complete understanding of BYDV epidemiology and the dynamics of infections in the Valley of Mexico. We have shown that BYDV is prevalent in the Valley of Mexico and occurs regularly.

To enhance the possibility of natural BYDV infection in screening germ plasm for resistance, November or January sowings seem more suitable than May sowings. All five of the generally recognized BYDV serotypes were detected, with MAV predominating. This location is therefore a good one for testing the performance of cereal germ plasm exposed to natural infection by BYDV during the first steps of a selection program, but tolerance to MAV serotypes would be the predominant characteristic selected for. Thus, genotypes selected as tolerant under these conditions would need to undergo further testing by artificial infection with a panel of specific BYDV serotypes to determine their tolerance or sensitivity in more detail (3).

Our approach to assessing BYDV prevalence in these experiments was similar to that of Griesbach et al (9) in California and could be useful in any breeding program selecting for tolerance based on natural infection. Our approach should also help to clarify whether the selections made would be useful in other regions.

Table 2. Cumulative frequency of barley yellow dwarf virus infection among field-grown barleys and wheats during two summer and two winter cycles at Atizapan

Planting Sampling date[a]	Plants sampled (no.)	Serotype[b]					Percentage infected	Mixed infections (no.)
		PAV	MAV	SGV	RPV	RMV		
May 1988 wheat	147							
20 July		0	11	—	0	—	11	0
2 September		0	41	—	0	—	41	0
May 1989 wheat	160							
2 July		1	0	—	1	2	4	0
1 September		9	5	—	5	11	26	4
May 1988 barley	146							
20 July		1	15	—	1	—	16	1
2 September		2	84	—	9	—	86	9
May 1989 barley	98							
2 July		2	0	—	3	0	5	1
1 September		9	0	—	5	11	14	4
January 1990 barley	79							
10 April		38	18	3	6	28	51	27
12 June		49	70	9	15	49	88	41
November 1990 barley	83							
15 March		6	2	8	6	15	25	7
20 May		13	64	8	10	59	86	54

[a] Plants were sampled at the late-tillering and dough stages.
[b] We did not test for RMV or SGV serotypes in 1988 or for SGV serotypes in 1989.

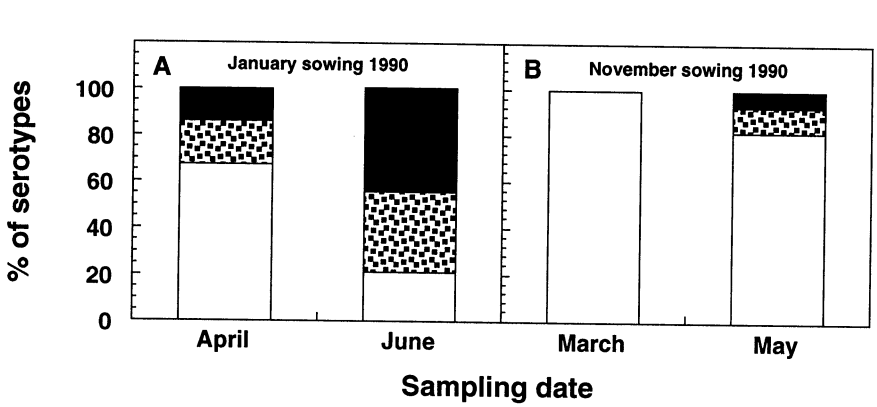

Fig. 2. Percentages of the mixed infections found in barley sown at Atizapan in January 1990 **(A)** and November 1990 **(B)** with two serotypes (open), three serotypes (stippled), and four serotypes (black) of barley yellow dwarf virus. Plants sown in January were sampled on 10 April and 12 June; those sown in November were sampled on 15 March and 20 May.

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