

Spring Beauty Latent Virus: A New Member of the Bromovirus Group

R. A. Valverde

Graduate assistant, Department of Plant Pathology, University of Arkansas, Fayetteville 72701. Present address, Department of Plant Pathology, University of California, Riverside 92521.

Published with the approval of the director of the Arkansas Agricultural Experiment Station.

Accepted for publication 15 October 1984.

ABSTRACT

Valverde, R. A. 1985. Spring beauty latent virus: A new member of the bromovirus group. *Phytopathology* 75:395-398.

Spring beauty, *Claytonia virginica*, was found to be infected with a latent virus in northwest Arkansas. This virus, designated spring beauty latent virus (SBLV), was similar to the members of the bromovirus group in particle size and shape, sedimentation coefficient, molecular weight, and number of RNA components. Nevertheless, it differed in host range, molecular weight of protein coat, and serology. By using Ouchterlony

double-diffusion tests, a very distant serological relationship to cowpea chlorotic mottle virus and brome mosaic virus could be demonstrated, but no relationship to broad bean mottle virus and melandrium yellow fleck virus was evident. Because of its properties, SBLV is proposed as a new member of the bromovirus group.

Additional key words: electrophoresis, serology.

Members of the bromovirus group, such as cowpea chlorotic mottle virus (CCMV), brome mosaic virus (BMV), and broad bean mottle virus (BBMV) have similar properties and some are serologically related (9,15,16). Melandrium yellow fleck virus (MYFV) has been proposed by Hollings and Horvath as a member of this group (6), although it is not serologically related and differs in some properties.

Spring beauty, *Claytonia virginica* L. (Portulacaceae), a widespread perennial wild plant, was found to be naturally infected with a latent virus referred to here as spring beauty latent virus (SBLV). Preliminary studies indicated that this virus was serologically related to BMV and CCMV.

The purpose of this investigation was to characterize SBLV and compare it with other members of the bromovirus group.

MATERIALS AND METHODS

Plants of *C. virginica* were collected from different lawns and wooded areas in northwest Arkansas and tested for the presence of SBLV by mechanical inoculation of a series of indicator plants. Symptomless flowers from infected spring beauty plants were ground in sodium phosphate buffer, pH 7.0, and mechanically inoculated to *Gomphrena globosa* L., a local lesion and systemic host for this virus. After three single local lesion transfers, the virus was mechanically inoculated to different plant species.

SBLV was purified from infected *G. globosa*. Infected tissue was harvested 3 wk after inoculation and stored at -20 C. Procedures commonly utilized for purification of other bromoviruses (8,16) were not satisfactory. Infectivity of sap extracts as well as purified virus preparations was greater when sodium phosphate buffer, pH 7.0, was used. Frozen tissue was homogenized in a Waring blender with 1 ml of 0.02 M sodium phosphate buffer, pH 7.0, and 0.5 ml of chloroform-butanol (1:1) per gram of tissue. The extract was stored overnight at 4 C and centrifuged at 5,000 g for 10 min. The aqueous phase was then centrifuged at 80,000 g for 90 min. Pellets were suspended in 0.01 M sodium phosphate buffer, pH 7.0, and centrifuged in a Beckman SW-27 rotor at 25,000 rpm for 150 min in

0.2-0.7 M sucrose gradients prepared in the same buffer. The band containing the virus was drawn off with a syringe and the virus was pelleted by centrifuging at 80,000 g for 90 min. The final pellets were suspended in 0.01 M sodium phosphate buffer.

The sedimentation coefficient was determined by centrifuging purified virus (5 mg/ml) at 32,000 rpm in the An-D rotor in a Beckman analytical ultracentrifuge with schlieren optics. The S value was calculated by the graphical method of Markham (11) and was based on the average of six runs.

Molecular weight of the coat protein was determined after eight trials by electrophoresis in 7.5% SDS-polyacrylamide gels in a vertical slab gel apparatus (10). Standards for molecular weight determinations were chymotrypsin (25,700) (18) and the coat proteins of BMV (20,000) (4), CCMV (19,200) (1), cucumber mosaic virus (24,500) (5), and *Pseudoplusia includens* densovirus (87,000, 64,000, 54,000, and 46,500) (Yu-Chan Chao, *personal communication*).

Ribonucleic acid was extracted from purified SBLV with SDS-NaClO₄ (19), denatured using glyoxal and analyzed by electrophoresis in 1% agarose gel (12) in a horizontal slab-gel apparatus. Standards used were RNAs from BMV (1.19, 1.07, 0.80, and 0.27 × 10⁶) (13), CCMV (1.21, 1.06, 0.79, and 0.25 × 10⁶) (13), BBMV (1.10, 1.03, 0.90, and 0.36 × 10⁶) (9), and southern bean mosaic virus (1.4 × 10⁶) (2). Gels were scanned in an ISCO model 659 gel scanner at 580 nm after staining with toluidine blue O. The molecular weights of SBLV RNAs were also calculated by electrophoresis in 2.9% polyacrylamide cylindrical gels under non-denaturing conditions by using Lane's method (7). Molecular weights were obtained from the average of 10 runs.

Purified virus was stained with 2% uranyl acetate, placed on a Formvar-coated grid, and viewed with the electron microscope.

Antiserum for SBLV was prepared as described previously (17). Ouchterlony double-diffusion tests were performed with 0.9% agarose in 0.01 M sodium phosphate buffer, pH 7.0, and some trials at pH 6.0. Antisera for CCMV, BMV, BBMV, and SBLV were reacted with all four antigens which consisted of purified virus (2.5 mg/ml) and crude sap from infected plants. MYFV and SBLV antisera also were reacted with their respective antigens. Antiserum and antigen to MYFV were kindly supplied by A. A. Brunt.

RESULTS

SBLV was isolated from 14 of 16 plants of *C. virginica* collected randomly from different lawns and from three of 18 plants from wooded areas. Infected plants were indistinguishable

TABLE 1. Plant species susceptible to spring beauty latent virus (SBLV) by mechanical inoculation^a

Plant species	Symptoms ^b
<i>Chenopodium quinoa</i>	LLn
<i>C. album</i>	LLn
<i>C. amaranticolor</i>	LLn
<i>Cucumis sativus</i> 'Model'	Chll
<i>Cucurbita pepo</i> 'Small Sugar'	LLn
<i>Commelina diffusa</i>	Smo
<i>Datura stramonium</i>	LLn
<i>Glycine max</i> 'Lee' and 'Bragg'	LLn
<i>Gomphrena globosa</i>	LLn, Smo, Sn
<i>Nicotiana megalosiphon</i>	LLn, Sn
<i>N. clevelandii</i>	LLn, Sn
<i>N. debneyi</i>	LLn, Smo
<i>Pisum sativum</i> 'Little Marvel'	Smo, Sn
<i>Phaseolus vulgaris</i> 'Pinto,' 'Bountiful,' and 'Black Valentine'	LLn
<i>P. aureus</i>	LLn
<i>Vigna unguiculata</i> subsp. <i>unguiculata</i> 'Monarch'	LLn
<i>Vicia faba</i>	Sn
<i>Vinca rosea</i>	Sn, Smo

^aPlants were inoculated with sap extracted from SBLV-infected *N. megalosiphon* in 0.01 M sodium phosphate buffer, pH 7.0.

^bLLn = necrotic local lesions, Chll = chlorotic local lesions, Sn = systemic necrosis, and Smo = systemic mottle.

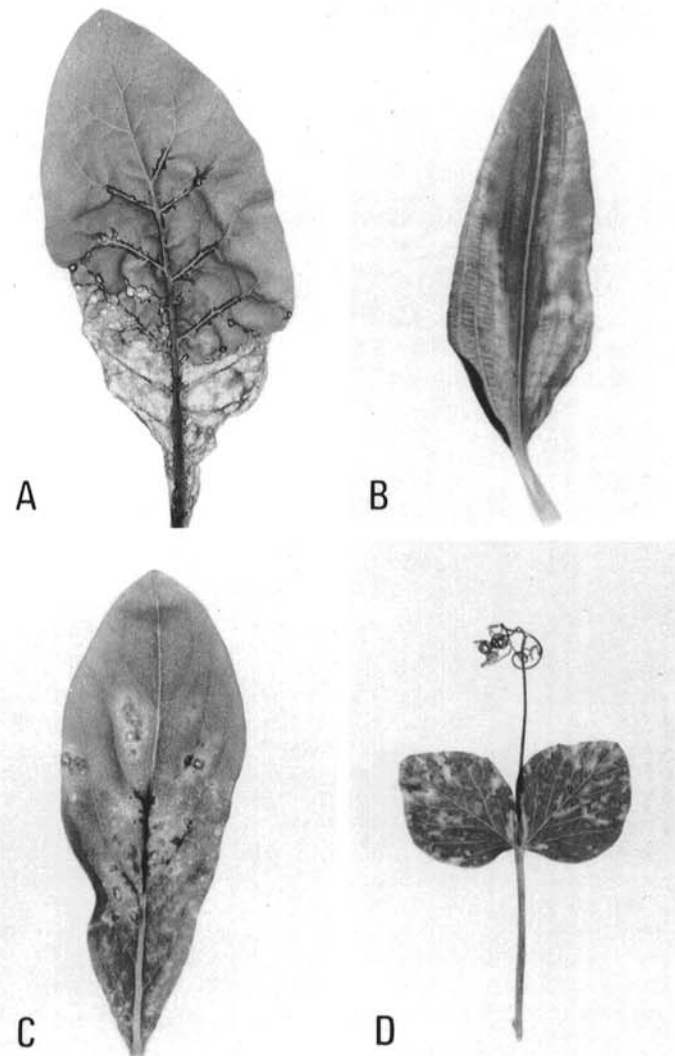


Fig. 1. Leaf symptoms of different plant species induced by spring beauty latent virus after mechanical inoculations. A, Vein necrosis on *Nicotiana megalosiphon*. B, Mottle on *Commelina diffusa*. C, Systemic necrosis on *Gomphrena globosa*. D, Mottle on *Pisum sativum*.

from healthy ones. The virus was more easily transmitted from flower tissue than from leaf tissue.

Host range. Plant species inoculated with SBLV and found to be susceptible are shown in Table 1. Symptoms on *G. globosa*, *Nicotiana megalosiphon*, *Commelina diffusa* Burm., and *Pisum sativum* L. are shown in Fig. 1. Nonsusceptible plant species were: *Capsicum annuum* L. 'California Wonder'; *Lycopersicon esculentum* Mill. 'Bonny Best'; *N. tabacum* L. 'Havana 38'; *Portulaca oleracea* L.; *Triticum aestivum* L. 'McNair'; *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'TVu 612,' 'Georgia 21,' and 'Crimson'; *Zea mays* L. 'Funk's G-4740,' and *Zinnia elegans* Jaq. Lack of infection was verified by back-inoculation to *G. globosa*.

Virus purification. One single band containing SBLV was obtained after centrifugation in a sucrose gradient. Yields of virus were approximately 10 mg/100 g of tissue.

Sedimentation coefficient. One single component was observed during ultracentrifugation with sedimentation coefficient of $90 \pm 1S$.

Protein. A single polypeptide was obtained from SBLV with a molecular weight of $22,000 \pm 500$ daltons (Fig. 2).

RNA. Four RNAs with molecular weights of 1.27, 1.13, 0.84, and 0.25×10^6 daltons were obtained from SBLV by electrophoresis under denaturing conditions. Electrophoresis of SBLV under nondenaturing conditions resulted also in four RNAs with molecular weights of 1.15, 0.89, 0.79, and 0.35×10^6 daltons (Fig. 3).

Electron microscopy. Examinations of purified virus preparations revealed icosahedral particles of approximately 28 nm in diameter (Fig. 4).

Serology. Purified SBLV reacted with antisera to SBLV, CCMV, and BMV but not with BBMV nor MYFV antisera at pH 7.0. These reactions were not observed at pH 6.0. No reaction was obtained between SBLV antiserum and CCMV, BMV, BBMV, and MYFV antigens. Serological reactions among BMV, CCMV, BBMV, and SBLV are shown in Fig. 5 (A-D). Lack of relationship between SBLV and MYFV is shown in Fig. 5 (E and F). Similar results were obtained with crude sap, and no reactions with sap extracts from healthy plants.

DISCUSSION

Properties of SBLV, such as molecular weight and number of RNAs, sedimentation coefficient, and particle morphology and

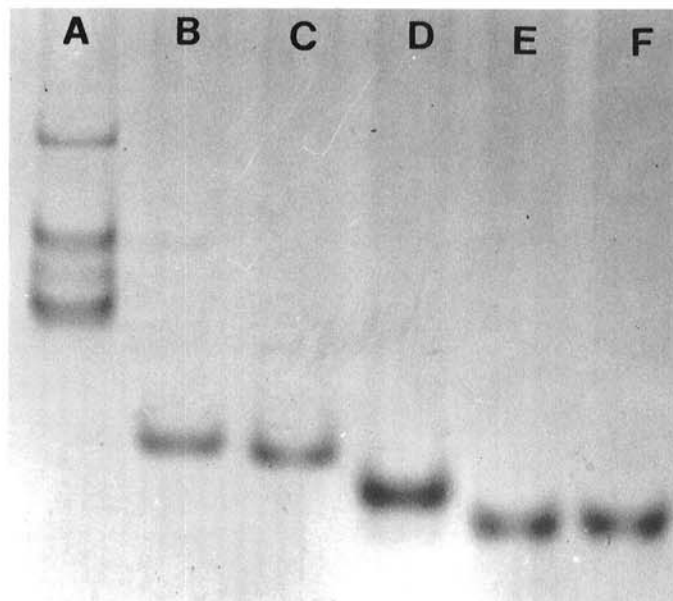


Fig. 2. Electrophoretic analysis of chymotrypsin and viral proteins in sodium dodecyl sulfate-polyacrylamide gels (7.5%): lane A, *Pseudoplusia includens* densovirus; lane B, chymotrypsin; lane C, cucumber mosaic virus; lane D, spring beauty latent virus; lane E, cowpea chlorotic mottle virus; and lane F, bromo mosaic virus.

size, are similar to those of the described bromoviruses. However, SBLV differed from three other bromoviruses in host range, molecular weight of coat protein, and serology. The molecular weight of the coat protein ($22,000 \pm 500$ daltons) was considerably higher than those reported for other bromoviruses. Despite that difference, the serological relationship between SBLV and CCMV

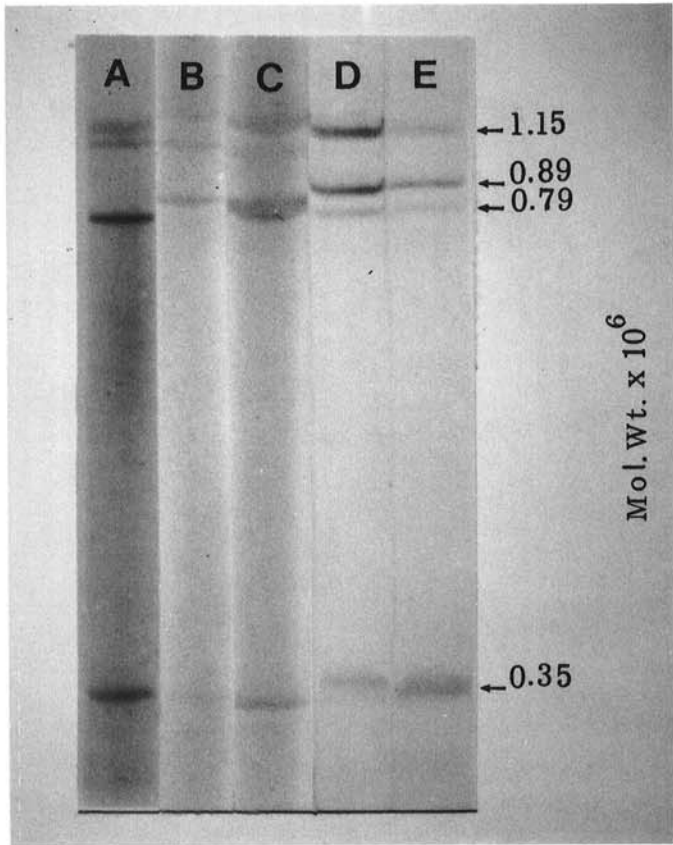


Fig. 3. Polyacrylamide gel (2.9%) electrophoresis of nondenatured ss-RNAs from: lane A, cowpea chlorotic mottle virus; lane B, broad bean mottle virus; lane C, brome mosaic virus; and lanes D and E, spring beauty latent virus.

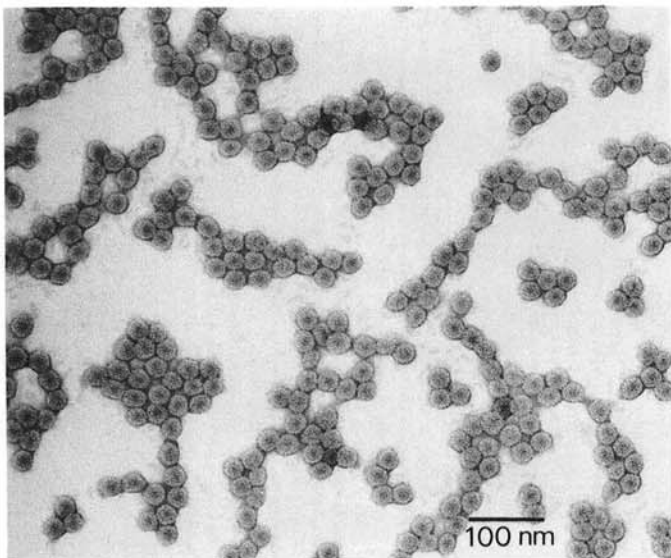


Fig. 4. Electron micrograph of purified spring beauty latent virus negatively stained with 2% uranyl acetate.

and BMV indicates protein coat similarity. Differences in molecular weight observed here may be due to differences in properties of SDS-protein complex as reported for two strains of tobacco rattle virus by Ghabrial and Lister (4). On the other hand, two members of the luteovirus group, beet western yellows virus and soybean dwarf virus, have protein coats with molecular weights of 24,000 and 22,000 daltons, respectively, and are also serologically related (3,14). The lack of serological relationship among BMV, CCMV, and SBLV at pH 6.0 suggests that the cross reactions at pH 7.0 may be due to swollen viruses. Similar results were obtained by Rybicki and Von Wechmar (15) with BMV and CCMV. The molecular weight of the four RNAs of SBLV differed slightly from those of other bromoviruses under denaturing conditions. However, when SBLV RNA was electrophoresed under nondenaturing conditions the molecular weights of the RNAs were considerably different from those of the other bromoviruses. This may be an indication of differences in the secondary structure of the RNAs. Preliminary studies on the double-stranded ribonucleic acids (dsRNAs) of the bromoviruses (R. A. Valverde and J. A. Dodds, *unpublished*) indicates that

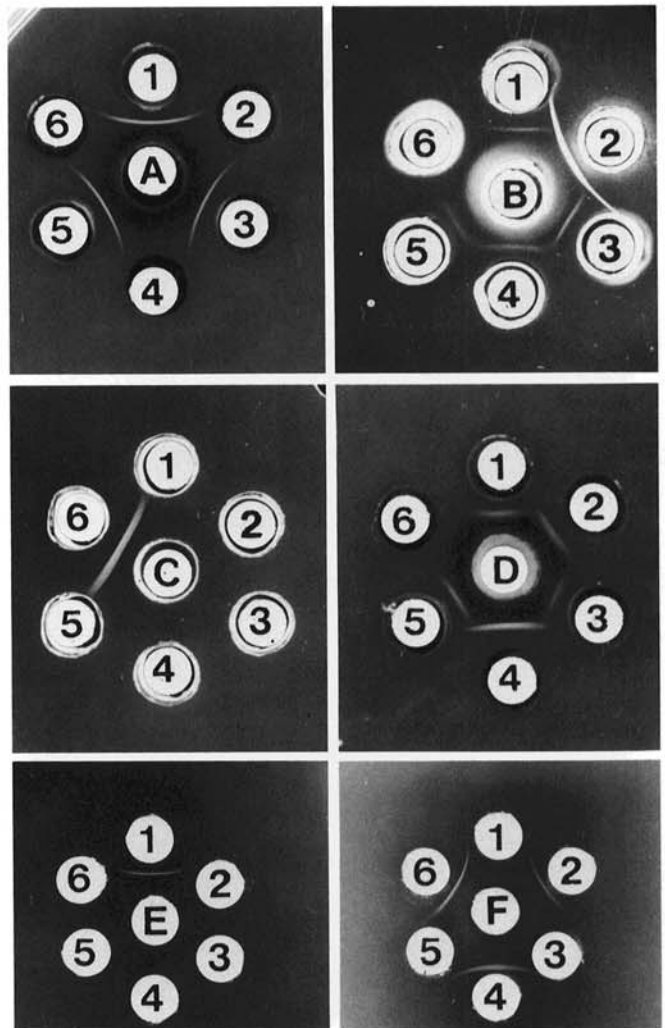


Fig. 5. Ouchterlony double-diffusion tests showing the reactions of different bromoviruses with their homologous and heterologous antisera. Gels were made of 0.9% agarose in 0.01 M sodium phosphate buffer, pH 7.0. Center wells contain antisera. **A**, Spring beauty latent virus (SBLV). **B**, Brome mosaic virus (BMV). **C**, Broad bean mottle virus (BBMV). **D**, Cowpea chlorotic mottle virus (CCMV). **E**, Melandrium yellow fleck virus (MYFV). **F**, SBLV. Peripheral wells in **A-D** contain purified virus: wells 1, 3, and 5, SBLV; well 2, BMV; well 4, CCMV; and well 6, BBMV. Wells in **E** and **F** contain sap extracts: well 1, from *Nicotiana clevelandii* infected with MYFV; wells 2, 4, and 6, from *N. clevelandii* infected with SBLV; and wells 3 and 5, healthy sap.

SBLV has four dsRNAs with molecular weights similar to those of the other bromoviruses.

The high incidence of SBLV in lawns may result from mechanical transmission during mowing. A similar dissemination mechanism has been proposed for BMV isolates infecting Gramineae and Commelinaceae in lawns in northwestern Arkansas (17). The occurrence of SBLV in wooded areas indicates that transmission by vectors, seed, or pollen may occur in nature. The known distribution of SBLV is northwestern Arkansas; however, since symptoms are not evident in infected *C. virginica*, it may be wider.

SBLV is similar to MYFV in host range, sedimentation coefficient, and molecular weight of the coat protein. However, the molecular weights of their RNAs are different, and unlike SBLV, MYFV is not serologically related to BMV or CCMV. Furthermore, no serological relationship was evident between these two viruses.

Based on the data presented, the characteristics of SBLV appear to closely resemble those of the bromoviruses, and it is proposed that this virus is a new member of this group.

LITERATURE CITED

1. Agrawal, H. O., and Tremaine, J. H. 1972. Proteins of cowpea chlorotic mottle, broad bean mottle, and brome mosaic viruses. *Virology* 47:8-20.
2. Diener, T. O. 1965. Isolation of infectious ribonucleic acid from southern bean mosaic virus. *Virology* 27:425-429.
3. Duffus, J. E. 1977. Serological relationship among beet western yellows, barley yellow dwarf, and soybean dwarf virus. *Phytopathology* 67:1197-1201.
4. Ghabrial, S. A., and Lister, R. M. 1973. Anomalies in molecular weight determinations of tobacco rattle virus protein by SDS-polyacrylamide gel electrophoresis. *Virology* 51:485-488.
5. Habili, N., and Francki, R. I. B. 1974. Comparative studies on tomato aspermy and cucumber mosaic viruses. I. Physical and chemical properties. *Virology* 57:392-401.
6. Hollings, M., and Horvath, J. 1981. Melandrium yellow fleck virus. No. 236 in: *Descriptions of Plant Viruses*. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 4 pp.
7. Lane, L. C. 1974. The components of barley stripe mosaic virus and related viruses. *Virology* 58:323-333.
8. Lane, L. C. 1977. Brome mosaic virus. No. 180 in: *Descriptions of Plant Viruses*. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 4 pp.
9. Lane, L. C. 1979. Bromovirus group. No. 215 in: *Descriptions of Plant Viruses*. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 4 pp.
10. Maizel, J. V. 1971. Polyacrylamide gel electrophoresis of viral proteins. Pages 179-246 in: *Methods in Virology*. Vol. 5. K. Maramorosh and H. Koprowski, eds. Academic Press, New York. 530 pp.
11. Markham, R. 1960. A graphical method for the rapid determination of sedimentation coefficients. *Biochem. J.* 77:516-519.
12. McMaster, G. K., and Carmichael, G. G. 1977. Analysis of single- and double-stranded nucleic acid on polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc. Nat. Acad. Sci. USA* 74:4835-4838.
13. Reijnders, L., Aalbers, A. M. J., Van Kammen, A., and Thuring, R. W. J. 1974. Molecular weights of plant viral RNAs determined by gel electrophoresis under denaturing conditions. *Virology* 60:515-521.
14. Rochow, W. F., and Duffus, J. E. 1981. Luteoviruses and yellows diseases. Pages 147-170 in: *Plant Virus Infections*. E. Kurstak, ed. Elsevier/North-Holland Biomedical Press, New York. 943 pp.
15. Rybicki, E. P., and Von Wechmar, M. B. 1981. The serology of the bromoviruses. I. Serological interrelationships of the bromoviruses. *Virology* 109:391-402.
16. Scott, H. A., and Slack, S. A. 1971. Serological relationship of brome mosaic and cowpea chlorotic mottle viruses. *Virology* 46:490-492.
17. Valverde, R. A. 1983. Brome mosaic virus isolates naturally infecting *Commelina diffusa* and *C. communis*. *Plant Dis.* 67:1194-1196.
18. Weber, K., and Osborn, M. 1969. The reliability of molecular weight determinations by dodecyl-sulfate-polyacrylamide-gel electrophoresis. *J. Biol. Chem.* 244:4406-4412.
19. Wilcockson, J., and Hull, R. 1974. The rapid isolation of plant virus RNAs using sodium perchlorate. *J. Gen. Virol.* 23:107-111.