A Satellitelike Virus Particle Associated with Maize White Line Mosaic Virus

R. E. Gingery and Raymond Louie

Research chemist and research plant pathologist, U. S. Department of Agriculture, Agricultural Research Service (USDA-ARS), and the Department of Plant Pathology, The Ohio State University (OSU), Ohio Agricultural Research and Development Center (OARDC), Wooster 44691

Cooperative investigation of USDA-ARS and OSU-OARDC.
Approved for publication as Journal Article 115-84 of OSU-OARDC, Wooster.
Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA or imply approval of it to the exclusion of other products that may also be suitable.
We gratefully acknowledge the excellent technical assistance of A. M. Gerig, J. J. Abt, and K. T. Lu.
Accepted for publication 13 March 1985 (submitted for electronic processing).

ABSTRACT

Gingery, R. E., and Louie, R. 1985. A satellitelike virus particle associated with maize white line mosaic virus. Phytopathology 75:870-874.

A satellitelike virus (SV) particle (isometric, 17 nm in diameter) was found in maize white line mosaic virus(MWLMV)-infected maize plants. The SV was serologically unrelated to MWLMV, to the satellite virus of tobacco necrosis virus, and to the satellitelike particle associated with panicum mosaic virus, but the SV was serologically related to a satellitelike particle associated with maize dwarf ringspot virus, a virus from France that is serologically related to MWLMV. The roots of all 34 MWLMV-infected plants examined also contained SV. The leaves of these plants had no particles, both particles, or only MWLMV, but never SV alone. Yields of purified SV from maize roots and leaves were usually 300–500 μg/g of tissue. The SV had a sedimentation coefficient of 48S, a buoyant density in CsCl of 1.355 g/ml, a 24.7-kdalton coat protein, and a single-stranded RNA genome with an M_r of 0.44×10^6. As with MWLMV, the SV could be transmitted to healthy maize plants only by growing the plants in soils that had previously contained SV-infected maize plants.

Maize white line mosaic virus (MWLMV), a soilborne (vector unknown), 30-nm isometric virus, was discovered in 1979 in New York (1) and has since been found in seven other northeastern and northcentral states (15). A variety of mottle and mosaic patterns are commonly observed on MWLMV-infected maize (*Zea mays* L.); discrete, chlorotic white lines are characteristic of the disease. Symptomless infections are common (14). Yield losses as high as 45% in field corn and 100% in sweet corn (all ears unmarketable) have been reported (15).

Sometimes after rate-zonal centrifugation steps in the purification of MWLMV, we observed a UV-absorbing zone that sedimented more slowly than the MWLMV particles. This zone contained small (17 nm) isometric particles that had not been seen in previous work with MWLMV in the U.S. and Italy (5–7,13). We later learned that Lapierre et al (11) had described a 17-nm particle associated with maize dwarf ringspot virus (MDRV) in France in 1976. MDRV is serologically related to MWLMV (5).

The small particles associated with MWLMV and MDRV are morphologically similar to the well-studied satellite virus of tobacco necrosis virus (TNV) (8), to the satellitelike particles associated with panicum mosaic virus (PMV) (4,19) including the St. Augustine decline strain (PMV-SAD) (12), and to a top component associated with cucumber necrosis virus (CNV) (20). In this communication, the 17-nm particle associated with MWLMV will be referred to as "satellitelike virus" (SV). We report here some properties of MWLMV and SV and the relationship of SV to other satellite and satellitelike particles.

MATERIALS AND METHODS

Virus source. SV and MWLMV were obtained from Seneca Chief sweet corn (*Zea mays* L. 'Saccharum') plants inoculated by sowing seeds in soils that formerly contained MWLMV-infected plants. These soils had been collected from two locations in Ohio. Infected plants were maintained in greenhouses at 27 ± 5 C day and 21 ± 3 C night temperatures, respectively.

Antisera. Antisera to the small and large particles of MDRV were prepared by H. Lapierre (INRA, Versailles, France) and obtained from M. Conti (Laboratorio di Fitovirologia, Turin, Italy). Antisera to TNV satellite virus strains 1 and C and to TNV-A were provided by D. A. Govier and B. Kassanis (Rothamsted Experimental Station, Harpenden, Hertsfordshire, England). Isolates of PMV, PMV-SAD strains H and N, and antisera to them were obtained from R. W. Toler (Texas A & M University, College Station). Additional PMV, PMV-satellite, PMV-SAD, and PMV-SAD-satellite antisera were supplied by C. L. Niblett (University of Florida, Gainesville). Antiserum to CNV was obtained from J. H. Tremaine (Agriculture Canada, Vancouver, BC).

Purification. MWLMV-SV-infected maize leaf or root tissue was ground in a blender with phosphate-citrate buffer, pH 6.0, plus 0.5% 2-mercaptoethanol (3 ml of buffer per gram of tissue). The buffer was prepared by mixing 0.2 M K_2HPO_4 and 0.1 M citric acid. Leaf homogenates were pressed through cheesecloth and clarified by emulsifying with 1/3 vol of chloroform. The aqueous phase was recovered after low-speed centrifugation (9,000 *g* for 15 min). The virus was pelleted by high-speed centrifugation (130,000 *g* for 2 hr) and resuspended in phosphate-citrate buffer (1/100 to 1/50 of the original extract volume). Concentrated virus was layered onto 10–40% linear sucrose gradients that were then centrifuged at 5 C in the Beckman SW 50.1 or SW 41 rotor at 40,000 rpm to an ω^2t of 1.0×10^{11} rad^2 sec^{-1} (about 100 min) or 1.5×10^{11} rad^2 sec^{-1} (about 150 min), respectively (Beckman Instruments, Inc., Palo Alto, CA). Centrifuged gradients were scanned at 254 nm and fractionated. The SV peak was diluted with phosphate-citrate buffer and the virus was pelleted, suspended, and treated with 1/10 vol of undiluted MWLMV antiserum (13) to remove contaminating MWLMV particles. The treated SV preparation was then sedimented through a second sucrose gradient, recovered by centrifugation, and used for characterization and antiserum production.

Serology. Antiserum to MWLMV was prepared previously (13). Antiserum to SV was raised in a rabbit by two intramuscular injections, spaced 2 wk apart, containing a total of 0.12 mg of SV. Before injection, the virus was suspended in 0.14 M sodium chloride, 0.01 M potassium phosphate, pH 7.0, (PBS), and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1985.

emulsified with an equal volume of Freund's complete adjuvant. Antiserum was collected 1 mo after the second injection, mixed with an equal volume of glycerol, and stored at −20 C.

Microprecipitin assays and enzyme-linked immunosorbent assays (ELISA) were performed as previously described (9 and 13, respectively). Gel double-diffusion assays were done in petri dishes (15-cm diameter) containing 12 ml of 0.5% Ionagar #2 in PBS or phosphate-citrate buffer. Reactions were observed after 24 and 48 hr.

For immuno-rate-zonal centrifugation assays, tissue was processed through the first high-speed concentration of the purification procedure. Gradients (SW 50.1 rotor) were prepared and run as for SV purification except that 0.1 ml of the antiserum to be tested was mixed with 0.1 ml of resuspended virus before layering onto gradients. Two gradients were run for each antiserum-virus combination.

Electrophoresis. Protein was released from purified SV and MWLMV by heating at 100 C for 2 min in 0.1 M tris, 1% SDS, and 1% 2-mercaptoethanol, pH 7.3. Samples were run on 12.5% polyacrylamide gel slabs (10). After staining with Coomassie Blue R-250, the lanes were excised and scanned at 580 nm. Reference proteins, treated similarly, were: bovine serum albumin, 68 kdaltons; ovalbumin, 43 kdaltons; carbonic anhydrase, 25 kdaltons; and pancreatic ribonuclease, 13.7 kdaltons.

RNA was extracted from SV, MWLMV, and the brome mosaic virus (BMV) and tobacco mosaic virus (TMV) standards by adding 1/10 vol of 10% SDS to virus suspensions in 0.09 M tris, 2.5 mM disodium EDTA, 0.9 M boric acid (tris-EDTA-borate buffer) (pH 8.0) and extracting with 2 vol of water-saturated phenol:m-cresol (9:1 v/v) containing 0.1% (w/v) of 8-hydroxyquinoline. The aqueous phase was recovered by centrifugation (3,000 *g* for 10 min). The phenol phase was reextracted with 1/2 vol of tris-EDTA-borate buffer. The combined aqueous phases were reextracted with 1 vol of phenol-cresol and this phenol phase then reextracted with 1/2 vol of tris-EDTA-borate buffer. The aqueous phases were combined and NaCl was added to 0.2 M. Three vol of cold 95% ethanol were added and the RNA was allowed to precipitate overnight at −20 C. The relative mass (M_r) of each RNA was estimated, after denaturation in glyoxal (16), by electrophoresis in agarose gels as described by Murant et al (18) except that 1% Sigma Type I, low EEO agarose (Sigma Chemical Co., St. Louis, MO 63178) was used in a vertical slab gel apparatus.

RNase and DNase sensitivity were determined by incubating SV-RNA and MWLMV-RNA separately with either DNase at 20 μg/ml in 0.14 M NaCl, 0.01 M potassium phosphate, 0.01 M $MgSO_4$, pH 7.0; pancreatic RNase at 20 μg/ml in 0.01 M potassium phosphate, pH 7.0, (low salt), or RNase at 20 μg/ml in 0.3 M NaCl, 0.01 M potassium phosphate, pH 7.0, (high salt) for 20 min at 37 C. Control samples were heated without enzyme. Enzymes were inactivated by incubating with proteinase K at 50 μg/ml for 20 min at 37 C. The samples were then electrophoresed in 1% agarose gels.

Sedimentation rate. The linear-log method of Brakke and Van Pelt (3) was used.

Buoyant density. Purified virus suspensions were adjusted to 1.35 g/ml with solid CsCl and centrifuged at 35,000 rpm for 48 hr in the Beckman SW 50.1 rotor at 10 C. Densities of 0.4-ml gradient fractions were determined by weighing 50-μl aliquots in a micropipette previously calibrated with water. Gradient density at the peak of virus-zone absorbance (254 nm) was considered the virus buoyant density.

Electron microscopy. Virus fractions were stained on carbon-coated Formvar grids with 2% ammonium molybdate adjusted to pH 7.0 with HCl and viewed in a transmission electron microscope. TMV was placed on some grids as a size reference (width = 18 nm).

RESULTS

Absorbance profiles of sucrose density gradients containing extracts from MWLMV-infected plants sometimes contained a UV-absorbing zone (I) above the MWLMV zone (II) (Fig. 1). Zone I contained mostly small isometric particles, some larger isometric particles filled with stain that appeared to be MWLMV particles devoid of nucleic acid, and a few MWLMV particles (Fig. 2A). Zone II contained the expected MWLMV particles, some stain-filled particles, and a few small particles (Fig. 2B). The diameters of the SV, larger stain-filled, and MWLMV particles were estimated to be 17, 28, and 30 nm, respectively.

Purification. The purification procedure for SV was a modification of that used for MWLMV (13). After treatment of Zone I with MWLMV antiserum followed by a second rate-zonal centrifugation, no MWLMV particles (stain-filled or not) were seen, and such preparations did not react with MWLMV antiserum in gel double-diffusion, microprecipitin, or ELISA tests.

High-speed centrifugation, polyethylene glycol (PEG) precipitation, and $(NH_4)_2SO_4$ precipitation were compared as concentration methods. The highest recovery of MWLMV was achieved by high-speed centrifugation, whereas the highest recovery of SV was by PEG precipitation. Precipitation with $(NH_4)_2SO_4$ gave high yields of both particles, but there was more host material in the preparations than after centrifugation or PEG precipitation. About half of the SV particles were lost during concentration from sucrose gradient fractions with either of the three methods. Crystalline arrays of SV particles were frequently observed by electron microscopy of purified SV, especially in concentrated preparations. Yields of SV varied between 300 and 500 μg/g of tissue as estimated by the Bio-Rad protein assay (2) with bovine serum albumin as the standard.

In a test of virus preservation, samples of leaf tissue containing both MWLMV and SV were frozen or lyophilized. After 1 and 5 wk, the samples were tested for virus. Neither treatment reduced the yield of SV as judged by measuring the peak area after rate-zonal centrifugation, but nearly all the MWLMV was lost after either period or treatment.

Association of MWLMV and SV. Because SV had not been observed in previous MWLMV studies (1,6,7,13), we investigated the frequency of association of SV with MWLMV. Forty-four plants grown in soil from two Ohio locations with a history of MWLMV were examined. Data from the two soils were combined. Because symptomless MWLMV infections are known (14), plants with and without symptoms were examined by rate-zonal centrifugation assay to see if the presence of SV affected symptom expression. The results are in Table 1. There was no apparent

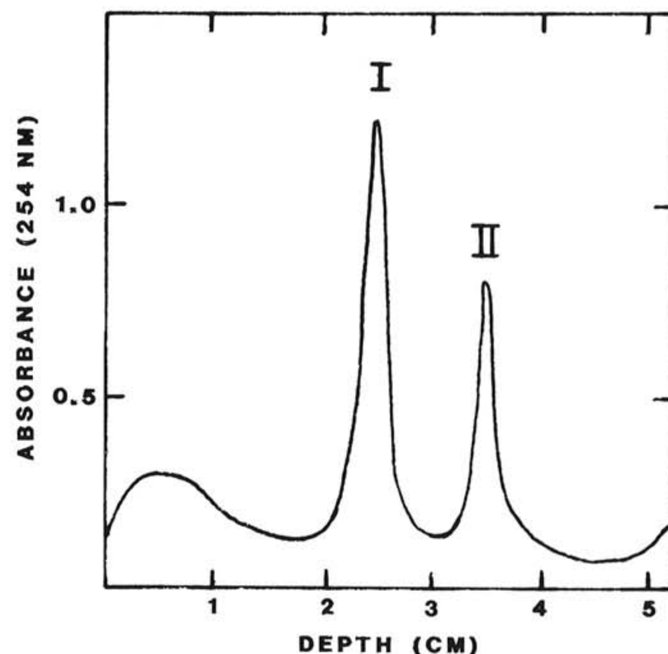

Fig. 1. Rate-zonal sucrose density gradient profile of a concentrated extract from 1 g of maize leaf tissue taken from a plant infected with satellitelike virus (SV) (Zone I) and maize white line mosaic virus (MWLMV) (Zone II). Centrifugation was to $\omega^2 t = 1.0 \times 10^{11}$ rad^{2}sec^{-1} at 40,000 rpm (about 100 min) at 5 C on a 10–40% linear sucrose gradient.

difference in symptoms between plants with one or both particles in the leaves, nor was there any consistent pattern in the relative amounts of SV and MWLMV in either roots or leaves, i.e., some samples had more SV and others had more MWLMV, as judged by the areas under the peaks after rate-zonal centrifugation.

Properties of SV. With MWLMV as the reference particle (117S [6]), the sedimentation rate of SV on linear-log gradients was about 48 ± 4S (mean value of separate determinations of three preparations).

Buoyant densities of SV and MWLMV in CsCl dissolved in phosphate-citrate buffer were 1.355 ± 0.003 g/ml and 1.337 ± 0.004 g/ml, respectively. Three measurements on each of two preparations for each particle were made.

Single coat proteins with M_r's of 24.7 ± 0.8 kdaltons and 35.5 ± 1.2 kdaltons were seen for SV and MWLMV, respectively, after SDS-polyacrylamide gel electrophoresis (mean values of separate determinations of four preparations).

Sensitivity to RNase in both high and low salt and insensitivity to DNase indicated that the genome of SV was single-stranded RNA. For six determinations in agarose gels after glyoxal denaturation, the M_r of SV-RNA was estimated to be $0.44 \pm 0.06 \times 10^6$. The M_r of MWLMV-RNA in the same experiments was $1.65 \pm 0.10 \times 10^6$.

The ultraviolet absorbance spectrum of SV was typical for a nucleoprotein with a maximum absorbance at 259 nm, a minimum at 239 nm, and an A_{260}/A_{280} of 1.7 ± 0.1 uncorrected for light scattering (mean of three separate determinations).

Serological relationship between SV and MWLMV. No cross reactions were observed between SV and MWLMV in either microprecipitin or gel double-diffusion tests. Lack of serological cross reactivity was confirmed by immuno-rate-zonal centrifugation tests in which only the homologous particles were removed from the gradients (Fig. 3). For these tests, a partially purified virus preparation with SV and MWLMV zones of about equal UV absorbance was used. Various amounts of each antisera were used. The gradients shown are those containing ten times the amount of antiserum needed to eliminate the homologous particle.

Serological relationship between SV and other viruses. Neither SV nor MWLMV reacted with antisera to TNV satellite virus-1 or -C, SAD-H, SAD-N, PMV, CNV, or TNV-A in immuno-rate-zonal tests. In a reciprocal test, no reaction was seen between SV antiserum and the SAD satellite whereas the antisera to SAD-H, SAD-N, and PMV removed the SAD satellite. The other antisera were not tested against their homologous particles. However, antisera to the large and small MDRV particles reacted with both SV and MWLMV in microprecipitin, gel double-diffusion, and immuno-rate-zonal tests. In gel double-diffusion tests with these antisera and extracts from MWLMV-SV-infected plants, two precipitin bands were seen; one fused with the band between SV and its antiserum, and the other fused with the band between MWLMV and its antiserum. These results indicated that both antisera to the MDRV particles contained antibodies to both particles.

TABLE 1. Presence of maize white line mosaic virus (MWLMV) and satellitelike viruses (SV) in plants grown in MWLMV-infested soils

Number of plants	Foliar symptoms	Presence[a] of particles in: Roots		Leaves	
		MWLMV	SV	MWLMV	SV
10	−	−	−	−	−
6	−	+	+	−	−
11	+	+	+	+	+
17	+	+	+	+	−

[a]Determined by the presence of UV-absorbing zones in rate-zonal centrifugation assays of partially purified preparations.

DISCUSSION

Although SV was associated with MWLMV in these experiments, it had not been seen previously except for the MDRV study (11). When SV was not found, it is possible that SV was not present or that it was present in the roots (not used in those studies) but not in the leaves, a common condition in the plants we examined. It is not known if MWLMV can infect maize roots in the absence of SV.

The SV occurred in high concentrations in infected tissue and was relatively easy to purify despite the 50% losses we sustained during the concentration steps. Kassanis (8) ascribed losses of TNV satellite virus in purified preparations to spontaneous crystallization. We do not know if a similar phenomenon occurred in SV preparations, although the crystalline arrays of SV particles seen by electron microscopy suggest this possibility.

According to Murant and Mayo (17), three criteria must be met before a particle can be classified as a true satellite: the particle must be unable to multiply in cells without the assistance of the helper virus; the particle must be unnecessary for multiplication of the helper virus; and there must be no appreciable sequence homology between the genomes of the particle and the helper virus. The size of SV and its association with MWLMV suggests that it is a satellite. However, because we cannot test criteria 1 and 2 until transmission of purified MWLMV and SV is possible, and because criterion 3 has not been addressed, we retain the designation "satellitelike". The vector(s) for MWLMV and SV is(are) unknown. Strictly

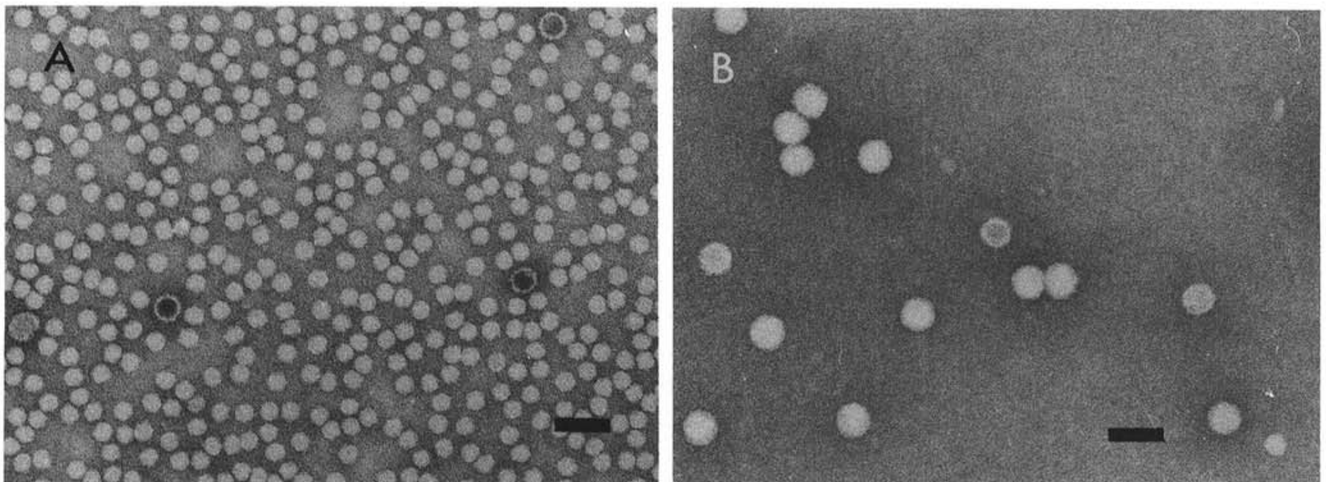

Fig. 2. Electron micrographs showing particles present in zones I and II (Fig. 1). The zones were collected, diluted 3- to 5-fold, and centrifuged 2 hr at 40,000 rpm in a Beckman SW 50.1 rotor. Pellets were resuspended in phosphate-citrate buffer. Samples were negatively stained in 2% ammonium molybdate, pH 7.0. The magnification bars represent 50 nm. **A,** Zone I. **B,** Zone II.

speaking, the MWLMV particle should be designated MWLMVLP (VLP = viruslike particle) because its infectivity has not been demonstrated. However, MWLMV is used for simplicity.

Our estimate of the size of SV-RNA ($M_r = 0.44 \times 10^6$) agrees reasonably well with the 0.4×10^6 value reported by Lapierre et al (11) for the MDRV satellitelike particle. The TNV satellite RNA also has an M_r of about 0.4×10^6 based on its nucleotide sequence (22). The M_r of 1.65×10^6 for MWLMV-RNA that we obtained under denaturing conditions is larger than that obtained by de Zoeten et al (6) under nondenaturing conditions (1.25×10^6), but similar to the 1.5×10^6 estimate for the RMA from the large MDRV particle (11) and the $1.3–1.6 \times 10^6$ value for TNV-RNA (21).

The serological relationships between MWLMV and MDRV and their respective satellitelike particles, indicate that they represent separate isolations of the same or related viruses. Our observation that the antisera to MDRV and its associated satellitelike particle both contain antibodies to MWLMV and SV explains why Conti (5) observed a reaction with both of these antisera to his preparations of MWLMV that did not contain SV.

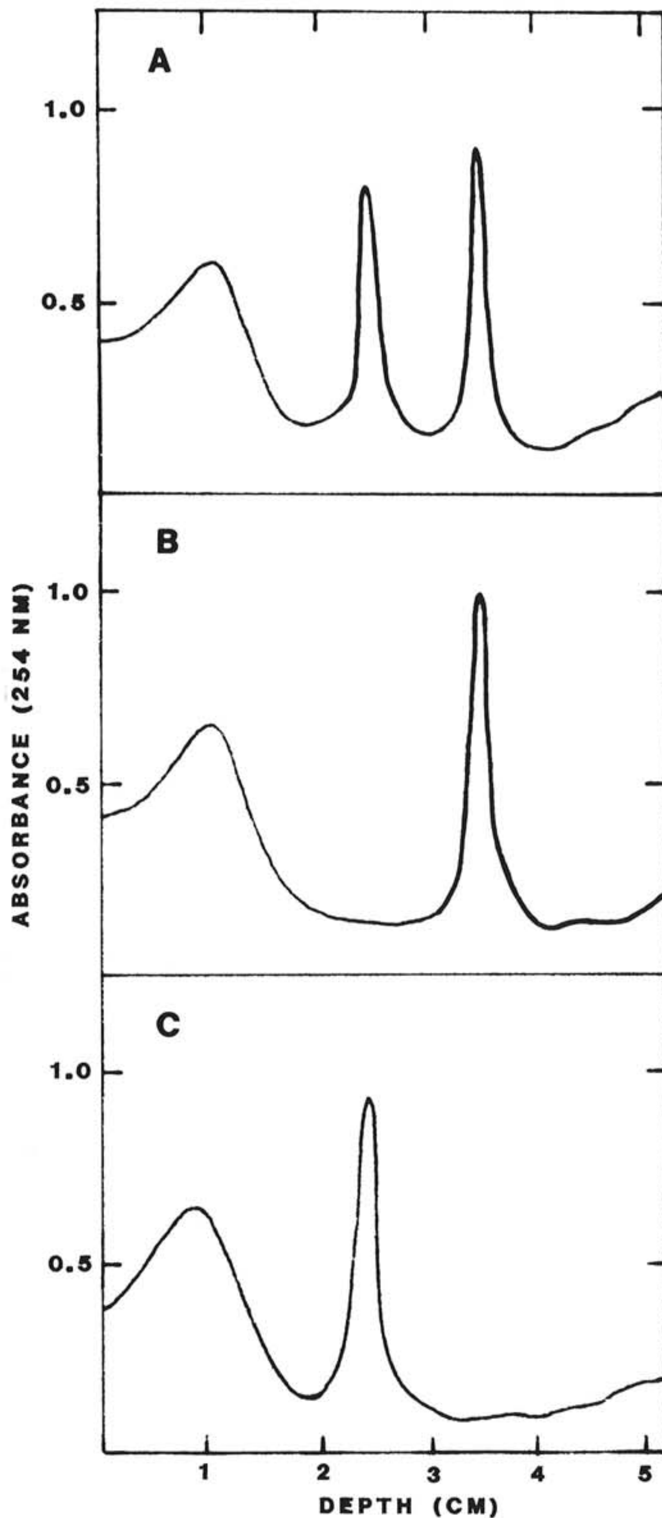

Fig. 3. Immuno-rate-zonal centrifugation profiles of concentrated extracts of leaves from an MWLMV-SV-infected plant. Aliquots (0.1 ml) of the extract were treated with 0.1 ml of various antisera diluted 1:10 and then centrifuged to $\omega^2 t = 1.0 \times 10^{11}$ rad^{2}sec^{-1} at 40,000 rpm (about 100 min) at 5 C on 10–40% linear sucrose gradients. **A,** Preimmune serum. **B,** SV antiserum. **C,** MWLMV antiserum.

LITERATURE CITED

1. Boothroyd, C. W., and Israel, H. W. 1980. A new mosaic disease of corn. Plant Dis. 64:218-219.
2. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.
3. Brakke, M. K., and Van Pelt, N. 1970. Linear-log sucrose gradients for estimating sedimentation coefficients of plant viruses and nucleic acids. Anal. Biochem. 38:56-64.
4. Buzen, F. G., Niblett, C. L., and Hooper, G. R. 1977. A possible satellite virus of panicum mosaic virus. Proc. Am. Phytopathol. Soc. 4:132.
5. Conti, M. 1983. Maize viruses and virus diseases in Italy and other Mediterranean countries. Pages 103-112 in: Proc. Int. Maize Virus Dis. Colloq. Workshop, 2-6 August 1982. D. T. Gordon, J. K. Knoke, L. R. Nault, and R. M. Ritter, eds. Ohio State University, Ohio Agric. Res. Dev. Center, Wooster.
6. de Zoeten, G. A., Arny, D. C., Grau, C. R., Saad, S. M., and Gaard, G. 1980. Properties of the nucleoprotein associated with maize white line mosaic in Wisconsin. Phytopathology 70:1019-1022.
7. Gotlieb, A. R., and Liese, A. L. 1980. White line mosaic and stunt of field and sweet corn in Vermont associated with polyhedral virus infection. (Abstr.) Phytopathology 70:462.
8. Kassanis, B. 1970. Satellite virus. No. 15 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biologists, Kew, Surrey, England. 4 pp.
9. Knoke, J. K., Louie, R., Anderson, R. J., and Gordon, D. T. 1974. Distribution of maize dwarf mosaic and aphid vectors in Ohio. Phytopathology 64:639-645.
10. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-684.
11. Lapierre, H., Moreau, J. C., and Molin, G. 1976. Une nouvelle maladie a virus de mais: Mosaique d'anneaux foliaires du Bugey (France). Poljopr. Znan. Smotra 39:187-188. (In French).
12. Lee, T. A., Jr. 1973. Isolation, purification and characterization of the virus causing St. Augustine decline. Ph. D. dissertation. Texas A & M University, College Station. 66 pp.
13. Louie, R., Gordon, D. T., Knoke, J. K., Gingery, R. E., Bradfute, O. E., and Lipps, P. E. 1982. Maize white line mosaic virus in Ohio. Plant Dis. 66:167-170.
14. Louie, R., Gordon, D. T., Madden, L. V., and Knoke, J. K. 1983. Symptomless infection and incidence of maize white line mosaic. Plant Dis. 67:371-373.
15. Louie, R., Knoke, J. K., Gordon, D. T., and Gingery, R. E. 1983. Maize white line mosaic and maize subtle mosaic viruses. Pages 139-140 in: Proc. Int. Maize Virus Dis. Colloq. Workshop, 2-6 August 1982. D. T. Gordon, J. K. Knoke, L. R. Nault, and R. M. Ritter, eds. Ohio State University, Ohio Agric. Res. Dev. Center, Wooster.
16. McMaster, G. K., and Carmichael, G. G. 1977. Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. Proc. Nat. Acad. Sci. USA 74:4835-4838.
17. Murant, A. F., and Mayo, M. A. 1982. Satellites of plant viruses. Annu. Rev. Phytopathol. 20:49-70.
18. Murant, A. F., Taylor, M., Duncan, G. H., and Raschke, J. H. 1981. Improved estimates of molecular weight of plant virus RNA by agarose gel electrophoresis and electron microscopy after denaturation with glyoxal. J. Gen. Virol. 53:321-332.
19. Niblett, C. L., and Paulsen, A. Q. 1975. Purification and further

characterization of panicum mosaic virus. Phytopathology 65:1157-1160.
20. Tremaine, J. H. 1972. Purification and properties of cucumber necrosis virus and a smaller top component. Virology 48:582-590.
21. Uyemoto, J. K. 1981. Tobacco necrosis and satellite viruses. Pages 123-146 in: E. Kurstak ed., Handbook of Plant Virus Infections and Comparative Diagnosis. Elsevier/North Holland. Amsterdam.
22. Ysebaert, M., van Emmelo, J., and Fiers, W. 1980. Total nucleotide sequence of a nearly full-size DNA copy of satellite tobacco necrosis virus RNA. J. Mol. Biol. 143:273-287.