Oat Blue Dwarf Virus in Its Plant Host and Insect Vectors

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ABSTRACT

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Latex serological tests were used to show that 0.9 and 1.6% of the barley plants in North Dakota growers' fields were infected with oat blue dwarf virus (OBDV) in 1982 and 1983, respectively. Enzyme-linked immunosorbent assays were used to show that 15% of the early-migrating *Macrosteles fascifrons* leafhoppers in North Dakota tested positive for OBDV in 1983. The level of leafhoppers testing positive for the virus decreased to near zero in August. OBDV is probably transported into the state each year from areas to the south.

Additional key words: ELISA, leafhopper migration

Oat blue dwarf virus (OBDV) is an isometric virus transmitted by the aster leafhopper (*Macrosteles fascifrons* Stål.) and is found on barley in North Dakota each year. Before the development of serological methods by Banttari (1), the economic significance of the oat blue dwarf disease was difficult to assess because of the lack of suitable assay methods. Detection of the virus in the past was based on visual symptoms in the field. Such detection methods were inadequate because plants with mild or no symptoms often outnumbered those with symptoms (R. G. Timian, *unpublished*). The use of leafhoppers to assay plants for OBDV is impractical because of the time and space involved. The serological procedures used in these studies made it possible to estimate the percentage of infected plants in growers' fields and also the percentage of leafhoppers carrying the virus.

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MATERIALS AND METHODS

Antiserum production. OBDV was purified from infected oat plants by column chromatography and density-gradient centrifugation according to methods previously reported (2). The amount of purified virus available was limited, and immunization procedures were modified from those previously reported (1). A New Zealand white rabbit was bled for normal serum, and 1 wk later, it was injected with 0.5 mg of purified OBDV suspended in 0.5 ml of 0.05 M Tris-HCl (pH 7.0) with 0.001 M EDTA + 0.5 ml of Freund's complete adjuvant for the initial injection. No reaction was obtained with normal serum when tested against OBDV antigen in microprecipitin and agar double-diffusion experiments. A combination of intramuscular (0.5 ml) and multiple subcutaneous (0.1 ml at each of five sites) injections was used on each injection day. Freund's incomplete adjuvant was used in three additional weekly injections. After a 4-wk rest period, booster injections (as described) were given. The rabbit was bled 7 days later and at weekly intervals for an additional 3 wk. Four weeks later, the rabbit was given another booster and bled weekly for an additional 4 wk. The blood fractions were separated, and the sera from each of the two series of bleedings were combined and stored at −15 C. Titer of the antisera as determined

by microprecipitin tests was 1:256.

Latex tests. Latex beads (0.797 μm, Sigma Chemical Company, St. Louis, MO) were sensitized with the gamma globulin fraction of OBDV antiserum using methods given by Bercks et al (3) as modified by Banttari (1). Higher concentrations of latex beads as reported by Banttari (1) did not increase the sensitivity in these tests.

Plant samples for testing were ground 1:10 (w/v) in buffered saline (0.05 M Tris-HCl [pH 7.0] + 0.85% NaCl). Sensitized latex beads (10:1) were mixed for 15 min with 20 μl of the sample suspension in 100-μl disposable micropipettes on a rotating mixer at 8 rpm. Flocculation was determined by visual observations with a ×20 dissecting microscope.

Enzyme-linked immunosorbent assay (ELISA). The double-antibody sandwich method of Clark and Adams (4) was used for ELISA. A concentration of 1 μg/ml of coating gamma globulin and a 400-fold dilution of enzyme-gamma globulin conjugate were optimal in these studies. Individual leafhoppers were ground in 175 μl of PBS-Tween (pH 7.4) (8 g of NaCl, 0.2 g of KH_2PO_4, 2.9 g of $Na_2HPO_4 \cdot 12H_2O$, 0.2 g of KCl, 0.2 g of NaN_3, and 0.5 ml of Tween 20 per liter) in drop plates, and 150 μl of the crude extract was used for each test. Absorbance measurements ($A_{410\ nm}$) were made with a Dynatech Microelisa Minireader MR590 (Dynatech Labs. Inc., Alexandria, VA). Samples were considered positive when their absorbance values were at least three times that of known virus-free leafhoppers included in each plate. Virus-free leafhoppers were maintained on barley plants in insectproof cages in a growth chamber. During the past 10 yr, there has been no indication of OBDV infection in the leafhopper host plants, and leafhoppers withdrawn from the population on a periodic basis have never transmitted OBDV to indicator plants.

Surveys. Surveys for OBDV in barley fields were made in 1982 and 1983, but leafhopper populations were tested in 1983 only. From early June through August, leaves from 10 consecutive plants in a row at 10 random locations in each barley field were tested for OBDV with the latex test. Those fields selected for sampling on each collection date were the latest planted in a particular area. Known virus-free and virus-infected plant samples were tested as controls with each group of 20 field samples. Four fields were sampled each week. Fields for sampling were selected in four areas of the Red River Valley in eastern North Dakota. Most of the early-season collections were from the southern areas, and the later collections were mostly from the northern part of the valley.

Leafhopper collections were made weekly in the area where plant samples were collected. The number of leafhoppers per 100 sweeps was recorded and up to 100 leafhoppers were tested for virus per week by ELISA. Leafhoppers known to be free of virus and leafhoppers that had fed on known OBDV-infected plants for 18 or more days before testing were included on each ELISA plate. Some leafhopper collections were stored at −15 C for as long as 14 days before testing.

RESULTS AND DISCUSSION

The average percentage of OBDV-infected plants in commercial barley fields in 1982 was 0.9%, with the highest level of infection (1–1.2%) in the northern part of eastern North Dakota (Table 1). In 1983, the average percentage of infected plants in tested fields in eastern North Dakota was 1.6%, and again, infection was highest in the northern areas. Infection levels were higher in plants collected in June than in plants collected later in the season (Table 2). These results indicate that infection occurred at a higher rate in fields planted early than in fields planted later.

Leafhoppers were tested for OBDV in 1983 only. ELISA results showed the range of absorbance ($A_{410\ nm}$) for virus-free leafhoppers to be 0.00–0.12 with an average of 0.02. The absorbance values for the infected leafhoppers in the collected samples ranged from 0.20 to 1.82 with an average of 0.62. In May and June, 15.2 and 14.6%, respectively, of leafhoppers tested positive for OBDV (Table 3). By mid-August, none of the leafhoppers tested positive. The level of OBDV in the leafhopper population increased again in September and October.

The number of leafhoppers that tested positive in ELISA for OBDV was not indicative of the number that would transmit the virus at that time. Tests in this laboratory have shown that about 30% of a leafhopper population will transmit OBDV after acquisition feeding on infected barley plants. The field survey results should, however, reflect the relative OBDV transmission potential of the leafhopper population being sampled.

According to R. K. Chapman (*personal communication*), the *M. fascifrons* that migrate into North Dakota overwinter in northwestern Louisiana, western Arkansas, and along the border of Missouri and Kansas on winter cereals. Leafhopper nymphs hatch in the spring, and adults are carried northward by low-level jet winds when the ambient temperature reaches 16 C. These leafhoppers usually reach North Dakota in May, although the time of arrival varies with climatic conditions. In 1983, *M. fascifrons* nymphs were not found in North Dakota until June; therefore, leafhoppers tested (adults) through June were apparently from the migrating population. The migrating population would have acquired OBDV from either their overwintering plant hosts (probably winter oats or wheat) or other OBDV-infected plants while en route. Westdal (5) reported 18 plant species susceptible to OBDV. OBDV has not been recovered from native perennial plants in North

Table 1. Detection of oat blue dwarf virus infection by latex agglutination in plants from commercial barley fields in four areas in eastern North Dakota in 1982 and 1983

Year tested	Area	Total plants tested	No. infected with OBDV	Percent infection
1982	Fargo	600	3	0.5
	Hillsboro	900	8	0.9
	Grafton	900	11	1.2
	Langdon	1,451	14	1.0
Total or av.		3,851	36	0.9
1983	Fargo	600	5	0.8
	Hillsboro	740	4	0.5
	Grafton	900	25	2.8
	Langdon	1,036	18	1.7
Total or av.		3,276	52	1.6

Table 2. Relationship between sampling period and amount of oat blue dwarf virus detected by latex agglutination in plants from commercial barley fields in eastern North Dakota in 1982 and 1983

Month	Total plants tested	No. infected with OBDV	Percent infection
June	3,040	50	1.6
July	3,436	35	1.0
August (1982 only)	651	3	0.5

Table 3. Percentage of aster leafhoppers (*Macrosteles fascifrons*) collected during the summer of 1983 in eastern North Dakota that tested positive for oat blue dwarf virus by enzyme-linked immunosorbent assay[a]

Month	No. tested	Percent positive
May	250	15.2
June	535	14.6
July	672	5.5
August	403	0.7
September	101	9.9
October	48	6.3

[a] Range of absorbance readings for virus-free leafhoppers: 0.00–0.12 (av. 0.02). Range of absorbance readings for infected leafhoppers in samples collected: 0.20–1.82 (av. 0.62).

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These preliminary results indicate that the severity of OBDV in barley in North Dakota is largely dependent on the migrating population of leafhoppers. Since an incubation period of 2–3 wk is required after acquisition of OBDV, the source of the virus could be its overwintering hosts in the Louisiana, Arkansas, Missouri, and Kansas area. The presence of OBDV on winter oats in Arkansas has recently been confirmed by both visual and serological means (R. G. Timian, *unpublished*). Studies to determine the overwintering hosts of OBDV are planned.

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