Reliable Detection of Barley Yellow Dwarf Viruses in Field Samples by Monoclonal Antibodies

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ABSTRACT

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Monoclonal antibodies in triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) and polyclonal antibodies in double-antibody sandwich ELISA (DAS-ELISA) were used for detection of three serotypes of barley yellow dwarf viruses (BYDVs) in field collected oat and wheat leaf samples. Agreement between the two ELISA systems was >99% for 900 and 1,050 samples collected in 1989 and 1990, respectively, indicating that the monoclonal antibodies were detecting highly conserved viral epitopes. In both years, PAV and RPV serotypes of BYDVs were present in oat and wheat fields in Illinois. The overall incidence of PAV serotypes was higher than that of RPV serotypes (8.3 vs. 1.7% in 1989 and 16.6 vs. 2.5% in 1990). No MAV serotypes were detected. The results demonstrate the usefulness of monoclonal antibodies in field surveys for BYDVs.

Barley yellow dwarf (BYD), a disease of worldwide importance in cereals and grasses, is caused by several strains of at least two viruses in the luteovirus group (11). Barley yellow dwarf viruses (BYDVs) are small (approximately 25 nm) isometric particles that are transmitted in a persistent manner by aphid vectors (2). Strains of BYDVs originally were differentiated by the species of aphids that transmit them (5,9). Thus, the MAV strain was named for its principal vector, *Sitobion avenae* (Fabricius), the RPV strain for *Rhopalosiphum padi* (L.), and the PAV strain for both of these aphids. The MAV and PAV strains are closely related. The RPV strain is distantly related to either MAV or PAV and is considered by some to be a separate virus or a strain of another luteovirus, beet western yellows virus (3,8).

Isolates of the MAV, RPV, and PAV strains have been identified in previous surveys of small grains in Illinois by aphid transmission (10) and by serology (1). Two other strains of BYDVs (RMV, transmitted by *R. maidis* (Fitch), and SGV, transmitted by *Schizaphis graminum* (Rondani)) have been isolated only twice and never, respectively, in Illinois. We have begun a long-term survey of the oat- and wheat-producing regions of Illinois to monitor the temporal incidences and spatial distributions of the three most common strains of BYDVs.

The usefulness of monoclonal antibodies for field surveys has been

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questioned because each antibody represents only a single viral epitope and may fail to detect field isolates on which that epitope is altered or missing (7). We have tested the reliability and sensitivity of two serological assays for detection of BYDVs in a large number of field samples: triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA), in which each virus serotype is detected by a single monoclonal antibody, and double-antibody sandwich ELISA (DAS-ELISA), in which each virus is detected by a range of antibodies. We report here that monoclonal antibody-based serological assays were equal in reliability and sensitivity to polyclonal-based assays for detection of BYDVs in 1,950 field samples of oats and wheat collected in Illinois over 2 yr.

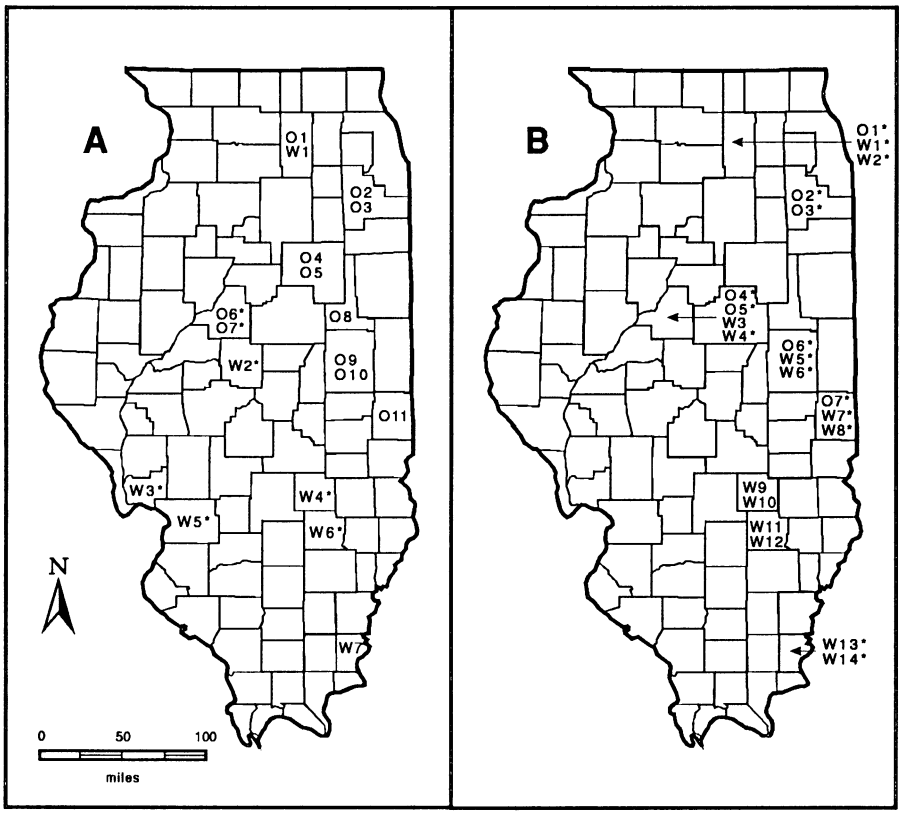

Fig. 1. Location of Illinois oat (O) and wheat (W) fields sampled for three serotypes of barley yellow dwarf viruses in **(A)** 1989 and **(B)** 1990. Fifty random leaf samples were collected in each field; symptomatic samples were collected in fields marked with an asterisk.

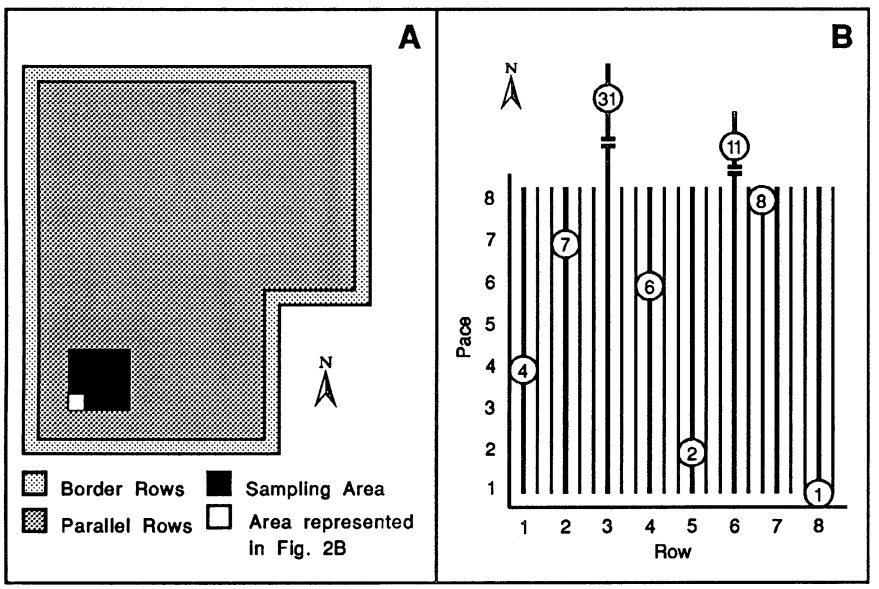

Fig. 2. Schematic field diagrams of **(A)** a sampled area (shown in black) measuring 150 rows by 50 paces located in the southwest corner of each field and **(B)** part of the sampling plot in **(A)** in which one leaf sample was collected from every third row. Circled numbers correspond to the number of paces from the end of a row where the sample was taken.

MATERIALS AND METHODS

Spring oat (*Avena sativa* L.) fields (11 in 1989 and seven in 1990) and winter wheat (*Triticum aestivum* L.) fields (seven in 1989 and 14 in 1990) were sampled across the small grains production areas of Illinois (Fig. 1). Four oat cultivars were sampled. Don was sampled in one field in 1989, Hazel was sampled in one field in 1990, Larry was sampled in three fields in 1989 and three fields in 1990, and Ogle was sampled in seven fields in 1989 and three fields in 1990. Wheat surveys were conducted exclusively in fields of the cultivar Cardinal in 1989 and in equal numbers of fields of Cardinal and Caldwell in 1990. Each field was sampled once during May or June each year.

The sampling area in each field was 150 rows wide by 50 paces long, located in the southwest corner of the field about 6–12 m from the field edge to eliminate possible border effects on virus incidence (Fig. 2A). A single leaf sample was collected from every third row in the sampling area for a total of 50 rows sampled. Selection of the plant to be sampled in a given row was made by randomizing the numbers 1 to 50, without replacement, and assigning a number to each row to be sampled. This number corresponded to the number of paces to be stepped off down the row from the south or west end of the field (Fig. 2B). In addition to these randomly chosen plants, two to five plants showing symptoms of possible BYD infection were selected for testing from some of the same oat and wheat fields in both years (Fig. 1).

Leaf samples were placed in individual paper envelopes, grouped in plastic bags, and kept on ice during transport to Urbana. All samples were frozen at −80 C for 3–9 mo and tested for virus infection in ELISA.

For ELISA, leaf samples of approximately 0.1 g were extracted on a leaf and germ juice extraction press (Erich Pollahne, Germany) with 0.75 ml of phosphate-buffered saline, pH 7.4. Each sample was tested for MAV, RPV, and PAV serotypes of BYDVs in DAS-ELISA and TAS-ELISA (Table 1). In DAS-ELISA, immunoglobulins (Igs) from polyclonal antisera were used as the coating antibodies on Immulon 1 microtiter plates (Dynatech Laboratories, Inc., Chantilly, VA) and, as the detecting antibodies, conjugated to alkaline phosphatase. In TAS-ELISA, polyclonal Igs were used to coat Linbro microtiter plates (Flow Laboratories, Inc., McLean, VA),

monoclonal antibodies MAV-NY-1, RPV-IL-5, or PAV-IL-1 (4) were used as secondary antibodies, and rabbit anti-mouse antibodies labeled with alkaline phosphatase were the tertiary antibodies. After addition of *p*-nitrophenyl phosphate substrate, the absorbance of each well was read at 405 nm. A sample was considered positive if the absorbance was greater than two times that of the mean absorbance value of 18 control wells of extracts from uninoculated, greenhouse-grown oat plants. Positive controls of extracts from greenhouse oats infected with MAV, RPV, or PAV were included on each plate.

RESULTS

The overall agreement between the BYDV-PAV DAS-ELISA and TAS-ELISA systems was 99.9% (899/900) in 1989 and 99.8% (1,048/1,050) in 1990 (Table 2). Of 75 samples positive for PAV serotypes in 1989, the two systems both detected virus in 74 (98.7%). The PAV TAS-ELISA system detected virus in one sample not positive in the DAS-ELISA system. In 1990, 172 samples were positive for PAV serotypes in both systems (98.9% agreement). One sample was positive in each system and negative in the other.

Over all samples, the DAS-ELISA and TAS-ELISA systems for detection of BYDV-RPV serotypes agreed for 99.1% (892/900) in 1989 and 99.7% (1,047/1,050) in 1990. Of 15 positive samples in 1989, only eight (53.3%) were positive in both systems (Table 2). The RPV TAS-ELISA system detected virus in seven samples not positive in the DAS-ELISA system. In 1990, agreement between the two RPV systems was better. Twenty-three samples of 26 positive (88.5%) were positive in both systems. One sample was positive only in the TAS-ELISA system, and two samples were positive only in the DAS-ELISA system.

In 1989, the incidence of BYDV-PAV serotypes in the randomly selected samples ranged from 0 to 40% in oat fields and from 0 to 2% in wheat fields (Table 3). BYDV-PAV-infected plants were found in randomly selected plants from fields from northern and central Illinois but not from southern Illinois, where wheat was predominantly sampled. All four Larry oat plants and 14 of 20 Cardinal wheat plants that had been specifically selected from fields in central and southern Illinois because of their BYD-like symptoms were positive for PAV serotypes of BYDV.

In 1990, the incidence of PAV serotypes in the randomly selected samples ranged from 4 to 60% in oat fields and from 0 to 60% in wheat fields (Table 4). PAV serotypes were found in all fields sampled except three wheat fields in Clay and Tazewell counties (W3, W11, and W12 in Fig. 1B). Of the nonrandomly selected symptomatic plants, 23 of 25 oat plants and 17 of 24 wheat plants were positive for PAV serotypes of BYDV.

The incidence of BYDV-RPV serotypes ranged from 0 to 8% in oat fields and from 0 to 2% in wheat fields in 1989 (Table 3). No RPV serotypes were detected in the 24 nonrandomly selected symptomatic plants. In 1990, the incidence of RPV serotypes ranged from 2 to 6% in oat fields and 0 to 6% in wheat fields (Table 4). Only four of the 25 nonrandomly selected symptomatic oat samples and none of the 24 wheat samples were positive for RPV serotypes. No field samples were positive for BYDV-MAV serotypes in either assay in either year of the survey (Tables 3 and 4).

In 1989, 97.8% (88/90) of the samples positive for BYDV serotypes were oats (Table 3). Oats comprised 61.1% (550/900) of all samples. The incidence of PAV and RPV serotypes in oats in 1989 was 13.5 and 2.5%, respectively. In wheat, the incidence of both PAV and RPV serotypes was only 0.3%. Over all 900 samples from these small grains fields, the incidence of PAV and RPV serotypes

Table 1. Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and triple-antibody sandwich ELISA (TAS-ELISA) protocols used to analyze Illinois oat and wheat field samples for BYDV serotypes in 1989 and 1990

Step	DAS-ELISA	TAS-ELISA
Polyclonal antibody coat	1–2 μg/ml, 2 hr, 22 C	1–2 μg/ml, 1 hr, 37 C
Block	0.5% milk, 2 hr, 37 C	0.1% milk, 1 hr, 22 C
Sample extract	Overnight, 4 C	Overnight, 4 C
Monoclonal antibody	...	1:1,000 dilution, 2 hr, 37 C
Alkaline phosphatase conjugated antibody	1:400-1:800 dilution in sap, overnight, 4 C	1:1,000 dilution, 2 hr, 37 C
Substrate	0.5 mg/ml, 0.5–1 hr, 22 C	0.5 mg/ml, 1 hr, 22 C

Table 2. Number of samples positive or negative in double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and triple-antibody sandwich ELISA (TAS-ELISA) analyses of randomly collected samples[a] from Illinois oat and wheat fields in 1989 and 1990

	PAV			RPV		
Year	DAS-ELISA	TAS-ELISA	Both	DAS-ELISA	TAS-ELISA	Both
1989						
Positive	74	75	74	8	15	7
Negative	826	825	825	892	885	885
1990						
Positive	173	173	172	25	24	23
Negative	877	877	876	1,025	1,026	1,024

[a]900 samples were analyzed in 1989 and 1,050 samples were analyzed in 1990.

Table 3. Incidence of barley yellow dwarf virus serotypes in 50 random leaf samples from oat and wheat fields in Illinois in 1989

		Serotype incidence (%)		
Field[a]	Cultivar	PAV	RPV	MAV
Oat				
O1	Ogle	0	0	0
O2	Ogle	0	0	0
O3	Larry	22	6	0
O4	Larry	40	4	0
O5	Ogle	4	0	0
O6	Larry	16	2	0
O7	Ogle	12	0	0
O8	Ogle	26	8	0
O9	Don	28	2	0
O10	Ogle	0	6	0
O11	Ogle	0	0	0
Wheat				
W1	Cardinal	0	2	0
W2	Cardinal	2	0	0
W3	Cardinal	0	0	0
W4	Cardinal	0	0	0
W5	Cardinal	0	0	0
W6	Cardinal	0	0	0
W7	Cardinal	0	0	0

[a]See Figure 1A for field locations.

Table 4. Incidence of barley yellow dwarf virus serotypes in 50 random leaf samples from oat and wheat fields in Illinois in 1990

		Serotype incidence (%)		
Field[a]	Cultivar	PAV	RPV	MAV
Oat				
O1	Ogle	8	2	0
O2	Hazel	60	6	0
O3	Larry	20	4	0
O4	Larry	16	2	0
O5	Ogle	4	6	0
O6	Larry	44	6	0
O7	Ogle	34	6	0
Wheat				
W1	Caldwell	4	0	0
W2	Cardinal	8	4	0
W3	Caldwell	0	0	0
W4	Cardinal	60	2	0
W5	Caldwell	4	0	0
W6	Cardinal	4	0	0
W7	Caldwell	12	4	0
W8	Cardinal	12	4	0
W9	Caldwell	8	0	0
W10	Cardinal	4	0	0
W11	Caldwell	0	0	0
W12	Cardinal	0	0	0
W13	Caldwell	28	6	0
W14	Cardinal	18	0	0

[a]See Figure 1B for field locations.

was 8.3 and 1.7%, respectively, in 1989.

In 1990, 54.5% (109/200) of the samples positive for BYDV serotypes were oats (Table 4). That year, oats comprised only 33.3% of all samples. The incidence of PAV serotypes in 1990 was 26.6% in oats and 11.6% in wheat. The incidence of RPV serotypes was 4.6% in oats and 1.4% in wheat. The incidence of PAV and RPV serotypes in all 1,050 samples in 1990 was 16.6 and 2.5%, respectively.

DISCUSSION

As we planned a long-term survey to monitor the incidence and distribution of BYDVs in small grains in Illinois, we could find no reports of a large-scale study of the reliability of monoclonal antibodies for detection of BYDVs in field samples. Because the monoclonal antibodies had the advantages of being unlimited in supply and constant in nature, they were preferable as detection agents. However, we were concerned that an assay based on monoclonal antibodies might fail to detect a significant number of BYDV field isolates, which were only slightly altered from those against which the monoclonal antibodies had been produced. Therefore, we compared the sensitivity and reliability of monoclonal antibodies in TAS-ELISA with their more widely tested counterpart, polyclonal antibodies in DAS-ELISA.

The overall agreement between the TAS-ELISA and DAS-ELISA systems in both years was excellent (>99%). The TAS-ELISA systems detected virus in a few samples that were negative in the DAS-ELISA systems. False negatives in the polyclonal antibody-based systems are probably attributable to higher backgrounds in these systems. When background in the RPV DAS-ELISA system was reduced in 1990 by cross absorption of the conjugate with sap from older uninoculated plants (6), fewer false negatives occurred in the DAS-ELISA system and agreement between the two systems improved. The three 1990 samples positive in DAS-ELISA systems but negative in the monoclonal antibody-based TAS-ELISA systems may be infections caused by virus isolates that lack the epitope represented by the monoclonal antibodies used. Because we did not do aphid transmissions from these samples, the serological results were not confirmed by a biological assay.

PAV and RPV serotypes of BYDVs were detected in oats and wheat from most areas of Illinois sampled in 1989 and 1990. The counties sampled were distributed across the major small grains production areas of the state. In randomly selected plants, PAV incidences were consistently higher than RPV incidences, a pattern that is in agreement with the results of two earlier surveys (1,10). In those studies, a low incidence of MAV was reported, but no MAV serotypes were detected in the current surveys.

The low incidence of BYDVs in wheat samples in 1989 may have been attributable to the stage of growth of the plants at the time of sampling. Flag leaves of wheat plants were sampled during heading. Virus titer in these plants may have been higher earlier in the growing season. Certainly, the leaves were tougher than the younger oat leaves sampled, and virus extraction was probably less efficient. In 1990, wheat samples were collected earlier in the season, before plants were heading. A higher incidence of both PAV and RPV serotypes was found in these samples. Although a higher serotype incidence also was found in oat samples in 1990, the increase was only twofold over 1989, whereas in wheat samples, the increase in virus incidence was greater.

Over the 2 yr of this study, 85.9% of BYD infections found in randomly selected small grain samples from Illinois were PAV serotypes and 14.1% were RPV serotypes. The serotype distribution in 58 plants collected because they displayed BYD-like symptoms was 93.5% PAV and only 6.5% RPV serotypes. This is probably because PAV isolates tend to be somewhat more severe than RPV isolates, and, thus, the symptoms are more noticeable (9). Fifteen plants selected as having BYD-like symptoms were not positive for any of the three serotypes. It is possible that these plants were infected with other serotypes of BYDVs, but it is more likely that the symptoms displayed were attributable to other biotic and/or abiotic stresses.

The usefulness of monoclonal antibody ELISA systems for field surveys for BYDVs is demonstrated by the results of this study. The monoclonal antibodies used here detect virus isolates of similar serotype from around the world (4) and, therefore, represent highly conserved epitopes of these viruses. Monoclonal antibodies such as these are likely to be useful in field surveys, whereas antibodies that represent rare or unstable epitopes would not be useful. The high agreement between the two systems has allowed us to select the TAS-ELISA, which provides clearer results and is more conservative of polyclonal antibodies, for our continued study of the incidence and distribution of BYDV serotypes in Illinois.

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