Brome Mosaic Virus Isolated in Manitoba, Canada

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ABSTRACT

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Brome mosaic virus (BMV) was isolated from trial plots of spring wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and bromegrass (*Bromus inermis*) along roadways at Portage la Prairie and Glenlea, Manitoba. Symptoms were stunting, mosaic, and streaking. The Portage isolate of BMV (BMV-P) was serologically identical to the Nebraska type isolate (BMV-N), but differed in the symptoms it induced in *Datura stramonium*, *Chenopodium quinoa*, and *C. amaranticolor*, and in the pattern of double-stranded RNA species isolated from infected tissue. In greenhouse tests, BMV-P markedly reduced biomass, tillering, and yield in several commercial bread and durum wheat cultivars.

Symptoms of stunting, mosaic, and streaking were observed in spring wheat plants in row- and single-hill trial plots at Portage la Prairie, Manitoba, at heading in July 1985. A mechanically transmissible virus was isolated from affected plants, purified, and identified as the causal agent. In June 1986, a similar virus was isolated from barley and bromegrass (*Bromus inermis* Leyss.) at Portage la Prairie, and from barley and bromegrass 90 km distant in experimental plots at Glenlea, Manitoba. These isolates proved to be brome mosaic virus (BMV), designated here as the Portage isolate (BMV-P). The Portage isolate was compared with the Nebraska type isolate (BMV-N).

Until recently, BMV has not been reported to cause economic losses in cereals (16), although it is widespread and readily infects cereals (4–6). Because BMV-P was first noticed by the losses it caused in wheat at one location (Portage la Prairie), this study also included greenhouse infection tests to examine the potential of BMV-P for causing economic losses in some current western Canadian cultivars and breeding lines of wheat, barley, and oats. This is the first description of a BMV isolate from Canada. A preliminary report has appeared as an abstract (2).

MATERIALS AND METHODS

Surveyed locations are shown in Figure 1. Samples of wheat, barley, oats, bromegrass, or ryegrass (*Lolium* spp.) plants were transplanted into peat fiber pots and analyzed in Winnipeg.

Infectious cultures were established by mechanical inoculation to cultivar Little Club wheat (LCW) (4). The following taxa were inoculated: wheat (*Triticum aestivum* L. 'Little Club'), barley (*Hordeum vulgare* L. 'Herta'), oats (*Avena sativa* L. 'Rodney'), maize (*Zea mays* L. 'Golden Beauty'), cucumber (*Cucumis sativus* L. 'National Pickling'), bean (*Phaseolus vulgaris* L. 'Top Crop'), wild tobacco (*Nicotiana rustica* L.), *Datura stramonium* L., *Gomphrena globosa* L., *Chenopodium amaranticolor* Coste & Reyn, and *C. quinoa* Willd. To determine potential economic effects of BMV-P, replicated sets of four seedlings per pot were inoculated at the two-leaf stage with either mock LCW inoculum or BMV-P from LCW source plants. Plants were grown in a random pattern in a greenhouse simulating natural conditions (i.e., 15–24 C under natural light supplemented by fluorescent light for 14–16 hr daylength).

Virus was propagated in LCW or cultivar Black Hulless barley (BHB) and purified according to Lane (5). Double-stranded (ds) RNA was isolated from infected LCW tissue as described by Morris and Dodds (9). DsRNA was characterized in three separate analyses by agarose gel electrophoresis according to Wakarchuk and Hamilton (17). Deoxyribonuclease I, ribonuclease T_1, and ribonuclease A used for characterization were from Sigma Chemical Co. DsRNA isolated from the Portage isolate was compared with that from several Nebraska isolates of BMV and an isolate of the S-strain of cucumber mosaic virus

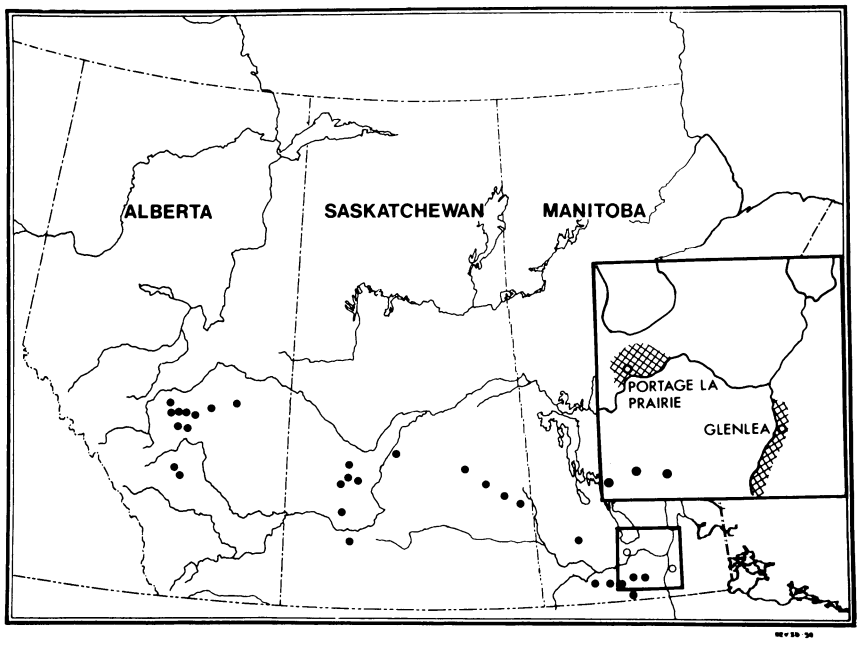

Fig. 1. Areas of three Canadian provinces examined for isolates of brome mosaic virus. Closed circles indicate locations covered in 1985 cereal virus survey. Open circles in main map and inset indicate locations where brome mosaic virus was isolated; crosshatched portions indicate nearby surveyed areas where BMV was not isolated.

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(CMV). Relative molecular masses of dsRNA species of BMV-P were estimated by a robust curve-fitting procedure (12), using dsRNA of CMV and dsRNA of other isolates of the type strain of BMV as size markers. The values for the relative molecular masses of these standards were obtained by doubling the reported relative molecular masses of their ssRNA species estimated under denaturing conditions (1,4). A Hind III restriction digest of bacteriophage lambda (10) was also run in these gels to show that there was a consistent relationship between migration distance and relative molecular mass of double-stranded nucleic acid species, but was not used to estimate relative molecular masses of dsRNA of BMV-P.

Some properties of purified virions. *Analytical sucrose density gradient centrifugation (SDGC).* Virus preparations were layered onto 10–40% linear sucrose gradients in 0.05 M sodium acetate (pH 5.0) buffer and centrifuged in a Beckman SW 40 rotor for 120 min at 31,500 rpm and 4 C. Runs were repeated three times. Gradients were scanned in an ISCO ultraviolet absorbance monitor at 254 nm. Sedimentation velocities were estimated by comparison, without correction for infinite dilution, with similar gradient scans of tobacco ringspot virus (TRSV) as a standard (14).

Electron microscopy. Sucrose gradient absorbance peak fractions from three sucrose gradient runs, and leaf dips made from tissues sampled from locations indicated in Figure 1, were stained and washed with 0.2% phosphotungstate (pH 7.2). Grids were examined in a Philips

Table 1. Host reactions[z] of Nebraska (N) and Portage (P) isolates of brome mosaic virus (BMV)

Host and cultivar	BMV-N symptoms	BMV-P symptoms
Triticum aestivum		
cv. Little Club	Systemic mosaic	Systemic mosaic
Hordeum vulgare		
cv. Herta	Systemic mosaic	Systemic mosaic
Avena sativa		
cv. Rodney	Systemic mosaic	Systemic mosaic
Zea mays		
cv. Golden Beauty	Chlorosis, wilt, death	Chlorosis, wilt, death
Chenopodium quinoa	Necrotic local lesions	Pinpoint lesions
C. amaranticolor	Necrotic local lesions	Pinpoint lesions
Datura stramonium	Faint chlorotic spots	Necrotic rings
Gomphrena globosa	No symptoms	No symptoms
Nicotiana rustica	No symptoms	No symptoms
Cucumis sativus	Necrotic local lesions	Necrotic local lesions (smaller than those of BMV-N)
Phaseolus vulgaris		
cv. Top Crop	No symptoms	No symptoms

[z]Sets of four plants of each assay host were rub-inoculated with infectious wheat tissue ground in 0.1 M sodium acetate buffer (pH 5). On dicotyledenous hosts, extracts of infectious tissue of the two isolates were rubbed on opposite half-leaves on two plants. On each of the remaining plants each isolate was rub-inoculated onto half-leaves whose opposite had been mock-inoculated with an extract from healthy wheat tissue.

Table 2. Effect of the Portage la Prairie isolate of brome mosaic virus (BMV-P) infection on seed yield in greenhouse studies[x]

		Comparison of seed yield (g) per pot (four plants)[y]			Comparison of numbers of seeds per pot (four plants)[z]		
Host and cultivar	Infection	Pot 1	Pot 2	Significance	Pot 1	Pot 2	Significance
Triticum aestivum							
cv. Neepawa	Control	6.4 a	7.3 a	2.98	205 a	225 a	3.13
	Infected	4.1 b	4.8 b		138 b	170 b	
cv. Columbus	Control	4.9 a	6.8 a	3.77	178 a	245 a	3.25
	Infected	1.0 b	2.5 b		52 b	101 b	
cv. Katepwa	Control	4.4 a	6.7 a	1.81	142 a	225 a	1.50
	Infected	2.6 a	3.8 a		96 a	134 a	
cv. HY320	Control	4.9 a	6.2 a	0.85	106 a	139 a	0.99
	Infected	3.6 a	5.4 a		76 a	132 a	
T. durum							
cv. Hercules	Control	3.3 a	4.9 a	5.81	76 a	113 a	4.79
	Infected	0.1 b	0.4 b		3 b	8 b	
Hordeum vulgare							
cv. Heartland	Control	5.2 a	8.4 a	0.87	171 a	274 a	1.06
	Infected	4.1 a	7.7 a		143 a	184 a	
cv. Argyle	Control	6.3 a	7.8 a	3.29	175 a	229 a	2.00
	Infected	5.2 b	5.2 b		130 a	155 a	
cv. Bedford	Control	6.1 a	7.2 a	5.38	210 a	216 a	6.22
	Infected	4.8 b	5.2 b		155 b	156 b	
cv. Bonanza	Control	5.5 a	7.6 a	0.20	181 a	224 a	0.71
	Infected	5.6 a	6.9 a		151 a	205 a	
Avena sativa							
cv. Dumont	Control	7.7 a	8.4 a	3.63	239 a	247 a	4.25
	Infected	4.5 b	5.9 b		131 b	173 b	
cv. Lamar	Control	5.1 a	6.8 a	0.00	164 a	248 a	0.87
	Infected	5.8 a	6.1 a		132 a	191 a	
cv. Ogle	Control	4.7 a	5.5 a	4.02	141 a	174 a	2.60
	Infected	3.1 b	3.5 b		113 b	116 b	
cv. OT 240	Control	6.3 a	6.6 a	0.77	169 a	198 a	1.88
	Infected	4.4 a	6.6 a		135 a	160 a	
cv. OT 749	Control	5.8 a	7.5 a	1.12	154 a	173 a	1.58
	Infected	3.7 a	6.2 a		127 a	169 a	

[x]Replicated sets of four seedlings per pot were inoculated at the two-leaf stage with mock Little Club wheat inoculum or BMV-P from Little Club wheat source plants. Pots were distributed randomly in a greenhouse simulating natural conditions (15–24 C under natural light supplemented by fluorescent light for 14–16 hr daylength).

[y]Seed yields followed by the same letter were not significantly different at $P = 0.05$ in one-tailed t test.

[z]Seed numbers followed by the same letter were not significantly different at $P = 0.05$ in one-tailed t test.

EM 420 electron microscope at 80 kV.

SDS-PAGE of virion protein subunits. Virion preparations from peak SDGC fractions of purified BMV-P and BMV-N were dissociated as described by Lane (5). Electrophoresis was performed (two repeats) according to Laemmli (3): 100 V was applied across a 12% polyacrylamide gel with 4% stacking gel (0.75 mm thick) in a discontinuous buffer system. Staining was with Coomassie Brilliant Blue dye (3). Migrations of standard size markers (phosphorylase-b, bovine serum albumin, ovalbumin, carbonic anhydrase, and β-lactoglobulin) were compared with those of virion protein subunits, and a robust curve-fitting procedure was used to estimate relative molecular mass (12).

Agarose gel electrophoresis of virion RNA. RNA was extracted from SDGC-purified preparations of BMV-N and BMV-P according to Lane (5). Horizontal submarine electrophoresis was performed (three repeats) in a Mini-Cell system (Bio-Rad, Inc., Richmond, CA) with 1.2% agarose gel in Tris-acetate-EDTA (ph 8.3) buffer at 40 V for 120 min. RNA was stained with ethidium bromide at 10 μg/ml.

Agar gel double-diffusion serology. Serological relationships of BMV-N and BMV-P were examined in reciprocal double diffusion in solidified agar on glass slides (8). The agar was made up with 0.1 M sodium acetate buffer (pH 5.2) to prevent dissociation of BMV particles in the course of immunodiffusion. Antibody preparations consisted of undiluted sera obtained from rabbits immunized with preparations of the respective purified BMV strains by intramuscular injection of 1 mg of virus emulsified in 2 ml of a 1:1 mixture of standard phosphate-buffered saline:Freund's incomplete adjuvant, followed by a similar injection 1 wk later. Rabbits were bled from alternating ears once a week for 8 wk following the second injection. Sera from the fifth bleed were used for double-diffusion analyses. Antigen samples for immunodiffusion were purified virus preparations of the two isolates at 0.25 mg/ml (estimated using $E^{0.1\%}_{10\ mm}$ at 260 nm = 5.2) (5) in 0.1 M sodium acetate buffer (pH 5.2). Controls were made by extracting healthy wheat sap 1:4 (w/v) with 0.1 M sodium acetate buffer (pH 5.2).

RESULTS

Host range and reactions. Host responses to BMV-N and BMV-P are shown in Table 1. Host ranges were indistinguishable. Differential reactions were observed in the local lesion hosts, *C. amaranticolor*, *C. quinoa*, and *D. stramonium* (Fig. 2). The Portage isolate of BMV induced smaller, pointlike lesions in the *Chenopodium* hosts and necrotic rings rather than faint spots in *D. stramonium*.

Effect of BMV-P infection on cereals. Infection with BMV-P reduced height, biomass, and seed yield per plant most markedly in some bread (*T. aestivum*) and durum wheat (*T. durum* Desf.) cultivars, but also in some barley and oat cultivars (Table 2). All lines initially showed streaking, mosaic, and stunting but began to recover as early as 15 days (Bonanza barley) or as late as 30 days after inoculation (Hercules durum wheat). The severity of initial infection and duration of acute symptoms were correlated with yield loss. In all lines, infectious virus could be recovered from infected plants at all times.

Isolation of dsRNA from infected tissue. Instead of the four usually reported (2.2, 2.1, 1.4, and 0.6 MDa) (6), five dsRNAs were observed following agarose gel electrophoresis of extracts from Little Club wheat or Black Hulless barley infected with BMV-P or BMV-N (Fig. 3), whereas no corresponding bands were detected in healthy extracts of Black Hulless barley or Little Club wheat (*data not shown*). The additional dsRNA (estimated size: 1.02 MDa) that migrated between the 1.4 and 0.6 MDa dsRNAs was especially prominent in extracts from BMV-P infected plants but barely visible in corresponding extracts from BMV-N infected plants. Failure of deoxyribonuclease I (10 μg/ml in 150 mM NaCl, 15 mM sodium citrate, 30 mM MgCl [pH 7.0]), ribonuclease T_1 (200 units/ml in 50 mM Tris-HCl, 10 mM EDTA [pH 8.0]), and ribonuclease A in high salt (3 μg/ml in 300 mM NaCl, 30 mM sodium citrate [pH 7.0]), but success of ribonuclease A at low salt (3 μg/ml in 15 mM NaCl, 1.5 mM sodium citrate [pH 7.0]) in hydrolyzing the material indicated its dsRNA nature (*data not shown*; 17).

Purification of BMV-P. Experimentally infected Little Club wheat and naturally infected bromegrass gave virus yields between 0.2 and 0.5 mg/g infected tissue as determined spectrophotometrically using an extinction coefficient of 5.2 (5). Purified BMV-N and BMV-P, collected from sucrose gradient zones, induced similar symptoms in diagnostic hosts to those caused by infectious plant sap. Electron microscopy of purified BMV-P showed 26-nm spherical particles with stain penetration that is characteristic of bromoviruses (Fig. 4A).

Fig. 2. Differential reactions between brome mosaic virus type isolate (BMV-N) and Portage isolate (BMV-P) mechanically inoculated to half-leaves of *Datura stramonium*.

Sucrose gradient centrifugation. The Portage isolate of BMV sedimented as a major peak in 10–40% sucrose gradients (Fig. 4B); a smaller, faster-sedimenting peak, when examined by electron microscopy, was found to contain partially swollen particles. Sedimentation was compared with that of top, middle, and bottom components of tobacco ringspot nepovirus (TRSV) sedimenting in similar sucrose gradients in the same centrifugation runs, and the sedimentation coefficient, $s_{20,w}$ (uncorrected for infinite dilution), was estimated as 85 ± 1 S (mean of three runs).

Coat protein subunit molecular weight. In SDS-polyacrylamide gels (Fig. 4C), BMV-N and BMV-P virion protein subunits migrated at the same rate. Mixtures of preparations of virion protein subunits made from the two isolates likewise migrated as a single entity with an estimated molecular weight of 20.2 kDa.

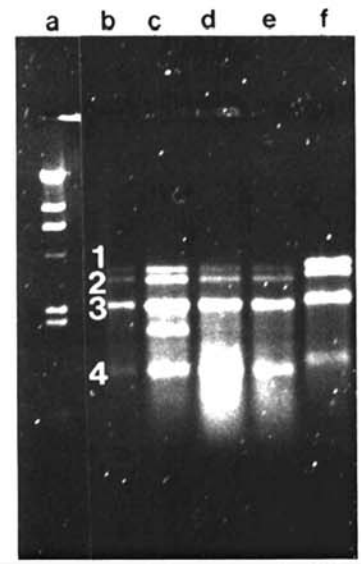

Fig. 3. Agarose gel electrophoresis of dsRNA from Black Hulless barley infected with brome mosaic virus type isolates (BMV-N) and Portage isolate (BMV-P). **(A)** Hind III digest of lambda phage dsDNA (size standard) in order of decreasing size: 15.0, 6.1, 4.3, 2.9, 1.5, 1.3, and 0.35 MDa. **(B)** BMV-N53 isolate dsRNA: 2.2, 2.1, 1.4, 0.6 MDa. **(C)** BMV-P isolate dsRNA: 2.2, 2.1, 1.4, 1.0, 0.6 MDa. **(D)** BMV-N315 isolate dsRNA: 2.2, 2.1, 1.4, 0.6 MDa. **(E)** BMV-N422 isolate dsRNA: 2.2, 2.1, 1.4, 0.6 MDa. **(F)** Cucumber mosaic virus dsRNA: 2.6, 2.2, 1.6, 0.66 MDa. Electrophoresis for 2 hr at 40 V. Gel was stained with ethidium bromide at 10 μg/ml.

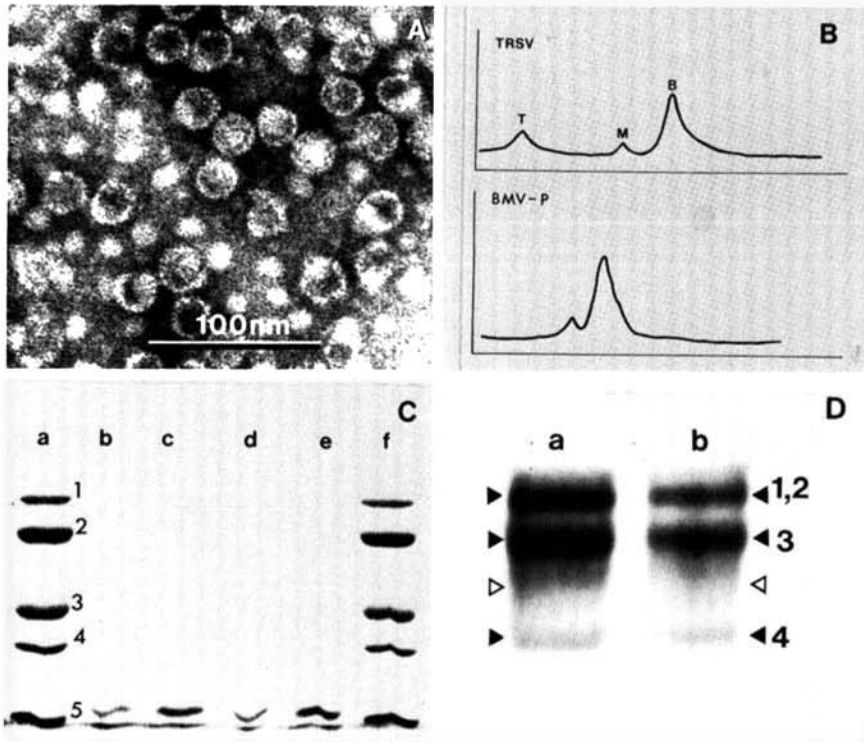

Fig. 4. Properties of virus particles of brome mosaic virus (BMV). **(A)** Electron micrograph of leaf dip of field specimen of wheat infected with Portage isolate of BMV stained and washed with 0.2% phosphotungstate (pH 7.2) and examined at 80 kV. **(B)** Sedimentation in 10–40% sucrose gradients. Upper panel, tobacco ringspot nepovirus (TRSV): top (T), 53 S; middle (M), 91 S; bottom (B), 126 S. Lower panel, Portage isolate of bromegrass mosaic bromovirus (BMV-P). **(C)** SDS-PAGE of BMV virion protein subunits. Lane a, protein size standards, 20 μg total protein: 1 = phosphorylase-b, 94 kDa; 2 = bovine serum albumin, 67 kDa; 3 = ovalbumin, 43 kDa; 4 = carbonic anhydrase, 30 kDa; 5 = β-lactoglobulin, 18.4 kDa. Lane b, BMV-N, 1 μg. Lane c, BMV-N + BMV-P, 2 μg. Lane d, BVM-N + BMV-P, 1 μg. Lane e, BMV-P, 2 μg. Lane f, protein size standards, 20 μg total protein. **(D)** Agarose gel electrophoresis of RNA extracted from purified virions. Lane a, BMV-N isolate. Lane b, BMV-P isolate. Each solid arrow indicates one or two bands corresponding to single-stranded (ss) RNAs: 1 = 1.1 MDa, 2 = 1.0 MDa, 3 = 0.7 MDa, 4 = 0.3 MDa. Open arrows indicate position of the ssRNA species (0.56 MDa), not detected, that would correspond to the additional double-stranded (ds) RNA migrating between dsRNA-3 and dsRNA-4 in lane c of Figure 3.

Virion RNA molecular weight. Four ssRNAs from purified virions of BMV-P comigrated in nondenaturing submarine agarose gels with those from BMV-N (Fig. 4D).

Double-diffusion serology. Reciprocal double diffusion in agar gel, using antisera prepared to either BMV-P or BMV-N against purified virions, produced confluent precipitin bands in reaction with either antigen (Fig. 5), indicating serological identity of the two virus isolates.

DISCUSSION

The virus isolated from spring wheat at Portage la Prairie, Manitoba, is BMV. To date, BMV-P has been found only in or near experimental plots at Portage la Prairie and Glenlea. The Manitoba isolates are indistinguishable from the Nebraska type isolate, except that in our tests the Manitoba isolates induced ring spot lesions on *D. stramonium* whereas the Nebraska type isolate did not. Extensive cereal virus surveys of the Canadian prairies in 1985 and 1987 (S. Haber, *unpublished*) failed to detect BMV at other locations. We have also not found BMV in bromegrass in other parts of eastern Manitoba and in barley and wheat fields near the Portage la Prairie and Glenlea plots. This suggests that BMV-P is a recent introduction rather than an endemic disease.

The infected spring wheat breeding plots from which BMV-P was first isolated showed visible damage. Although BMV is widespread in the world's cereal-growing regions, it generally does not cause economic losses (4,6). Recently, however, it has been shown that BMV may mimic barley yellow dwarf virus, a virus that causes considerable losses (16). Our field observations and greenhouse tests suggest that early infection with BMV reduces growth and seed yield per plant in certain cultivars of wheat, barley, and oats. It is unlikely, however, that BMV-P threatens commercial cereal cultivation in Manitoba. We have been unable to transmit BMV-P using the most common cereal aphids in Manitoba, the English grain aphid (*Sitobion avenae* Fabr.) and the oat-bird cherry aphid (*Rhopalosiphum padi* L.) (*data not shown*). This is in contrast with the reports of aphid transmissibility of BMV by von Wechmar and Rybicki (16). The pattern of infected plants in experimental plots is consistent with mechanical transmission (e.g., mowing and movement of implements). At present, the principal concern is that infection with BMV-P may confound evaluation of breeding plots (A. McKendrie, *personal communication*).

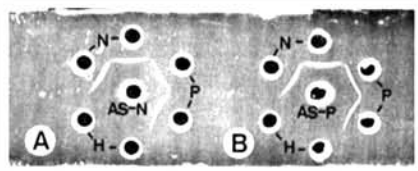

Fig. 5. Agar gel immunodiffusion. Central wells contain undiluted rabbit polyclonal antiserum made against purified brome mosaic virus (BMV) preparations. Peripheral wells loaded with purified virus (0.25 mg/ml) of BMV type isolate (N), Portage isolate (P), or extract of healthy plant sap (H). **(A)** Antiserum to BMV type isolate (AS-N). **(B)** Antiserum to Portage isolate of BMV (AS-P).

Distinct isolates of BMV have been obtained from *Commelina diffusa* Burm. and *C. communis* L. grown together in a contiguous lawn of perennial grasses in Arkansas (15). These Arkansas isolates were serologically indistinguishable from type BMV, but produced different symptoms in several dicotyledonous hosts. One of these BMV isolates (designated BMV-2) induced necrotic ringspots on *D. stramonium* similar to those induced by BMV-P. All BMV isolates collected in Manitoba induced only ring spot lesions in *D. stramonium*. The fifth dsRNA species, especially prominent in extracts from BMV-P infected plants, may correspond to the 0.56 MDa minor ssRNA found occasionally in BMV ssRNA preparations (7,11).

Seed transmission of BMV has been reported from southern Africa (13). We have failed to find BMV in excised seed embryos by inoculation tests or serology, but have occasionally observed transmission to as many as 40% of seedlings grown from batches of 200 seeds obtained by hand-threshing mature heads of BMV-infected plants (*data not shown*). Because we routinely recover virus from chaff and seed coats of mature infected barley or wheat plants, the observed virus transmission to seedlings probably occurs by mechanical inoculation. Such transmission, even if intermittent or inefficient, might account for the introduction into Manitoba of a distinct BMV strain.

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