

## Poa Semilalent Virus from Native Grasses

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### ABSTRACT

*Poa semilalent virus* (PSLV), discovered in *Poa palustris* and *Agropyron trachycaulum* from Alberta, Canada, caused mosaic, chlorosis, and blight on wheat (*Triticum aestivum*) and oats (*Avena sativa*), but only a mild transitory mottling on *P. palustris*. Other hosts include *Agropyron cristatum*, *Alopecurus aequalis*, *Avena* (16 spp), *Elymus canadensis*, *Hordeum marinum*, *H. vulgare* (11 cultivars), *Lolium multiflorum*, *Phleum pratense*, *Poa compressa*, *Secale cereale*, *Triticum durum*, and *Zea mays*.

The original isolate killed most wheat plants 2-3 weeks after inoculation. Nonlethal isolates were segregated from the lethal isolate, but when recombined in transmission tests, they suppressed the lethal effects of the latter on wheat.

Wheat and oat plants became infected when grown in pots of soil with naturally infected *P. palustris*. No infection occurred in tests for soil and seed transmission.

Highest infectivity and serological titers were obtained with juice from leaves that were 50% chlorotic. The virus was infectious in wheat juice at pH 4 to 10. Heat inactivation occurred between 65 and 67.5 C. Infectivity was lost in 2-4 months in juice and undried leaves stored in vials at 20, 5, or -15 C, but was undiminished after 1 year in leaves stored over CaCl<sub>2</sub> at -15 C.

Particles in dip preparations from plants infected with PSLV were stiff rods 25 nm in diam and 125-225 nm long, with a mean normal length of 162 nm, compared to 133 nm for barley stripe mosaic virus (BSMV). An antiserum prepared for PSLV had a precipitation dilution end point of 1/8,192 against PSLV, and 1/64 against BSMV. Absorption of either antiserum with the heterologous virus did not reduce its titer against the homologous virus.

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A virus that caused chlorotic mottling, blight, and death of inoculated plants of wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), and some other Gramineae, was isolated from *Poa palustris* L. found with mosaic symptoms near Nobleford in southern Alberta in 1966 (7). Subsequent tests showed that the mosaic symptoms observed on the original *P. palustris* plants were caused by *Hordeum mosaic virus* (8) and a virus resembling oat necrotic mottle (2), which were also present. The virus which caused the severe mottling and necrosis on wheat and oats caused only a mild mottling on inoculated *P. palustris* plants, but later these plants became symptomless carriers. Although the virus resembles barley stripe mosaic virus (BSMV) more than other known viruses on grasses, it appears to be distinct, and is designated "Poa semilalent virus" (PSLV).

This virus was subsequently isolated from *Agropyron trachycaulum* L. associated with infected *P. palustris* at the original location, and from one symptomless *P. palustris* plant collected near Portage Mountain, Alberta, (56° 03' N; 121° 55' W). The latter was the only infected plant from 20 *P. palustris* and 20 *A. trachycaulum* plants supplied by W. L. Pringle, Research Station, Canada Department of Agriculture, Beaverlodge, Alberta, in October 1969 from a collection of native plants from northwestern Alberta and northeastern British Columbia.

Some pathological and physical characteristics of PSLV are reported here.

**MATERIALS AND METHODS.**—Shoots from infected *P. palustris* and *A. trachycaulum* plants collected in the field were transplanted into pots of

soil and kept as original source material. PSLV was isolated from these plants by grinding about 1 g of leaves with 4 ml of water, adding Celite, and rubbing on leaves of Kent wheat or Clintland 60 oats. Although these hosts were susceptible at all stages from first leaf to stooling, they were normally inoculated in the two- to three-leaf stage. The inoculated plants were kept in a greenhouse at 18-25 C. Isolates of the virus, selected according to symptoms on wheat, were maintained in *P. palustris* plants, but the latter became symptomless carriers with very low virus titer. The virus from such plants was multiplied on wheat or oats before use in infection tests or for purification. The severe isolates killed wheat so quickly that they could be perpetuated on wheat only if frequently transmitted to new plants.

As most diseased wheat and oat plants developed extensive necrosis as well as chlorosis, disease severity was indicated by adding a 0-10 rating for degree of necrosis of leaves to a 0-5 rating for degree of chlorosis of areas that were not necrotic; thus, plants that were 50% necrotic, and the remaining tissues 50% chlorotic, would be rated 5 + 2.5 = 7.5.

Partially purified preparations of the severe isolate were obtained from Clintland 60 oats; and of the mild isolate, from Kent wheat. About 100 g leaves collected from diseased plants 10-12 days after inoculation were frozen overnight at -15 C, then thawed at about 5 C and ground with a food grinder, and the juice was squeezed from the pulp. One ml of 1.0 M phosphate buffer at pH 7.0 was added to each 9 ml juice. To each volume of this extract, 0.5

volume of a 2:1 mixture of chloroform and *n*-amyl alcohol was added while stirring (6). Stirring was continued for 15 min. The emulsion was centrifuged at about 3,000 g for 15 min. The aqueous supernatant was drawn off and recentrifuged. The supernatant from this was mixed with 0.5 volume of a saturated solution of  $(\text{NH}_4)_2\text{SO}_4$ , left undisturbed for 15 min, then centrifuged at about 35,000 g for 20 min. The precipitate was resuspended in distilled water equal to about one-third the volume of the original crude juice, shaken for 20 min, and centrifuged at about 3,000 g for 10 min. The supernatant fluid was centrifuged in the No. 40 rotor of the Spinco Model L centrifuge at 39,000 rpm for 1 hr. The precipitate containing the virus was resuspended in 2 ml of 0.01 M phosphate buffer at pH 7.0 with 0.85% NaCl (PBS), shaken for 20 min, then centrifuged at 3,000 g for 10 min to remove aggregated constituents.

BSMV for preparing an antiserum was partially purified from juice from 75 g of wheat leaves by heating, differential centrifugation, and density gradient centrifugation (3). The final precipitate was resuspended in 2 ml of PBS.

To prepare antisera, 1.5-ml portions of virus concentrates prepared as above were emulsified with an equal volume of Freund's incomplete adjuvant, and half of each preparation was injected into the muscle of each thigh of a rabbit. Each rabbit received a minimum of three injections at weekly intervals and an additional injection after 1 month.

The microprecipitin test (9) was generally used to determine serological reactions. Plant juice was clarified for serological tests by heating at 45 C for 10 min, then centrifuging at about 3,000 g for 15 min. Partially purified concentrates were prepared from clarified juice by centrifuging in a No. 30 rotor at 29,000 rpm for 1 hr, then resuspending the precipitate in 1 ml of PBS for each 10 ml of original plant juice (= x 10 concentrate), or 1 ml for each 40 ml of juice (= x 40 concentrate). For antiserum absorption tests, 0.5-ml portions of each antiserum were mixed with 1.5 ml of x 40 concentrates from virus infected or healthy plants. These proportions provided about x 2 the  $\alpha$ -optimal proportional amount of virus in the homologous mixtures (4). The mixtures were incubated in a water-bath at 40 C for 2 hr, kept overnight at +5 C, then centrifuged at 3,000 g for 10 min to remove antigen-antibody precipitates. The precipitation titers were then determined for the absorbed and nonabsorbed sera against the homologous and heterologous viruses. The titers of antisera and virus preparations are expressed as the reciprocals of the dilution end points in precipitin tests.

**RESULTS.—Symptoms.**—Six to 10 days after wheat was inoculated with a severe isolate of PSLV, a light-yellow to white chlorosis developed in large areas and sometimes over the entire area of the inoculated leaves, and the leaves drooped (Fig. 1). Usually, the chlorosis developed most rapidly from the leaf tips. Sometimes it occurred first in the middle of inoculated leaves while the tips were entirely green, or green at the margins part way down

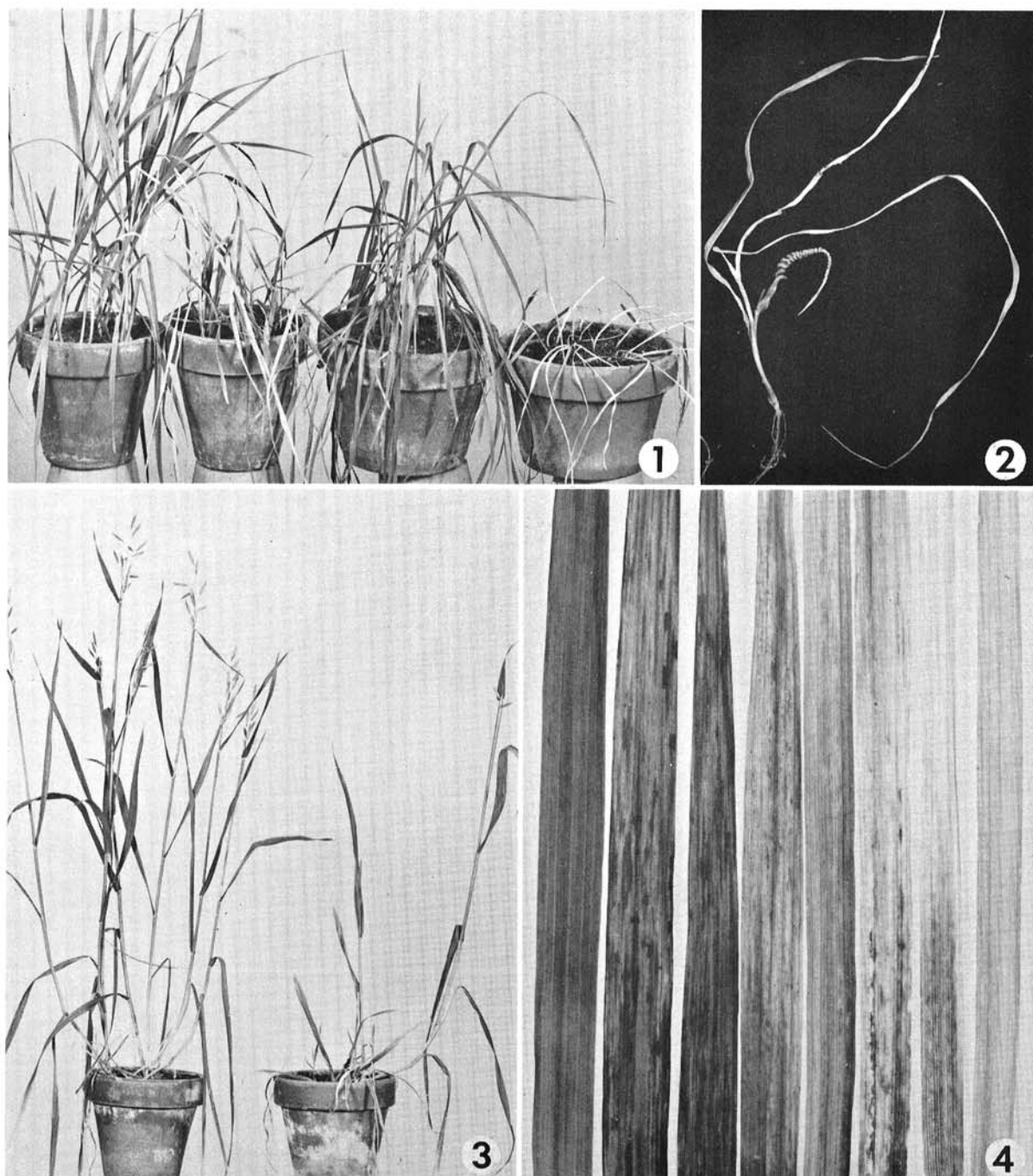
from the tip. The chlorotic tissue became flaccid, then desiccated, resulting in drooping and curling of affected portions. New leaves were usually crinkled and twisted (Fig. 2), and sometimes became entirely blighted when partially emerged. The chlorosis and death of tissue usually extended rapidly, and the plants died 10-15 days after inoculation. Wheat plants inoculated with milder isolates developed less extensive areas of chlorosis and less necrosis. These survived for several months in the greenhouse, stunted but with a mild chlorotic mottling.

Infected Clintland 60 oats developed chlorotic and necrotic symptoms similar to those on wheat (Fig. 1), except that sometimes the chlorotic areas appeared water-soaked. Fewer oat than wheat plants died because new tillers usually began to develop before the primary shoots died. The new tillers sometimes developed vigorously and without symptoms (Fig. 3), but usually some of the leaves became chlorotic from the tips downward, resembling plants infected with barley yellow dwarf virus, or they became mottled (Fig. 4).

Symptoms developed on less than 50%, and sometimes on none of the *P. palustris* seedlings inoculated manually. The symptoms included a mild mottling which developed in 10-14 days. After a few weeks, most or all of the infected plants were symptomless, but the virus could still be transmitted from them to wheat or oats.

**Hosts.**—Additional grasses, some plants of which developed chlorotic mottling and sometimes necrosis after manual inoculation with PSLV, and from which the virus was retransmitted to Kent wheat or Clintland 60 oats, include the following: *Agropyron trachycaulum* (Link) Malte; *A. cristatum* (L.) Gaertn.; *Alopecurus aequalis* Sobol.; *Avena abyssinica* (C.D.455, E28, 68-13); *A. barbata* (C.W.186.1, RL.1211, Israel); *A. brevis* Roth. (C.D.813, C.D.7848); *A. byzantina* C. Koch (C.W.553-5, Med. 147, Landhafer); *A. clauda* (one sample); *A. fatua* (C.D.8464, Regina sample); *A. hirtula* (C.I.3078, C.W.194); *A. longiglumis* (C.D.8029); *A. nudibrevis* (C.D.1124); *A. pilosa* (one sample); *A. sativa* L. (23 cultivars); *A. sterilis* L. (C.D.7948, S.F.63, C.W.486.2, Sterisel); *A. strigosa* Schreb. (C.D.3820, 4748); *A. tetras* (T.22, T3-1-5, T.1269-2); *A. ventricosa* (one sample); *A. wiestii* (C.D.6231); *Elymus canadensis* L.; *Dactylis glomerata* L.; *Hordeum marinum* Huds.; *H. vulgare* L. (11 cultivars); *Lolium multiflorum* Lam. (S22); *Phleum pratense* L.; *Poa compressa* L.; *Secale cereale* L. (Crown, Dominant, Select II, Sangaste, Slopske, Tetra Petkus); *Triticum durum* Desf. (Ramsey); *T. aestivum* L. (Spring types: Fortunata, Selkirk; winter types: Capelle des Prez, Genesee, Minter, Nebred, Richmond, Winalta); and *Zea mays* L. (Golden Cross Bantam).

Other grass species that were inoculated but did not develop symptoms include: *Agropyron intermedium* (Host) Beauv. (one sample); *A. repens* (L.) Beauv. (two samples); *Agrostis alba* L. (14 samples); *A. borealis* Hartm. (1 sample); *A. canina* L. (one sample); *A. palustris* Huds. (two samples); *A. stolo-*



**Fig. 1-4.** 1) Healthy and *Poa semilatifolia*, virus-infected Clintland 60 oats (left) and Kent wheat (right) 11 days after inoculation. Note the severe stunting, chlorosis, and blight of leaves of the inoculated oats, and death of most of the wheat plants. 2) A Kent wheat plant infected with *Poa semilatifolia* virus showing all leaves dead except the youngest, which is crinkled and twisted spirally. 3) Clintland 60 oats, healthy on the left, infected with *Poa semilatifolia* virus on the right, showing the death of all the early tillers, but the survival and almost normal growth of some of the younger tillers. 4) Leaves of Clintland 60 oats showing a healthy leaf and a range of mosaic, chlorosis, and necrosis symptoms caused by *Poa semilatifolia* virus.



*nifera* L. (7 samples); *A. tenuis* Sibth. (two samples); *Bouteloua gracilis* (H.B.K.) Lag. (one sample); *Bromus inermis* Leyss (one sample); *Festuca rubra* L. (one sample); *Hordeum jubatum* L. (four samples); and *Lolium perenne* L. (one sample).

*Differentiation of mild from severe strains.*—When the severe or blight-inducing isolates of PSLV were propagated in wheat, transmission to new plants was done from selected plants that were developing the blight syndrome of extensive chlorosis and drooping prior to death. Most of the wheat plants inoculated with the selected inoculum developed the blight syndrome, but some plants developed chlorosis and mosaic without killing the plants. Transmission from such plants induced a similar, relatively mild syndrome of symptoms. The milder, nonlethal isolates obtained in this way appeared to be variants from or components of the lethal isolate.

*Interaction of mixtures of mild isolates.*—To determine if the blight resulting in death of wheat could result from a synergistic interaction between two or more nonlethal isolates, the effects of combinations of different nonlethal isolates were tested. Wheat plants were inoculated with 6 nonlethal isolates separately and in combinations of two or three in mixed inoculum. The nonlethal isolates separately caused a range of symptoms including mottling, yellowing, and crinkling of leaves. Some caused moderate necrosis, but none caused death of plants during the first 2 weeks. None of the mixtures caused symptoms comparable with the severe or lethal strains from which they originated. Instead, the various combinations of isolates caused milder symptoms than were caused by the more severe components of each mixture alone.

*Interactions of severe and mild isolates.*—When Kent wheat and Clintland 60 oats were inoculated with a mild or nonlethal isolate (PSLV-M) or a severe or lethal isolate (PSLV-S) mixed with water or combined in a 1:1 ratio, the ratings for disease development, on a 1-10 scale, were as follows in a typical experiment: PSLV-M + water, 5.0 on wheat, 2.0 on oats; PSLV-S + water, 8.9 on wheat, 4.7 on oats; and PSLV-M + PSLV-S, 4.5 on wheat, 2.0 on oats.

The above and similar results with mixtures of other mild and severe isolates show that the milder isolate in each mixture suppressed the severe isolate.

Cross protection tests were done by inoculating Kent wheat with PSLV-M at times ranging from 4 days to immediately before inoculating with PSLV-S. Plants inoculated with PSLV-M before inoculating with PSLV-S developed less necrosis; hence, had milder disease ratings than comparable plants inoculated with PSLV-S alone, indicating that the milder isolates either protected plants from infection by the severe isolate or reduced its effects on wheat.

*Tests for natural means of transmission.*—PSLV has been found only at the two locations, but its occurrences in a number of plants of both *P. palustris* and *A. trachycaulum* show that some means of spread has operated in the field. Spread in the local area could have resulted merely from physical contact

including intermingling of roots. Confirmation that plants could become infected by contact with diseased plants was obtained by sowing Kent wheat and Clintland 60 oats in pots of soil with diseased *P. palustris* and *A. trachycaulum* transplanted from the field. Two of 69 wheat plants and 4 of 70 oat plants developed symptoms and were shown by manual transmission tests to be infected with PSLV. However, no test plants have become infected when grown in similar association with *P. palustris*, wheat, or oats infected by manual inoculation. Lack of transmission in this instance could be attributed to the absence of a transmitting agent that was present on diseased plants from the field.

Tests for infection from soil were made by sowing wheat, oats, *P. palustris*, and *A. trachycaulum* in soil obtained from around diseased plants at the site where the disease was found. Some of the plants were grown in the greenhouse at temperatures of 18-25 C, others were grown in rooms at temperatures of 15, 20, 25, and 30 C, with about 1,500 ft-c of light 16 hr/day for 2 months. No symptoms developed on any of the test plants.

No infection was detected, visually or by transmission tests, in about 200 *P. palustris* plants grown from seeds collected from naturally infected plants. Seeds were also collected from Clintland 60 oats infected artificially with PSLV and grown to maturity in the greenhouse. The seeds were poorly developed because of the severe effects of the disease on the plants, and only 6 plants grew from about 60 seeds planted. The virus was not detected in any of these plants.

*Infectivity and serological titers of juice from diseased plants.*—Inoculating with 1:4 dilutions of juice from leaves with chlorotic blotching or mosaic usually infected all wheat or oat test plants. Tests with leaves selected for comparable degrees of chlorosis and necrosis at different stages of disease development showed lower serological and infectivity titers for the isolates that killed the plants than for those causing the least necrosis. The dilution end point for infectivity was 1/256 for a severe isolate, and 1/1,024 for a mild isolate in leaves that were about 50% chlorotic 7-9 days after inoculation. Higher levels of infectivity (50% infection at a dilution of 1/256) occurred in older leaves with 50% chlorosis 10-15 days after inoculation than in younger leaves with only 10% chlorosis and no necrosis 5 days after inoculation. The highest serological titers in precipitin tests, 1/256 for the mild and 1/128 for the severe isolates, were also obtained from the older leaves that were 50% chlorotic. Both infectivity and serological titers were usually lower in roots than in leaves of the same plants.

*Effects of supplements on infectivity of inoculum.*—Results using 0.2 M borate buffer at pH 7.3, 0.5 M phosphate buffer at pH 7.0, or 0.1% Na<sub>2</sub>SO<sub>3</sub> were about equal to results with water as the diluting agent for inoculum from leaves. No infection developed on plants inoculated with a preparation including bentonite at the rate of 51.4 mg/ml of water.

*Leaf turgidity and light exposure in relation to plant susceptibility.*—Wheat and oat plants in the two- to three-leaf stage were given different water and light treatments prior to manual inoculation, including the withholding of water for 3 days to cause wilting, exposure in a mist chamber for 24 hr to induce maximum leaf turgidity, and retention in a dark room for 18 hr. None of these treatments caused a significant change in the susceptibility of plants.

*Effects of pH on infectivity.*—The pH of juice from oat plants infected with PSLV was usually between 5.8 and 6.2. When the pH of juice was adjusted downward with 0.5 N HCl or upward with 1.0 N KOH and tested for infectivity, infection resulted from juice at values ranging from pH 4.5 to 10.0, but not at pH 4.0. Wheat juice was infectious from pH 4.0 to 10.0.

*Effect of heat on infectivity and serological reaction.*—Infectivity of juice from diseased wheat was reduced by heating for 10 min at 60, 62.5, or 65 C, and was destroyed at 67.5 C or higher. Juice heated at 40 C for 10 min before cooling and centrifuging at 3,000 g was not so clear as juice heated at higher temperatures, but the serological titer was 1/128 as compared with 1/64 after treatment at 45 C, 1/32 at 50 and 55 C, and 1/16 at 60 C. No serological activity was detected in juice heated at 65 or 70 C.

*Stability of infectivity in juice and leaves stored at different temperatures.*—Undiluted juice from infected oats stored in 1-ml portions in 5-ml vials retained a low level of infectivity for 2 months, but was not infective after 4 months at 20, 5, or -15 C. Similarly, 1-g portions of leaf pieces stored undried in a capped vial at 20 C retained a low level of infectivity after 2 months, but were not infective after 4 months. The infectivity of leaf pieces placed in unsealed envelopes and stored over CaCl<sub>2</sub> in sealed jars was reduced to about half the original after 1 year at 20 C, but was undiminished after 1 year at -15 C.

*Particle measurements.*—Rod-shaped particles about 25 nm in diam by 50-600 nm long were found in shadowed dip preparations from wheat, oats, and *P. palustris* infected with PSLV. Measurements of 100 particles in three samples each of a severe and a mild isolate, prepared from wheat about 10 days after inoculation, showed that most particles were 125-225 nm long. The mean lengths of particles in this range were 163 nm for the severe isolate and 161 nm for the mild isolate. An isolate of barley stripe mosaic virus (BSMV) from Compana barley was similarly multiplied in wheat, and 100 particles from each of three preparations were measured by the same procedures. The particles were mainly 100-200 nm long. The means for this range were 133 nm. These measurements indicate that particles of PSLV are 20-22% longer than the particles of this strain of BSMV, but similar in length to particles of wheat (soil-borne) mosaic virus (1).

*Serological specificity of PSLV.*—An antiserum developed by intramuscular injections of a rabbit with partially purified preparations of PSLV had an

end point of 1/8,192 in a twofold dilution series tested by the microprecipitin method. This antiserum also reacted with BSMV, but only in dilutions up to 1/64. An antiserum for BSMV with a dilution end point of 1/2,048 against BSMV had a dilution end point of 1/16 against PSLV. These reactions were not changed by absorption of the antisera with healthy plant constituents. After absorption with the homologous virus, neither antiserum reacted with either virus, even at the lowest dilution tested (1/4). However, cross-absorption of each with the heterologous virus did not affect its titer against the homologous virus.

The above results show that although there is a low level of cross reaction between the PSLV antiserum and BSMV, and between the BSMV antiserum and PSLV, cross absorption tests do not indicate a close serological relationship between PSLV and BSMV.

**DISCUSSION.**—To date, PSLV has been isolated from only two locations in Alberta, but this does not indicate that the virus is rare. The two locations were widely separated; and in each case the hosts were symptomless carriers; hence, its discovery on them was fortuitous. There have been few attempts to find it elsewhere. It could be common but inconspicuous in native grasses in certain areas. Also, it could cause severe symptoms attributed to other causes on cereals in the field.

Particles of PSLV are similar in thickness to particles of wheat (soil-borne) mosaic virus and BSMV. In symptomatology and host range, it resembles BSMV more closely than other viruses (5). However, the lack of close serological relationship, as indicated by cross absorption tests, discourages the initial suggestion that PSLV could be a strain of BSMV; but further comparisons are necessary to determine possible relationships with the above viruses.

The partial cross reactions between the non-absorbed antisera and the heterologous viruses probably resulted because the antisera were prepared from rabbits injected 4 times with virus preparations, rather than just once as recommended for the most specific antisera (4).

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