Use of Antiserum to a New York Isolate of Wheat Spindle Streak Mosaic Virus to Detect Related Bymoviruses from North America, Europe, and Asia

J. E. CARROLL, Graduate Research Assistant, S. M. GRAY, Research Plant Pathologist, USDA-ARS, and G. C. BERGSTROM, Associate Professor, Department of Plant Pathology, Cornell University, Ithaca, NY 14853-5908

ABSTRACT

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Wheat spindle streak mosaic bymovirus (WSSMV) is a widespread and damaging pathogen of winter wheat in North America. The diagnosis of this viral disease has relied primarily on the observation of transient symptoms that may be confused with other biotic and abiotic stresses. Virus was purified from field-infected wheat plants grown in New York and a polyclonal antiserum was produced that by enzyme-linked immunosorbent assay detected as little as 0.25 ng WSSMV/μL leaf or root sap. The antiserum reacted to bymoviruses from North America, Europe, and Asia, including isolates of WSSMV, wheat yellow mosaic virus, and barley yellow mosaic virus, but did not detect oat mosaic virus or barley mild mosaic virus. This corroborates previous findings on serological relationships within the bymovirus group. The antiserum did not react with other cereal viruses tested, including soilborne wheat mosaic furovirus, wheat streak mosaic rymovirus, and barley yellow dwarf luteovirus. The availability of this antiserum will facilitate detection of bymoviruses and enhance research on biology, epidemiology, and management of the diseases caused by these fungal-vectored viruses.

Wheat spindle streak mosaic bymovirus (WSSMV) is a prevalent and damaging pathogen of winter wheat (*Triticum aestivum* L.) in central and eastern North America (5,11,17,23,25,26,31,39, 40). The virus is transmitted by the Plasmodiophoromycete *Polymyxa graminis* Ledingham, an obligate parasite of wheat roots (30). Resting spores (cystosori) of *P. graminis* form in the root cortex and can remain dormant for many years. Fungal-vectored viruses are thought to remain infectious in cystosori for over 10 yr in the absence of the virus's plant host (1).

Viruses from two groups, bymoviruses and furoviruses, are transmitted by *P. graminis*. In wheat, the bymovirus, WSSMV, is often found in association with the furovirus, soilborne wheat mosaic virus (SBWMV) (23). Due to coinfection with SBWMV, it has been difficult to routinely obtain suitable preparations of WSSMV from field-grown plants for antiserum production (23). The absence of SBWMV and the prevalence of WSSMV in central New York (24) enabled us to purify WSSMV from field-infected winter wheat and to produce an antiserum without risk of reactivity to SBWMV.

The objectives of the current study were to purify WSSMV from field-infected plants, to produce a polyclonal antiserum against WSSMV, and to evaluate the antiserum for its sensitivity and specificity to WSSMV and other bymoviruses.

All requests for antiserum should be sent to G. C. Bergstrom.

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MATERIALS AND METHODS

Virus origin and purification. For antiserum production, virus was obtained from symptomatic leaves of the winter wheat cv. Augusta collected from a plot in Ithaca, NY, during May 1992. For use as standards in the serological assays and for characterization of the antiserum, WSSMV was maintained in Augusta and cv. Frankenmuth by mechanical inoculation. Symptomatic leaf tissue was ground in a mortar on ice, at a ratio of 1:3 (w/v), in 0.1 M potassium phosphate buffer, pH 7.0, containing 0.001 M potassium cyanide and a small amount of carborundum. Seedlings, at the two- to three-leaf stage, were inoculated by rubbing the homogenate onto leaves (20,29). Inoculated plants were maintained in a growth chamber at 13 C day/8 C night, 12 hr day/12 hr night and observed for 4 mo for symptom development.

Virus was purified from symptomatic tissue collected from the field or from mechanically inoculated plants in a manner similar to that of Usugi and Saito (36) with the following modifications. Fresh tissue was ground in liquid nitrogen to a fine powder and homogenized at 4 C in extraction buffer (1:3 w/v) (0.1 M ammonium citrate, pH 6.5, 0.1% diethyldithiocarbamic acid, 0.037% iodoacetamide). Materials used in all subsequent steps were maintained at 4 C or kept on ice. Homogenate was strained through two layers of cheesecloth and centrifuged 15 min at 9,630 *g*. Triton X-100 was added to the supernatant to a final concentration of 2% (v/v); the mixture was stirred 10 min at 4 C, and centrifuged 10 min at 6,160 *g*. The supernatant was layered over a 4-ml, 20% sucrose pad buffered in 0.1 M ammonium citrate, pH 6.5, and centrifuged 50 min at 45,000 rpm in a 50.2 Ti rotor. Pellets were resuspended in 0.05 M ammonium citrate buffer, pH 6.5, to 1/10 original volume and the suspension was centrifuged 10 min at 5,018 *g*. An ultracentrifugation of the supernatant was repeated as above and the resulting pellets were resuspended overnight on ice in 500 μL of 0.05 M ammonium citrate buffer, pH 6.5. Resuspended pellets were centrifuged 10 min at 8,000 *g*. The supernatant was layered onto 10–40% sucrose gradients buffered in 0.05 M ammonium citrate, pH 6.5, and centrifuged for 3 hr at 25,000 rpm in an SW 28 rotor. Gradients were photometrically scanned (ISCO Density Gradient Fractionator, Lincoln, NE) and fractions with an absorbance (A) at 254 nm were collected. Virus was pelleted by centrifugation, 60 min at 45,000 rpm in a 65 Ti rotor. Pellets were covered with 100–200 μL of 0.05 M ammonium citrate buffer, pH 6.5, and allowed to resuspend overnight on ice.

An extinction coefficient for A_{260} of 2.5 was used to calculate virus concentration (14).

Purified preparations of WSSMV were placed on a Formvar carbon-coated 300-mesh copper EM grid and stained with a 3:1 (v/v) mixture of 2% potassium phosphotungstate (pH 6.9) and 1% vanadatomolybdate (pH 3.0), and examined by transmission electron microscopy for the presence of flexuous rods and the absence of contaminating materials. Proteins associated with purified virus preparations (5 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (21) and stained with Coomassie blue. Coat protein relative molecular mass (M_r) was determined by extrapolation from plots of $\log_{10}$ protein mass of SDS-7 marker proteins (SIGMA Chemical Co., St. Louis, MO) versus R_f (migration distance of protein/migration distance tracking dye).

Antiserum production. Purified preparations of WSSMV from field-infected plants were submitted to the College of Veterinary Medicine, Cornell University, for polyclonal antiserum production in a white New Zealand rabbit. An initial injection of 500 μg of WSSMV in 0.05 M ammonium citrate buffer, pH 6.5, plus Freund's complete adjuvant (SIGMA), was followed by two booster injections at 3 wk and 5 wk, each containing 150 μg of WSSMV in the citrate buffer, plus Freund's incomplete adjuvant (SIGMA). Test bleeds taken after the second and third boosters were screened for reactivity against WSSMV and total serum was collected 1 mo after the third booster.

The preimmune serum was immunoblot and enzyme-linked immunosorbent assay (ELISA) tested (see methods below) for reactivity to healthy wheat proteins and WSSMV. WSSMV antiserum was cross-adsorbed by mixing it 1:1 (v/v) with a clarified 1:4 (v/v) dilution of uninfected wheat leaf sap in phosphate-buffered saline (PBS), pH 7.4. This was incubated 2 hr at 37 C and the precipitate was pelleted at 8,000 *g* for 15 min. Supernatant was mixed with 0.5× volume of uninfected leaf extract, incubated, and supernatant collected as before. This procedure was repeated until no pellet was observed (usually once or not at all). The immunoglobulins were precipitated with 36% ammonium sulfate and collected by centrifugation (4,000 *g*, 15 min). The pellet was resuspended in 15–20 ml of 18% ammonium sulfate and the precipitated immunoglobulins collected by centrifugation as before. The final pellet was resuspended in 1–5 ml of 0.1 M sodium phosphate, pH 8.0, and dialyzed overnight against the resuspension buffer. The IgG fraction was purified using a MAPS II Kit (Bio-Rad Laboratories, Richmond, CA), following the manufacturer's instructions, and adjusted to 1 mg/ml. Purified IgG was conjugated with alkaline phosphatase (SIGMA) using a one-step glutaraldehyde method (3).

Serological assays. Each assay included sap from uninfected wheat or barley leaves and sap from New York WSSMV-infected wheat leaves as negative and positive standards, respectively. When necessary, WSSMV-infected leaf tissue that had been dessicated over calcium chloride at 4 C was used as a positive standard. Whenever possible, indirect ELISA and double antibody sandwich (DAS)–ELISA were performed on the same leaf sap preparations and on the same microtiter plate.

Immunoblots. Plant samples were prepared at 1:10 dilution in 0.1 M ammonium citrate buffer, pH 6.5, clarified with 0.5× chloroform, mixed 1:1 with 2× dissociation buffer (21), held at 100 C for 3–5 min, and chilled on ice. Proteins were resolved using SDS-PAGE (12.5% polyacrylamide) and electroblotted onto nitrocellulose membranes (34). Membranes were incubated in 0.02 M Tris, pH 6.5, with 0.5 M sodium chloride (TBS) containing nonfat dry milk (1%) for 1 hr at room temperature (RT). Anti-WSSMV IgG (2 μg/ml) was added to the blocking buffer and the membranes incubated 1 hr at RT. Membranes were washed (3 × 10 min) in TBS containing 0.05% Tween 20 and incubated 2 hr at RT in goat, antirabbit, alkaline phosphatase-conjugated IgG (SIGMA) diluted 1:2,000 in TBS containing 1% nonfat dry milk. The membranes were washed as described above and immunodetectable proteins were visualized using an NBT/BCIP development solution (SIGMA). When testing bymovirus samples received from cooperators (see below), either undiluted sap or homogenate from dessicated tissue ground in 0.1 M ammonium citrate buffer, pH 6.5, at 1:15 (w/v) was clarified with 0.5× chloroform. After addition of dissociation buffer and heating as described above, samples were frozen (−20 C) until use.

Indirect ELISA. Assays were performed essentially as described by Lommel et al (22) with the following modifications. Plant sap was clarified with 0.5× chloroform, diluted 1:40 in 0.1 M ammonium citrate buffer, pH 6.5, loaded into duplicate wells of a microtiter plate (Dynatek Immulon II) and incubated overnight at 4 C. Plates were washed with PBS containing 0.05% Tween 20 between all incubation steps. Anti-WSSMV IgG (0.3 μg/ml) in PBS containing 2% nonfat dry milk was incubated 2 hr at 37 C. Goat, antirabbit, alkaline phosphatase-conjugated IgG (SIGMA) diluted 1:2,000 in PBS containing 2% nonfat dry milk was incubated 2 hr at 37 C. Results were based on the average obtained from the values of A_{405} for the duplicate wells loaded for each sample. Samples were considered positive for virus antigen if the A_{405} value was greater than or equal to three times the A_{405} value of uninfected standards. Because of the varied nature of the samples received from cooperators, sap or tissue homogenate, prepared as described under immunoblots, was assayed in two buffers, 0.1 M ammonium citrate buffer, pH 6.5, and 0.05 M carbonate buffer, pH 9.6, 0.01% sodium azide, at dilutions of 1:5, 1:10, 1:20, 1:40, 1:80, and 1:160.

DAS-ELISA. Assays were performed as per Clark and Adams (10) with the following modifications. Wells of the microtiter plates were coated with anti-WSSMV IgG (0.5 μg/ml) in 0.05 M carbonate buffer, pH 9.6, 0.01% sodium azide, and incubated 2 hr at 37 C. Plates were washed as for indirect ELISA between incubation steps. Plant samples were prepared and incubated as for indirect ELISA. Alkaline phosphatase-conjugated anti-WSSMV IgG was diluted 1:2,000 in PBS containing 2% nonfat dry milk. Results were based on the average A_{405} value of the duplicate wells loaded for each sample. Samples were considered positive for virus antigen if the A_{405} value was greater than or equal to 0.100 or three times the A_{405} value of uninfected standards. Because of the varied nature of the samples received from cooperators, sap or tissue homogenate, prepared as described under immunoblots, was assayed at dilutions of 1:20, 1:40, 1:80, and 1:160 in 0.1 M ammonium citrate buffer, pH 6.5.

Characterization of the antiserum. The sensitivity of the WSSMV antiserum was tested in immunoblots, indirect ELISA, and DAS-ELISA against decreasing amounts of purified WSSMV. Uninfected leaf or root sap, prepared as for ELISA (1:40 dilution), was mixed with purified WSSMV. A two-fold dilution series from 1,000 to 0.12 ng virus per lane of an SDS-PAGE immunoblot or per well for ELISA was assayed.

Symptomatic leaves, of similar age, removed from chamber-grown plants were grouped into five categories based on the appearance of streaks: V0 = none to mild; V1 = mild to light; V2 = bright yellow; V3 = orange; and V4 = orange to brown. Sap from leaves in each category was prepared for ELISA, as described above, and diluted 1:5, 1:20, 1:80, 1:320, 1:1,280, 1:5,120, 1:20,480, and 1:81,920 in 0.1 M ammonium citrate buffer, pH 6.5. The sap dilutions for each symptom category were tested in indirect and DAS-ELISA, as described above.

The WSSMV antiserum was also tested for potential cross-reactivity with other wheat-infecting viruses, including SBWMV, wheat streak mosaic rymovirus (WSMV), and two barley yellow dwarf luteovirus isolates (RMV-NY and PAV-NY), and the potyvirus, potato virus Y (PVY).

Detection of bymoviruses. To test whether the WSSMV antiserum would

react with other bymoviruses, we requested virus-infected samples of leaf tissue from various cooperators throughout the world as shown in Table 1. We referred to the virus from New York as WSSMV and to viruses from other locations by the name given by the cooperator submitting the material. Fresh, dessicated, and lyophilized leaf tissues were received and tested by at least one of three methods: immunoblot, indirect ELISA, and DAS-ELISA, as described above. The ATCC material was tested both directly and from mechanically inoculated plants that developed symptoms. Immunoblots were repeated at least twice to avoid false negatives.

RESULTS

Virus purification. Fractionation of sucrose gradients yielded two distinct, but inseparable, peaks with absorbance at 254 nm (Fig. 1). Concentrated fractions contained WSSMV particles with minimal contaminating materials (Fig. 2). Of seven virus purifications, the average yield of WSSMV from leaves of field-grown plants was 1.1 μg virus/g leaf tissue (range of 0.5 to 1.9 μg/g). The A_{260}/A_{280} ratio averaged 1.33. WSSMV symptom expression in chamber-grown plants occurred 2–4 mo after mechanical inoculation of leaves. The average yield of WSSMV purified from leaves of chamber-grown plants was 3.64 μg virus/g leaf tissue (range of 2.3 to 6.1 μg/g). The A_{260}/A_{280} ratio averaged 1.24 for WSSMV purified from chamber-grown plants.

The relative molecular mass (M_r) of the coat protein was estimated, from 16 WSSMV preparations, to be 33.1 kD. The M_r of the additional peptides visible below the coat protein and assumed to be coat protein breakdown products (Fig. 3, lanes 1, 3, 4) was estimated to be 26.6 kD, 25.3 kD, and 24.8 kD. The amount of coat protein breakdown products increased as average daily air temperatures, at 1.5 m aboveground, increased from 7 to 19 C during May (Fig. 3). Virus preparations with minimal coat protein breakdown products, as shown in the May 7 lane in Fig. 3, were used for antiserum production. Purifications from symptomatic tissue collected on May 19, 22, and 24 yielded virtually no virus. In such plants, symptoms were absent on newly emerging leaves.

Characterization of the antiserum. In immunoblots, the WSSMV antiserum detected down to 2 ng virus/μL leaf sap and 8 ng virus/μL root sap. In indirect ELISA, the antiserum detected down to 2 ng virus/μL leaf sap and 70 pg virus/μL root sap. In DAS-ELISA, the antiserum detected down to 0.25 ng virus/μL of both leaf and root sap (Fig. 4). In indirect ELISA and DAS-ELISA, A_{405} values of sap from uninfected plants were consistently below 0.060 and 0.020, respectively, while A_{405} values of sap from symptomatic leaves were routinely 10 times higher than the uninfected con-

Table 1. Reaction of New York wheat spindle streak mosaic virus antiserum to bymoviruses

Virus[b]	Location[c]	Cooperator	Indirect ELISA[a] pH 9.6	Indirect ELISA[a] pH 6.5	DAS-ELISA[d]	Immunoblot[e]
North American Bymoviruses						
WSSMV-type[f]	Ontario, Canada	ATCC	0.6	1.9	0.2	+
WSSMV	Ontario, Canada	W. L. Seaman	0.8	2.5	0.2	+
WSSMV	Ontario, Canada	W. L. Seaman	0.1	1.0	−	−
WSSMV	Alabama, USA	R. T. Gudauskas	NT	NT	NT	+
WSSMV	Arkansas, USA	E. A. Milus	0.8	1.4	0.5	+
WSSMV	Georgia, USA	B. M. Cunfer	−	0.1	−	−
WSSMV	Illinois, USA	G. E. Shaner	2.3	1.0	2.4	+
WSSMV	Indiana, USA	G. E. Ruhl	NT	0.5	NT	+
WSSMV	Kansas, USA	R. L. Bowden	0.6	4.5	0.2	+
WSSMV	Kansas, USA	R. L. Bowden	0.9	6.3	2.7	+
WSSMV	Kentucky, USA	D. E. Hershman	4.1	2.0	2.1	+
WSSMV	Kentucky, USA	D. E. Hershman	0.4	1.8	−	NT
WSSMV	Michigan, USA	D. W. Fulbright	0.8	0.4	0.8	+
WSSMV	Virginia, USA	R. L. Grayson	1.3	7.9	2.7	+
OMV	North Carolina, USA	S. A. Lommel	−	−	−	−
European and Asian Bymoviruses						
WSSMV	France	P. A. Signoret	0.1	−	−	−
WSSMV	Italy	C. Rubies-Autonell	−	−	0.8	+
WSSMV	P.R. of China	Q. Hou	0.6	1.1	0.08	+
WYMV	France	M. Meyer	NT	0.3	NT	+
WYMV	Japan	S. Kashiwazaki	1.0	1.4	−	+
BaYMV	England	M. J. Adams	NT	0.5	NT	−
BaYMV	France	P. A. Signoret	0.5	0.9	−	+
BaYMV	France	P. A. Signoret	0.2	0.4	−	−
BaYMV	Italy	C. Rubies-Autonell	1.6	2.4	0.06	+
BaYMV	Italy	C. Rubies-Autonell	0.7	1.3	NT	+
BaYMV	Italy	C. Rubies-Autonell	0.3	0.4	−	−
BaYMV I-1	Japan	S. Kashiwazaki	2.6	3.5	0.05	+
BaYMV II-1	Japan	S. Kashiwazaki	2.7	3.4	−	+
BaYMV	P.R. of China	Q. Hou	0.3	0.4	−	+
BaYMV	P.R. of China	J. Chen	0.2	0.5	−	−
BaMMV	Various[g]		−	−	NT	−

[a] Enzyme-linked immunosorbent assay. Numbers are sample virus absorbance at 405 nm value divided by New York wheat spindle streak mosaic virus absorbance at 405 nm value. A negative result is indicated by − for absorbance at 405 nm values < 3× the healthy absorbance at 405 nm value. NT = not tested. Data shown are for samples diluted 1:40 in either carbonate buffer, pH 9.6, or 0.1 M ammonium citrate buffer, pH 6.5, (left column and right column, respectively).

[b] WSSMV, wheat spindle streak mosaic virus; WYMV, wheat yellow mosaic virus; OMV, oat mosaic virus; BaYMV, barley yellow mosaic virus; BaMMV, barley mild mosaic virus.

[c] Location of collection of virus-infected material.

[d] Double antibody sandwich ELISA. Numbers as for indirect ELISA. Absorbance at 405 nm of < 0.1 considered negative. Data shown are for samples diluted 1:40 in 0.1 M ammonium citrate buffer, pH 6.5.

[e] Immunoblot results: +, a coat protein band was obtained; −, no coat protein band obtained; NT, not tested.

[f] American Type Culture Collection WSSMV isolate # PV 116, deposited by J. T. Slykhuis in 1970s.

[g] England, M. J. Adams; France, M. Meyer; Japan, S. Kashiwazaki.

trols. In immunoblots, when either leaf sap or root sap contained 500 to 1,000 ng virus, coat protein breakdown products were detected and an upper, virus-specific, band of unknown origin was also detected.

We compared the magnitude of ELISA A_{405} values of New York WSSMV-infected leaf sap as affected by two-fold dilution series from 1:5 to 1:160 in either 0.1 M ammonium citrate, pH 6.5, or 0.05 M carbonate buffer, pH 9.6, in indirect ELISA and from 1:20 to 1:160 in 0.1 M ammonium citrate, pH 6.5, in DAS-ELISA, for several experiments. There was a high degree of plate-to-plate variability, as shown by the standard error values given below, probably associated with variability in the antigen content of the WSSMV-infected tissue used for each of the experiments. There was a trend of decreasing A_{405} values with increasing dilution of leaf sap for indirect ELISA using carbonate buffer (from $A_{405} = 1.067 \pm 0.148$ for 1:5 dilution to $A_{405} = 0.745 \pm 0.07$ for 1:160 dilution) and for DAS-ELISA using ammonium citrate buffer (from $A_{405} = 1.236 \pm 0.158$ for 1:20 dilution to $A_{405} = 0.570 \pm 0.117$ for 1:160 dilution). But with ammonium citrate buffer in indirect ELISA, the trend was for A_{405} values to increase slightly with increasing dilution (from A_{405} 0.786 ± 0.097 for 1:5 dilution to $A_{405} = 0.89 \pm 0.075$ for 1:160 dilution). For samples with low titer of virus and at the higher dilutions, we found improved detection of WSSMV and serologically related viruses when using ammonium citrate buffer rather than carbonate buffer in indirect ELISA.

The response of the antigen to dilution and the resulting A_{405} value in DAS-ELISA (Fig. 5A) and in indirect ELISA using ammonium citrate buffer (Fig. 5B) varied with the category of WSSMV foliar symptoms. Overall, symptom categories V1, V2, V3, and V4 yielded antigens with similar dilution end-points of 1:5,120 in indirect ELISA and 1:1,280 in DAS-ELISA. Antigen from leaves in symptom category V0 had dilution end-points of 1:1,280 in indirect ELISA and 1:320 in DAS-ELISA. The shape of the curves for the severe symptom categories, V3 and V4, was similar in DAS-ELISA and indirect ELISA; the shape of the curves for the milder symptom categories (V0, V1, and V2) was similar in each of the two assays. In both serological assays, antigen from the severe symptom categories, V3 and V4, gave maximum A_{405} values at 1:80 and 1:320 sap dilutions. In DAS-ELISA, antigen from leaves in symptom categories V0, V1, and V2 had maximum A_{405} values at the lowest, 1:5, dilution. In contrast, the indirect ELISA A_{405} values were affected little by dilution of sap from leaves in the V0, V1, and V2 categories; their response curves appear as a horizontal line. In indirect ELISA, the most severe, V4, symptomatic tissue contained antigen that gave substantially higher A_{405} values at sap dilutions from 1:20 to 1:1,280 than the antigen from tissue in other symptom categories; this was not found in DAS-ELISA.

We found that the WSSMV antiserum did not react with SBWMV-infected tissue, either in immunoblots (Fig. 6) or in indirect ELISA (SBWMV A_{405} = 0.035, uninfected wheat $A_{405} = 0.026$, and New York WSSMV A_{405} = 0.481). In indirect ELISA, WSMV-infected wheat gave negative results (WSMV A_{405} = 0.026, uninfected wheat $A_{405} = 0.054$ and, New York WSSMV $A_{405} = 0.617$). Tests of the WSSMV antiserum for detection of barley yellow dwarf luteovirus isolates RMV-NY and PAV-NY, commonly found in wheat in New York, also gave negative results with indirect ELISA (RMV-NY + PAV-NY mixture A_{405} = 0.035, uninfected wheat $A_{405} = 0.035$, and New York WSSMV A_{405} = 0.310) and with DAS-ELISA (RMV-NY A_{405} = 0.008, PAV-NY $A_{405} = 0.019$, uninfected wheat A_{405} = 0.001, and New York WSSMV A_{405} = 1.949). We tested the WSSMV antiserum against the potyvirus potato virus Y (PVY) because bymoviruses are related to potyviruses. In indirect ELISA, PVY was not detected by WSSMV antiserum (PVY A_{405} = 0.018, uninfected wheat $A_{405} = 0.035$, and New York WSSMV $A_{405} = 0.310$). Also, in DAS-ELISA, our antiserum did not react with PVY (PVY A_{405} = 0.005, uninfected wheat $A_{405} = 0.001$, and New York WSSMV $A_{405} = 1.949$).

Detection of bymoviruses. The antiserum to New York WSSMV did not react with the bymoviruses oat mosaic virus (OMV) or barley mild mosaic virus (BaMMV). The antiserum was able to

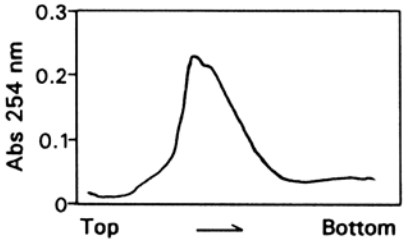

Fig. 1. Typical sedimentation profile of wheat spindle streak mosaic bymovirus scanned during fractionation of a 10–40% sucrose gradient in 0.05 M ammonium citrate buffer, pH 6.5.

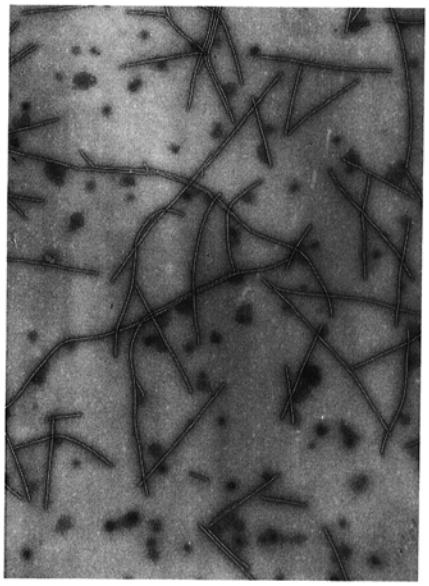

Fig. 2. Transmission electron micrograph of purified wheat spindle streak mosaic bymovirus stained with 3:1 (v/v) mixture of 2% potassium phosphotungstate (pH 6.9) and 1% vanadatomolybdate (pH 3.0) (×37,200). Virus incubated overnight at 4 C at 0.15 μg/μL in 0.1 M ammonium citrate, pH 6.5, prior to observation.

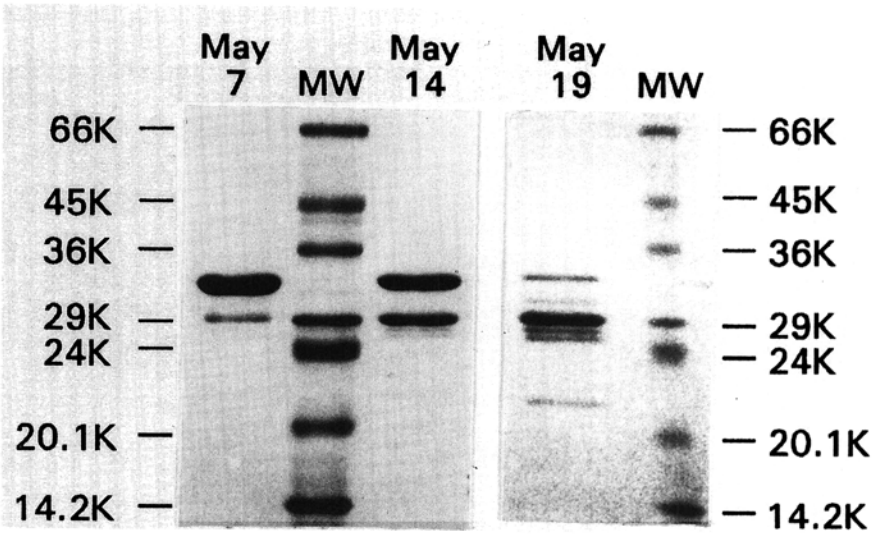

Fig. 3. Coomassie blue-stained sodium dodecyl sulfate–polyacrylamide gel electrophoresis of wheat spindle streak mosaic bymovirus (WSSMV) purified, on dates indicated above lanes, from field-grown wheat in Ithaca, NY, in 1992. Each lane contained 5 μg of WSSMV as determined spectrophotometrically (absorbance at 260 nm) using an extinction coefficient of 2.5. MW lanes contained molecular weight markers of sizes indicated.

detect WSSMV in samples from locations outside of New York as well as the bymoviruses wheat yellow mosaic virus (WYMV) and barley yellow mosaic virus (BaYMV) (Table 1). The antiserum was also used to detect WSSMV in wheat and rye (*Secale cereale* L.) plants in Poland (M. Jeżewska, *personal communication*). Rye has not been reported as a host for WSSMV in North America.

All North American, European, and Asian wheat bymovirus samples reacted with the antiserum in at least one of the assays used. For some wheat samples, the reaction relative to New York WSSMV in indirect ELISA using ammonium citrate buffer was very high, but in DAS-ELISA, the reaction relative to New York WSSMV was very low or negative. In most cases, the reactivity of the WSSMV or WYMV samples relative to New York WSSMV was greater in the ammonium citrate buffer than in the carbonate buffer in indirect ELISA. It is interesting that the values for the ELISAs presented in Table 1 for the ATCC material deposited by Slykhuis are very similar to those for material collected 20 yr later from the same province in Canada. In immunoblots, the North American WSSMV coat proteins comigrated with New York WSSMV (*data not shown*). Differences in migration rate of the coat protein of the European and Asian wheat bymoviruses relative to New York WSSMV were observed in immunoblots (Fig. 7). Three immunoblots gave similar results, with New York WSSMV coat protein migrating faster than the Japan, China, and Italy bymoviruses from wheat.

The BaYMV samples reacted with the WSSMV antiserum in all the indirect ELISAs. By contrast, the majority of these samples reacted negatively or gave a weak positive reaction relative to New York WSSMV in DAS-ELISA. In immunoblots, we found the BaYMV samples contained coat proteins that comigrated with each other, but migrated slightly slower than the coat protein of New York WSSMV (*data not shown*).

DISCUSSION

We were successful in producing an antiserum against WSSMV that is sensitive to WSSMV in immunoblots, indirect ELISA, and direct DAS-ELISA. Success primarily resulted from obtaining a sufficient amount of virus of high purity from field-infected plants for the production of the antiserum. This was made possible by three factors: *1)* spring 1992 was cool and conducive for disease development; *2)* SBWMV is absent in New York; and *3)* the purification procedure we used yielded moderate amounts of highly pure virus. Our purifications were free of detectable contaminating plant proteins as seen in SDS-PAGE stained with Coomassie blue (Fig. 3). It has been found that the buffer used during the extraction of the virus affects yield greatly; Huth et al (15) and Usugi and Saito (36) obtained best yields with 0.1 M citrate buffer, pH 7.0. Yields of 5.5–8.0 mg of WSSMV/kg leaf tissue were obtained in a protocol developed for BaYMV by Chen et al (9) that uses 0.5 M phosphate buffer, pH 7.0, containing mercaptoethanol and EDTA as the extraction buffer (7). Low yield of virus may also result from tissue harvested from the field that, although

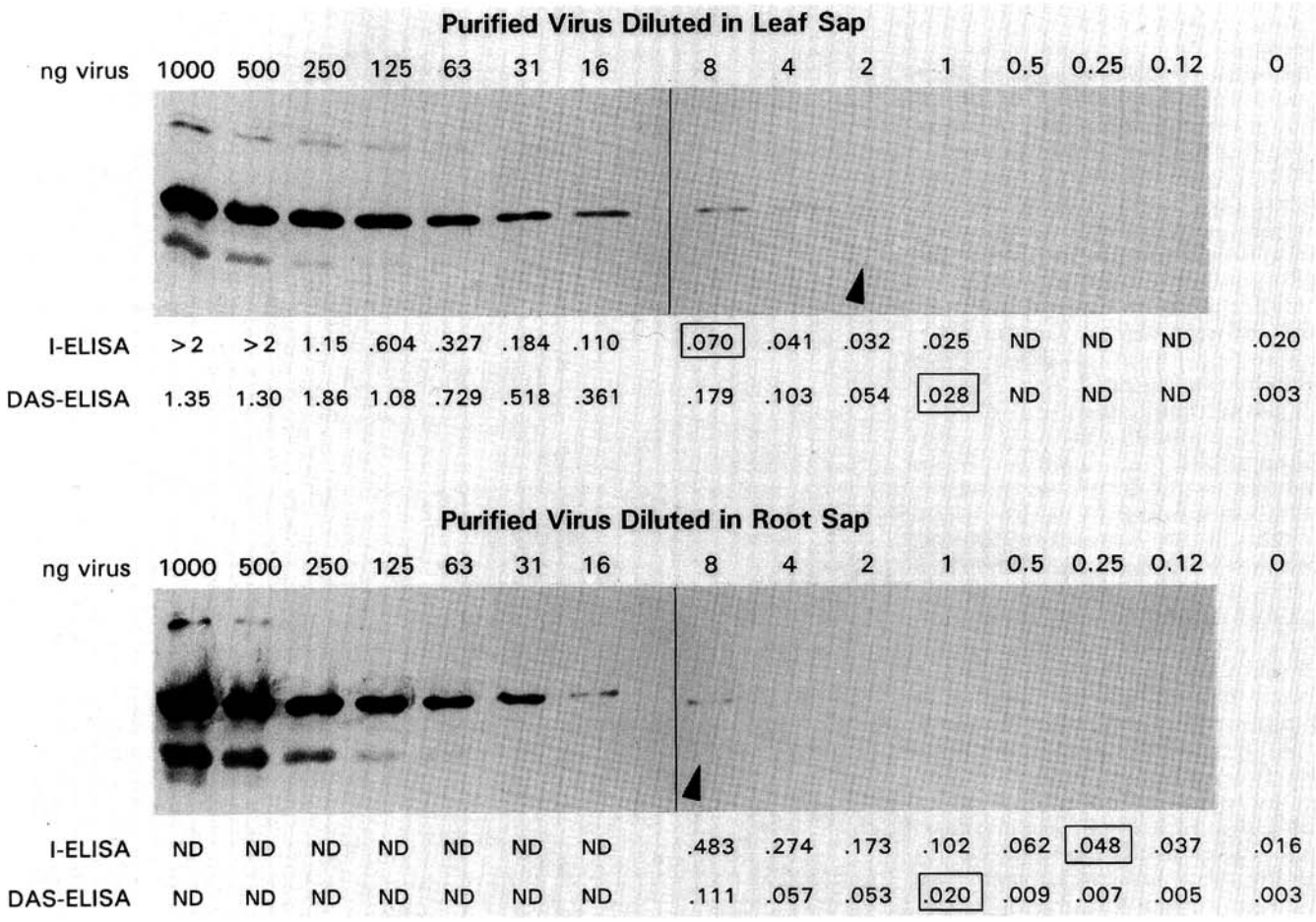

Fig. 4. Detection limits of wheat spindle streak mosaic bymovirus (WSSMV) antiserum for New York WSSMV in immunoblots, indirect enzyme-linked immunosorbent assay (I-ELISA), and double antibody sandwich (DAS)–ELISA. Detection limits shown by arrows on immunoblots and boxes around ELISA absorbance at 405 nm values. Data from three experiments for immunoblotting and indirect ELISA and one experiment for DAS-ELISA. Amount of virus given at top of each panel is ng loaded per lane of sodium dodecyl sulfate–polyacrylamide gel electrophoresis and per well of ELISA plate. Bands visible on immunoblots were virus-specific; middle, prominent band is 33.1 kD coat protein. Absorbance at 405 nm values represent average of two wells loaded with same sample on one plate. ND = not determined. For indirect ELISA, positive readings were $\geq 3\times$ healthy sap. For DAS-ELISA, positive readings were $> 3\times$ healthy sap.

symptomatic, yields negligible amounts of intact virus. We found that symptoms must be active (i.e., developing on the young, emerging leaves, as well as evident on the fully expanded leaves of field-grown plants) in order for acceptable virus yields to be obtained. Purifications of WSSMV from symptomatic leaves taken from plants without active symptoms yielded low amounts of virus with virtually no intact coat protein, as seen in lane 4 of Fig. 3.

Our estimate of 33.1 kD for the size of the WSSMV coat protein fit other estimates of between 30 kD and 36 kD for WSSMV and WYMV coat proteins (7,13,23,35). Variation in the estimated sizes may result from differences in experimental conditions. Haufler and Fulbright (13) and Lommel et al (23) estimated the capsid protein of WSSMV from Michigan and Kansas, respectively, to be 36 kD. In immunoblots, the capsid proteins of these three isolates of WSSMV comigrated. Recent sequence data for WSSMV from France (32) predicts the size of the coat protein of that isolate to be 31.9 kD.

Reaction of the preimmune serum to proteins from uninfected wheat led to a slight elevation in ELISA A_{405} values, but this did not interfere with the ability of the crude antiserum to detect WSSMV from infected leaves. The cross-adsorbed antiserum retained a superior capacity to detect WSSMV in the various serological assays tested.

Michigan WSSMV antiserum (produced against an isolate from Indiana) gave nonspecific activity in DAS-ELISA and protein A DAS-ELISA that could not be prevented by cross-adsorption with uninfected leaf sap (41). When tested against WSSMV-infected, symptomatic leaf tissue, the Michigan WSSMV antiserum and a Georgia WSSMV antiserum had infected/uninfected ratios of 2 and 2 to 14, respectively, in DAS-ELISA (11,41), while the New York WSSMV antiserum had infected/uninfected ratios ranging from 50 to 2,000. The Michigan WSSMV antiserum had a detection limit in DAS-ELISA of 28.6 ng virus (41) compared with the New York WSSMV antiserum detection limit in DAS-ELISA of 1 ng virus.

Dilution of leaf or root sap in ammonium citrate buffer, rather than PBS containing Tween 20 (PBS-T) used by Zagula et al (41), gave better results with the antiserum produced in this study. It is interesting to note that Usugi et al (38), using antiserum to Japan WYMV in ELISA, were unable to detect WYMV and BaYMV in root extracts diluted in PBS-T but did detect the bymoviruses in root extracts diluted in 0.1 M citrate buffer, pH 7.0 (the viruses were detected in leaf extracts diluted in either buffer). When comparing carbonate buffer and citrate buffer in indirect ELISA, we found that the citrate buffer was better in antigen detection at the higher dilutions. To test if this could be related to virus particle dissociation in the pH 9.6 carbonate buffer, we incubated purified virus overnight at 4 C in this buffer and in pH 6.5 ammonium citrate buffer and then observed the virus with transmission electron microscopy. Virus particles incubated in the different buffers appeared much the same and no particle dissociation was evident, as seen in Fig. 2.

The titer of antigen and its reactivity in DAS-ELISA and indirect ELISA varied with symptom severity. In leaves with the most severe symptoms (category V4) there appears to be very high titer of antigen, as seen in Fig. 5B with indirect ELISA. This is not reflected in the DAS-ELISA results (Fig. 5A) perhaps because of saturation of antibody binding sites or conformational changes in the virus caused by antibody binding that masks epitopes. Slykhuis (28) found that WSSMV was more readily transmitted from severely chlorotic leaves with islands of necrotic tissue than from leaves with light green mosaic. These results may be related to an abundance of virus in the severely symptomatic leaves as indicated by the indirect ELISA results. The maximum A_{405} values in both serological assays at the 1:80 and 1:320 dilutions obtained with the severely symptomatic leaf categories V3 and V4 suggest that there may be compounds in these leaf extracts that inhibit, interfere with, or compete with the antigen-antibody reaction at the lower sap dilutions. The horizontal dilution response curves in indirect ELISA for the V0, V1, and V2 symptom categories are difficult to interpret, particularly when the DAS-ELISA curves for these categories show a typical linear trend of decreasing A_{405}

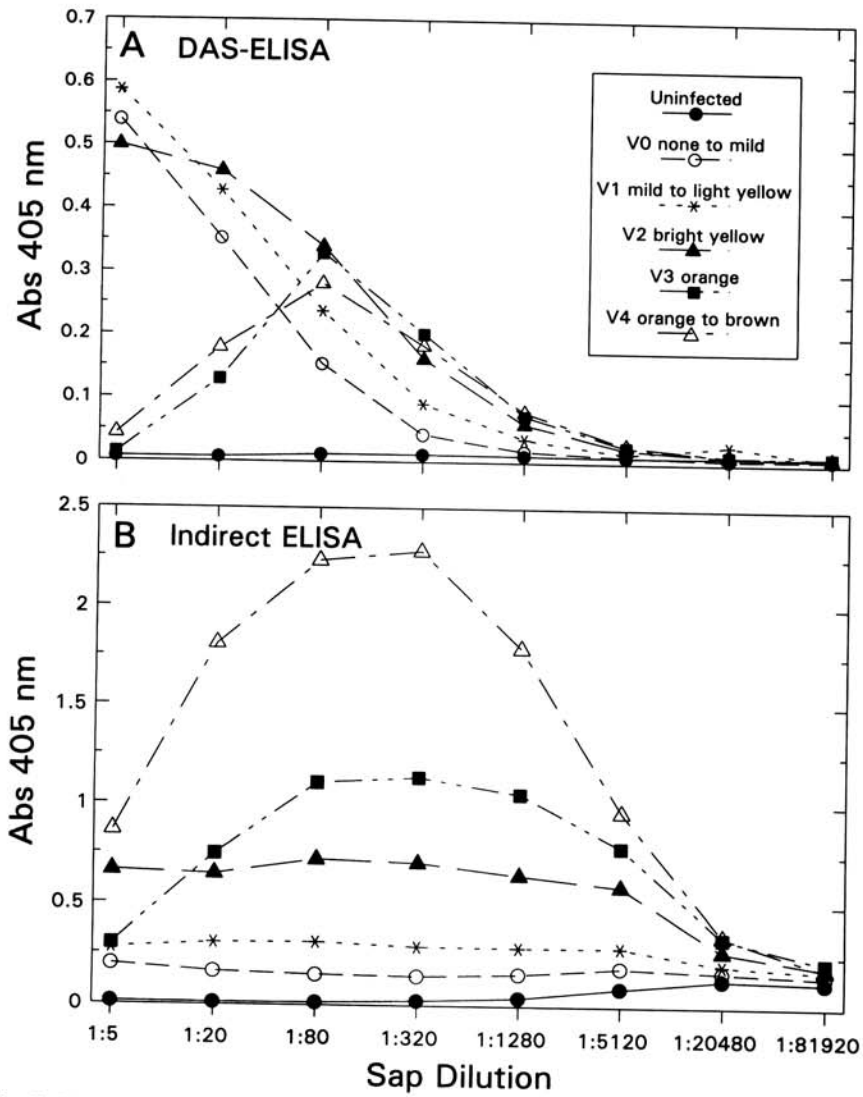

Fig. 5. Magnitude of enzyme-linked immunosorbent assay (ELISA) absorbance at 405 nm (A_{405}) values varying by dilution of sap prepared from uninfected leaves and leaves infected with wheat spindle streak mosaic bymovirus from New York. Infected leaves grouped into 5 different symptom categories based on appearance of streaks : V0 = none to mild; V1 = mild to light yellow; V2 = bright yellow;, V3 = orange; and V4 = orange to brown. **(A)** Double antibody sandwich-ELISA A_{405} values at 30 min. **(B)** Indirect ELISA, using 0.1 M ammonium citrate buffer, A_{405} values at 1 hr 15 min.

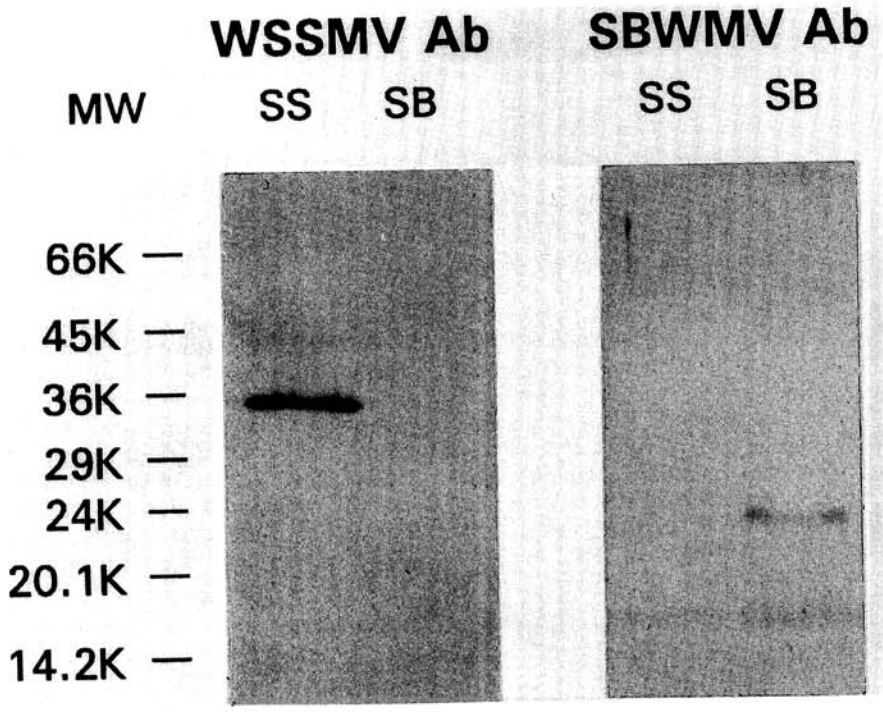

Fig. 6. Immunoblots of proteins extracted from plants infected with either wheat spindle streak mosaic bymovirus (SS) or soilborne wheat mosaic furovirus (SB). Polyclonal antiserum to wheat spindle streak mosaic virus (WSSMV Ab, 1 mg/ml) was used at 1:500; polyclonal antiserum to soilborne wheat mosaic virus (crude serum, SBWMV Ab) was used at 1:500.

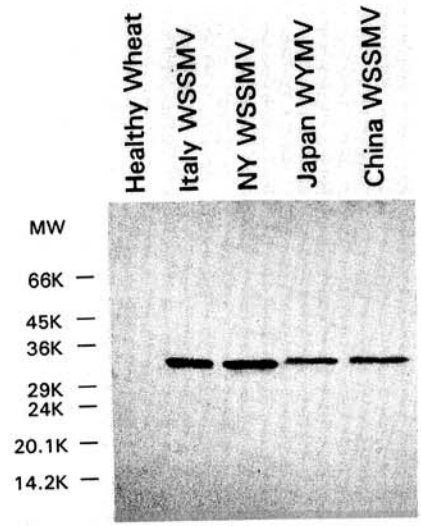

Fig. 7. Immunoblot of wheat spindle streak mosaic bymovirus (WSSMV) from New York, Italy and China and wheat yellow mosaic bymovirus (WYMV) from Japan. Samples loaded in lanes as indicated. All samples consisted of desiccated leaf tissue ground in 0.1 M ammonium citrate buffer pH 6.5 (1:15, w/v) and prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting as described in Materials and Methods. MW are molecular weight markers as shown.

with increasing dilution.

The New York antiserum reacted with bymoviruses, but not with any other cereal viruses tested in this study. Michigan WSSMV antiserum was found to react with SBWMV in immunoblots by Lommel et al (23) but not in direct ELISA by Bays et al (4). We found our antiserum to be specific for members of the bymovirus group that are serologically related as determined by Chen and Adams (8). Given the longevity in vitro of 1 hr at 20 C determined for WSSMV by Slykhuis (28), we were surprised that the antiserum was able to detect viral antigen in dessicated, frozen and thawed, and senescent and moldy tissues received from cooperators. We have since found that the antigen survives well in dessicated or fresh tissue stored at 4 C, but not as well in tissue that has been frozen (−20 to −80 C). WSSMV from ATCC deposited by Slykhuis in the 1970s (29) retained both antigenicity and infectivity.

The DAS-ELISA results presented in Table 1 suggest that there may be strain differences among WSSMV isolates in North America, because several samples gave very low DAS-ELISA absorbance values compared with the indirect ELISA absorbance values. Tests with more sensitive methods that compare nucleic acid sequences are required for confirmation of the existence of strains of this bymovirus. Wheat spindle streak mosaic virus may be a separate virus, a strain of, or synonymous with WYMV (37), first described in Japan (16). The genomes of bymoviruses are bipartite (12,18,19,33) and the size of WSSMV RNA-2 is reported as being slightly smaller than that of WYMV (35). The complete nucleotide sequences of WYMV and WSSMV are needed to compare their relatedness accurately. We found the migration rate of the coat proteins of these two viruses to differ slightly and this result is also suggested in Fig. 2 of Usugi et al (35). This may indicate that the coat proteins differ in the number, as well as, the composition of amino acids. The capsid protein is the 3′ terminal protein encoded by RNA 1 as determined from sequence data obtained for an isolate of WSSMV from France (32). As additional sequence data become available, strain comparisons within the wheat bymoviruses can be made.

The New York WSSMV antiserum detected BaYMV very well in indirect ELISA using both carbonate and ammonium citrate buffers. Therefore, the antiserum can be utilized to monitor barley (*Hordeum vulgare* L. emend. Bowden) for BaYMV in North America, where it does not occur. Barley yellow mosaic virus has spread throughout Europe (2,27). In Japan, strains have arisen that are able to infect previously resistant barley cultivars (20).

The WSSMV antiserum we have produced will facilitate research on the epidemiology, management, and biology of the diseases caused by bymoviruses on cereals. We have used the antiserum to study the progress of WSSM in field plots and have found that it is able to detect viral antigen in plants prior to the onset of symptoms (6). Because current management of this viral disease in wheat relies exclusively on the use of resistant cultivars, the antiserum will have utility in the screening of resistance in cultivars and breeding lines. Little is known about the processes of virus acquisition, retention, and transmission by the vector, *P. graminis,* or the proportion of the vector population that carries the virus. Because the New York WSSMV antiserum detects virus in roots, and virus is transmitted to roots by *P. graminis,* the antiserum will facilitate work on virus-vector-plant interactions.

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